MICROBIAL PROCESSES AND THEIR RELATIONSHIPS WITH ENVIRONMENTAL TRAITS IN DEGRADED TROPICAL PEATLANDS

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Declaration

I hereby declare that the thesis is my original work and it has been written by me in its entirety. I have duly acknowledged all the sources of information which have been used in the thesis.

This thesis has also not been submitted for any degree in any university previously.

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Shailendra Mishra

(December, 2014)

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Publications

Journal

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Conference

Invited talk as keynote speaker

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Poster

- Yung P Y, Umashankar S., Xie C., **Mishra S.**, Lu F., Drautz D., Benke P., Sudiana I. M., Idris A., Thomas T., Schuster S., and *Swarup S*.: *"Metagenomic analysis of microbial community in tropical low-lying peatlands"*, 13th EMBO conference Aquatic Microbial Ecology (SAME), 8-13th Sep 2013, Italy
- **Mishra S.**, Reuben, S., Lee, W. A., Hooijer, A., Idris, A., Jianjun W., Liew S.C., Sudiana, I. M., Yung P. Y. and Swarup, S.: *"Land use changes in tropical peatlands affect microbial and metabolic processes"*, International Conference, International Society of Microbial Ecology (ISME) – 14, Aug 2012, Copenhagen, Denmark

Contents

Summary

Southeast Asian peatlands are undergoing degradation through drainage and deforestation, accompanied with forest fires at unprecedented rates, for agriculture and human settlement purposes. Exposure of carbon-rich peat to air has resulted in enhanced microbial-mediated peat oxidation, emitting copious amounts of potent greenhouse gases (GHGs). In addition, peat oxidation results in land subsidence, thus, increasing the risk of flooding that can affect the livelihoods of millions of people.

The composition and quantity of GHGs, as well as microbial processes responsible for this peat oxidation, are different from temperate and tropical regions. As peat oxidation is extremely temperature–dependent, higher decomposition profile and rate are expected for tropical climates, compared to temperate. It is, therefore, critical to uncover the microbial processes leading to GHGs from tropical regions, which is as yet unexplored. Hence, *the overall aim of this study is to provide an in-depth understanding of how land-use change and peat management practices, affect the microbial ecology and physicochemical processes, leading to peat oxidation and release of GHGs*.

The first part of this study was aimed to understand the associations of microbial and physicochemical profiles with land-use patterns, hydrology and key environmental traits. We adopted molecular marker–based approaches (microbial and metabolic profiles) that revealed the profiles

were most influenced by variations in water table and land-use patterns, followed by age of drainage and peat thickness in that order. The sites with low water table depths undergo rapid fluctuations in water level leading to frequent drying-wetting, than those with high water table, thus, providing insights of increased peat oxidation in low water table regions. This part of the study also demonstrated that mixed crop plantations sites, (i) had high DOC, (ii) a high diverse metabolic profile, (iii) support a more diverse microbial community structure and (iv) importantly experience a lower rate of peat subsidence, a proxy for oxidation-led peat loss. Therefore, the findings led to evidence that subsidence is linked with two aspects, (i) it increases due to drying-wetting which is frequent in low water table sites and (ii) it reduces while mixed cropping practices are adopted.

In the second part of this study, we aimed to determine the functional potential of peat microbiome that is associated with peat oxidation. The outcomes using next-generation sequencing approach, revealed that one-carbon metabolism or methane metabolism was positively correlated with subsidence rate and carbon dioxide emissions. Metagenome analysis indicated that *Actinobacteria* was found abundant in degraded forest and oil palm plantations sites that had high peat oxidation rates in forms of subsidence and CO_2 emissions. These species are known to play an important role in aerobic cellulose degradation and other organic plant materials, including lignin and chitin. Hence, these findings possibly

explain why CO_2 and not CH_4 is the major GHG emitted from tropical peatlands, unlike those in temperate regions.

The last part of this study was a laboratory-based controlled study, which was performed to validate the hypothesis generated based on findings from first part of this study. This microcosm study was aimed to understand the physiological responses of peat microbiome that lead to gas emissions before and after rewetting. It positively validated the hypothesis from the first part of the study that low water table that undergo frequent drying-wetting cycles, had accelerated amount of CO_2 emissions (lab data) leading to continued high subsidence (field data) upon rewetting as well. It also revealed that microbial metabolic functions, such as, metabolism of aromatic compounds, amino acids, xenobiotics and carbohydrate metabolism that are distributed among diverse taxa are likely to govern the changes (non-reduced CO_2 emissions) upon rewetting, rather than specific microbial assemblages.

The overall findings of this study will be useful in peatland management by providing a basis to focus early efforts on hydrological interventions, especially in low water table regions to reduce repeated drying-wetting and improving sustainability of oil palm plantations by adopting mixed cropping practices.

List of Abbreviations

Abbreviation	Explanation
Gt	Gigatonnes
Mha	Million hectares
Mt	Million tonnes
$tCO_2\ ha^{-1}\ yr^{-1}$	Tones of CO2 per hectare per year
$g\ C\ m^{-2}\ yr^{-1}$	Gram Carbon per square meter per year
CO_{2e}	CO_2 equivalent
km^2	Square kilometer
cm	Centimeter
m	Meter
min	Minutes
ng	Nanogram
µL	Microliter
mL	Milliliter
$cmyr^{-1}$	Centimeter per year
gcm^{-3}	Gram per cubic centimeter
mm	Millimeter
µg/gm	Microgram per gram
m^3	Cubic meter
ppm	Parts per million
kDa	Kilo Dalton
GHG	Greenhouse gas
CO_2	Carbon dioxide
CH_4	Methane
N_2O	Nitrous oxide
IPCC	Intergovernmental Panel on Climate Change
SE Asia	Southeast Asia
PCR	Polymerase chain reaction
MS	Mass spectrometry
gDNA	Genomic Deoxyribonucleic acid
TRF	Terminal restriction fragment
DOC	Dissolved organic carbon
SOC	Soil organic carbon
T-RFLP	Terminal restriction fragment length polymorphism
MWCO	Molecular weight cut – off

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Chapter 1: Introduction

Southeast Asia is rich with natural resources that support both human lives and natural ecosystems. The World Climate Change Report Intergovernmental Panel on Climate Change (IPCC 2007; Table 10.11) has listed Southeast (SE) Asia as one of the most vulnerable regions in the world for climate change. Land degradation is one of the most vulnerable targets, which is resulting in large amount of greenhouse gas emissions (GHGs). In SE Asia, the largest contribution to these emissions is from peatlands. Other important contributions are from tropical land-use change and rapid expansion of urbanization.

Natural peatlands are formed by the accumulation of partially decayed vegetation matter over millennial timescales in low-lying areas that are frequently waterlogged due to heavy rainfall and/or periodic inundation (Anderson, 1964). At a global scale, peatlands are a highly vulnerable natural resource that cover 50-70% of global wetlands (Finlayson et al., 1999) and sequester one-third of the world's soil carbon (Freeman et al., 2012). In Southeast Asia, peatlands cover an area of nearly 25 million hectare (Mha) and store approximately 69 Gt of carbon, which is 77% of the world's tropical peatland carbon pool (88.6 Gt), of which 65% (57.4 Gt of carbon) is in Indonesia itself, distributed within 23.4Mha of peatland (Page et al., 2011). These pristine peatlands sequester net-carbon,

slowing the release of CO_2 and thereby, countering anthropogenic impacts on the Earth's atmosphere, and ultimately weather and climate systems.

Southeast Asian natural peatlands are undergoing drainage and deforestation, including forest fires at unprecedented rates, making way for commercial oil palm and Acacia plantations. These industrial-scale plantations on deforested, drained and burnt degraded peatland covers over 3.1Mha (approximately 20%) of the peatlands of Peninsular Malaysia, Sumatra and Borneo in 2010, an area expected to grow to 6.2Mha by 2020 (Miettinen et al., 2012a). Exposure of carbon-rich peat to air by drainage and deforestation has resulted in enhanced microbial mediated peat oxidation. A copious amounts of potent GHGs (carbon dioxide and methane), altering atmospheric quality is emitted due to this oxidation. The current carbon emissions are estimated at 230– 310 Mt CO_{2e} annually and predicted to rise to nearly 1 Gt CO_{2e} annually by 2020 (Miettinen et al., 2012b). This has led to GHG emissions from drained Southeast Asian peatlands, being considered the single largest anthropogenic environmental disaster at the regional scale, directly impacting global climate regimes. Forest fires and biomass burning linked to land-use change exacerbate these threats, by contributing additional GHGs and aerosols to the atmosphere. The peatland fires in 1997 approximately liberated between 0.81 Gt and 2.57Gt of carbon which is equivalent to 13% to 40% of mean annual global carbon emissions from fossil fuels (Page et al., 2002). In addition, loss of peat by microbial

oxidation results in land subsidence that increases the risk of flooding leading to unfavourable long-term socio-economic consequences apart from immediately affecting the liveability in that region. It is, therefore, critical to understand terrestrial activities that have such widespread impacts in the region.

Land-use changes and hydrological interventions have resulted in drastic decrease in water tables exposing the biomass sequestered in the peat to air. This atmosphere-exposed peat, full of live and dead roots and woody debris is highly prone to oxidation due to microbial activities. In most ecosystems, changes in land-use patterns impact both microbial diversity and their activity (Wardle et al., 1998; Ollivier et al., 2011; Putten 2012). While geochemical conditions affect microbial communities, they, in turn, affect the environment. It has been extensively reviewed in many ecosystems that environmental conditions affect the functional role as well as structure of microbial communities (Werner et al., 2011; Hanson et al., 2012). Temperature and genesis of peat, leading to varied peat types (reviewed in Chapter 2), are predominant factors that affect microbial community structure/functions and, in turn, are responsible for vast difference in the higher amount of CO_2 (Couwenberg et al., 2010; Murdiyarso et al., 2010; Page et al., 2011) and under-estimated amount of CH_4 from tropical regions (Jauhiainen et al., 2005, 2008, 2010). On the contrary, higher hot spots of CH_4 emission have been observed in temperate and boreal peatlands (Hendricks et al., 2007; Teh et al., 2011).

Hence, it is quite evident that peat oxidation as well as the nature of GHGs emitted due to this microbial-mediated oxidation is extremely temperature–dependent (Jauhiainen et al., 2014). In a recent field study (Jauhiainen et al., 2014), where a shading field experiment in the tropical peat was conducted, it was shown that heterotrophic respiration in drained tropical peat is greatly affected by temperature change. Based on above factors, it has been suggested that highest decomposition contributions are expected for tropical climates, compared to temperate (Page et al, 2011). With respect to microbial ecology of temperate peatlands, much is known about the relationship of microbial diversity to peatland functioning and greenhouse gas emissions (Opelt et al., 2007; Ausec et al., 2009; Tveit et al., 2012). In contrast, for tropical peatlands, we have a relatively poor understanding of the microbial ecology, their functional potential as well as their relationship with the GHG emission. It is, therefore, critical to uncover the unique, unexplored microbial processes leading to GHGs from tropical regions. With this motivation, the overall aim of this study is to provide an in-depth understanding of how land-use change and peat management practices affect the microbial ecology and physicochemical processes leading to peat oxidation and release of GHGs. To meet this overall goal, following specific objectives were set:

1. To understand the associations of microbial and physicochemical profiles with land-use patterns, hydrology and key environmental traits

2. To determine the functional potential of peat microbiome that is associated with peat oxidation in a field – based study
3. To understand the microbial physiological responses leading to gas emissions before and after rewetting of peat in a microcosm study

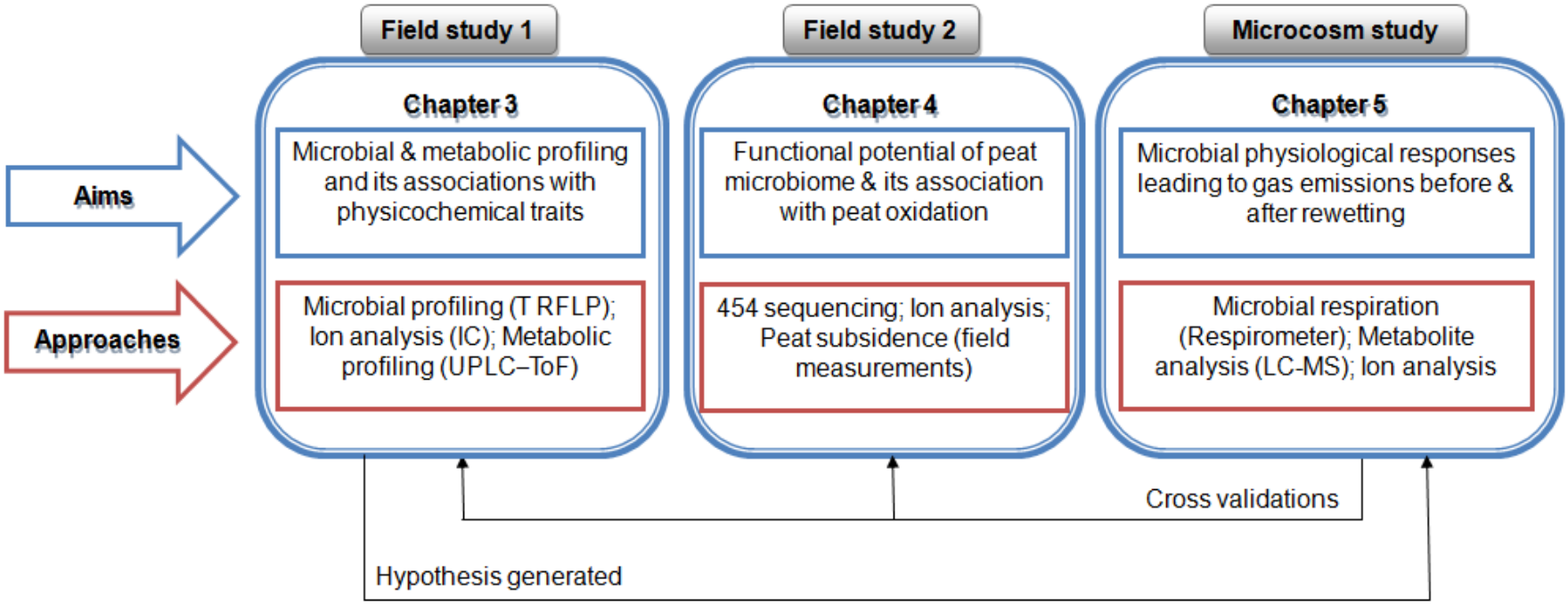
The overall project was divided into two field studies and one controlled microcosm study. While field studies 1 and 2 focused on first two objectives, respectively, microcosm study focused on objective 3.

- Chapter 1 gives a general introduction of the project, including the research gaps and provides an overview of the thesis organization. Chapter 2 reviews the literature and provides the necessary insights into the current state of research in the field.
- The results-based Chapter 3 demonstrates molecular marker–based approaches (microbial and metabolic profiles) that revealed the profiles were most influenced by variations in water table and land-use patterns, followed by age of drainage and peat thickness in that order. It also discusses better peat management practices that will help in the improved management and sustainability of tropical peatlands.
- Results-based Chapter 4, from field study 2, aims to determine the functional potential of peat microbiome that is associated with peat oxidation from contiguous land-use types: degraded forest, degraded land and oil palm plantation sites. The outcomes revealed that one-carbon metabolism is positively correlated with subsidence rate and CO_2 emissions, indicating methane oxidation, as one of the possibility

in the oxic zones of tropical peatlands. In degraded forest and oil palm plantations, *Rhizobiales* were abundant and were correlated with subsidence rates and ammonium concentrations, indicating linkages of these species with peat oxidation. *Actinobacteria* and *Firmicutes* were among the most abundant taxonomic groups, demonstrating their linkages in oxidation of tropical peatlands.

- Result-based Chapter 5, from microcosm study, aims to understand the microbial physiological responses leading to gas emissions before and after rewetting of peat. This study, revealed that the elevated carbon dioxide emissions and negligible amount of methane emissions are attributed to low water table depths. At such low depth, there would be higher frequency of rewetting during rainfall events. Thus, sites with low water depths that undergo frequent rewetting are predicted to have continuous and elevated CO_2 emissions. It also revealed that microbial metabolic functions, such as, metabolism of aromatic compounds, amino acids, xenobiotics and carbohydrate metabolism that are distributed among diverse taxa, are likely to govern the changes (non-reduced CO_2 emissions) upon rewetting, rather than specific microbial assemblages.
- Finally, Chapter 6 summarizes the major findings and highlights further questions, which can be addressed in future studies.

The overall organization of the study plan is shown below:



Chapter 2: Literature Review

2.1 Global climate change and scenario in Southeast Asia

The impacts of climate change are well evident from the recent synthetic report of Intergovernmental Panel on Climate Change (IPCC) published in 2014. During last 30 years, there has been successively warmer Earth's surface. The period from 1983 to 2012 was likely the warmest 30-year period of the last 1400 years in the Northern Hemisphere.

In context to global warming, anthropogenic greenhouse gas emissions (carbon dioxide, methane and nitrous oxide) have increased to an unprecedented rate in at least the last 800,000 years (Fig. 2.1a) (IPCC report, 2014). Between 1750 and 2011, cumulative anthropogenic CO_2 emissions to the atmosphere were 2040 ± 310 $GtCO_2$ (IPCC report, 2014). About 40% of these emissions have remained in the atmosphere (880 ± 35 $GtCO_2$); the rest were removed from the atmosphere and stored on land (in plants and soils) and in the ocean. About half of the anthropogenic CO_2 emissions between 1750 and 2011 have occurred in the last 40 years (Fig. 2.2b). Emissions of CO_2 from fossil fuel combustion and industrial processes contributed about 78% of the total greenhouse gas emissions increase from 1970 to 2010, with a similar percentage contribution for the increase during the period 2000 to 2010. As evident from recent climate change impacts, a warmer climate would increase the risk of floods.

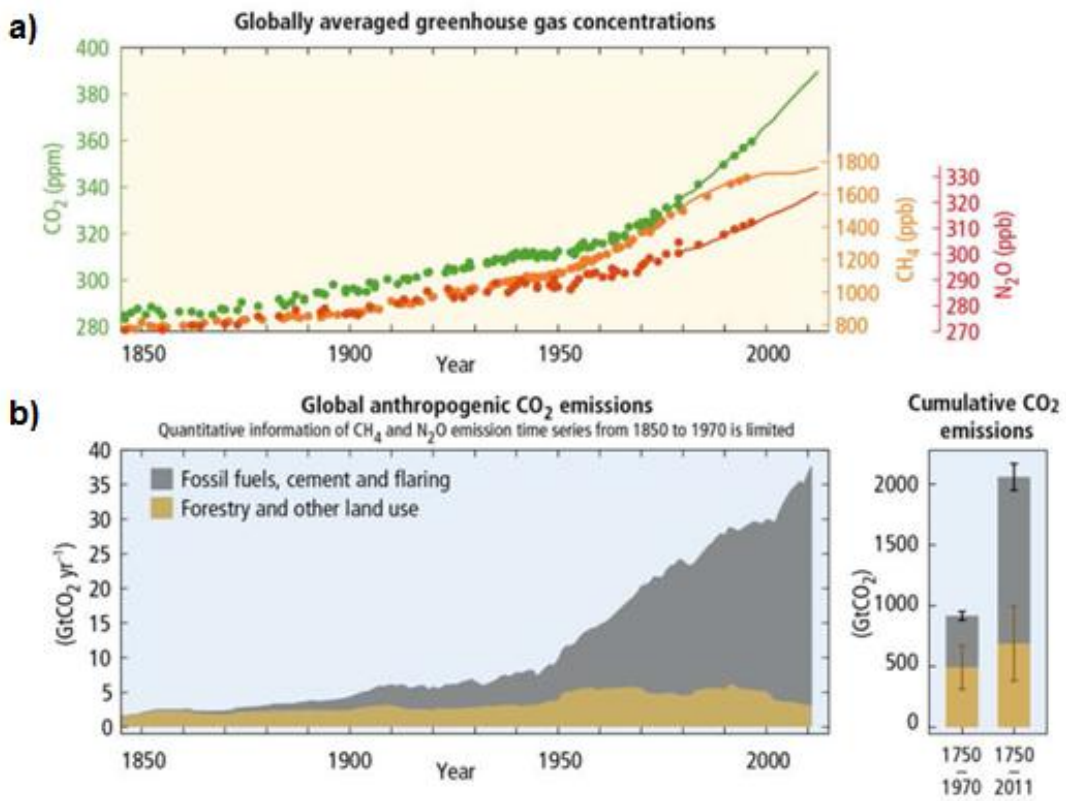


Fig 2.1: *(a) Atmospheric concentrations of the greenhouse gases carbon dioxide (CO_2, green), methane (CH_4, orange), and nitrous oxide (N_2O, red) determined from ice core data (dots) and from direct atmospheric measurements (lines). (b) Global anthropogenic CO_2 emissions from forestry and other land use as well as from burning of fossil fuel, cement production, and flaring. Cumulative emissions of CO_2 from these sources and their uncertainties are shown as bars and whiskers, respectively, on the right hand side. (**Source:** IPCC Climate Change Synthetic Report 2014)*

An ensemble of projections demonstrate a large increase in flood frequency in Southeast (SE) Asia, Peninsular India, Eastern Africa and the northern half of the Andes, with small uncertainty in the direction of change (Fig 2.2a) (Hirabayashi et al., 2013). In contrast, flood frequency will decrease in many regions of northern and eastern Europe, Anatolia, Central Asia, Central North America and southern South America. However, globally, flood frequency is expected to increase in 42% and decrease in 18% of the land grid cells (Hirabayashi et al., 2013). Out of 11

AOGCM (Atmosphere – Ocean General Circulation Model) models, the most consistent (10/11 and 11/11) likelihood of flood frequency is located in SE Asia demonstrating the apparent vulnerability of SE Asia to the global climate change. In a report submitted to Economy and Environment Program for Southeast Asia (EEPSEA), climate change vulnerability maps are shown for SE Asia (Yusuf and Francisco, 2009). While considering the five climate change related risks, such as, tropical cyclones, floods, landslides, droughts, and sea level rise, a climatic hazard maps for SE Asia predicts this region to be one of the most vulnerable part of the world (Fig. 2.2b).

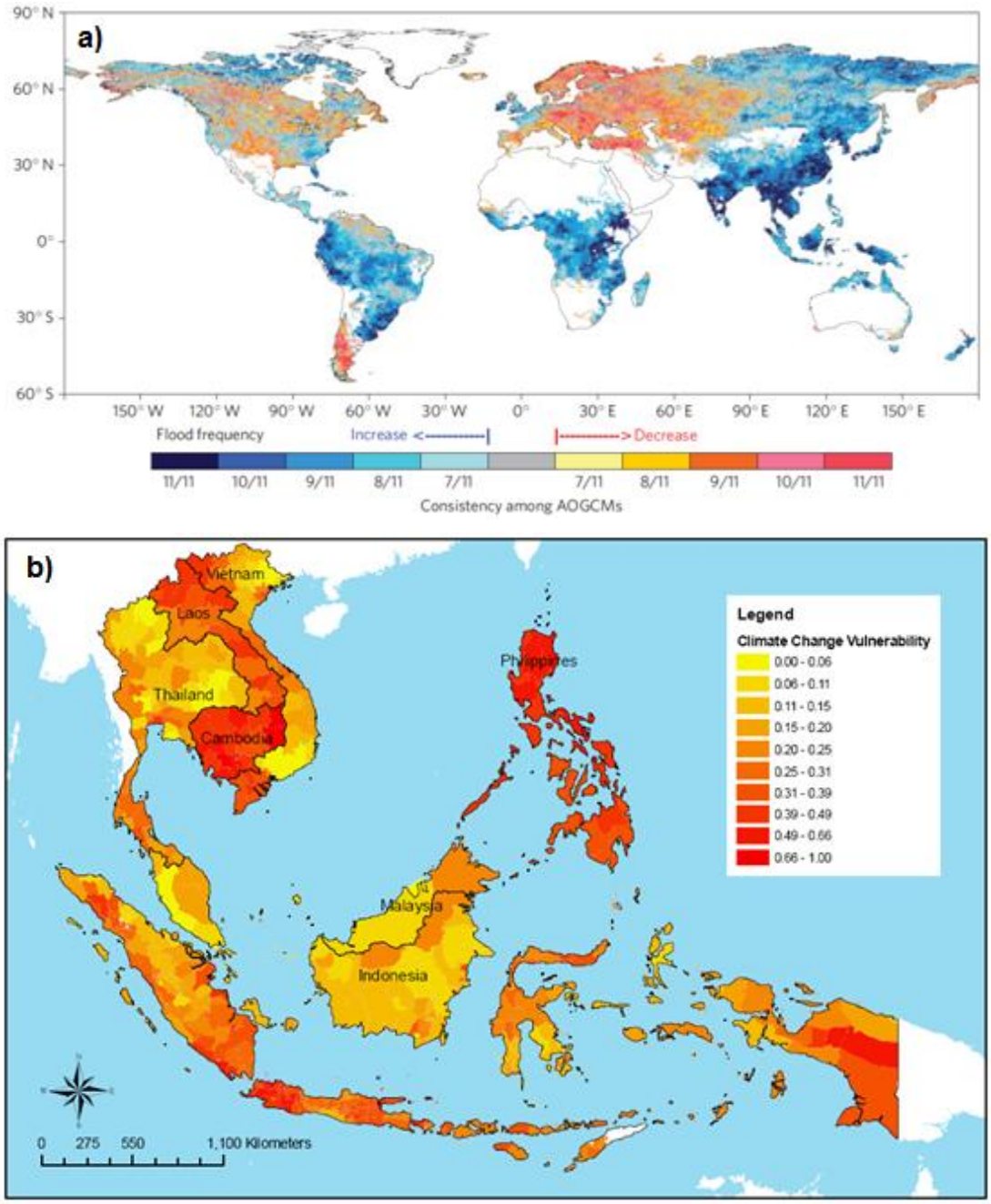


Fig 2.2: a) *Projected change in flood frequency. 11 AOGCMs was used to compute a global projection of changes in flooding and evaluate its consistency and spread (**Source:** Hirabayashi et al., 2013).* ***b)*** *Climate change vulnerability map of SE Asia the scale used is 0-1 indicating the lowest vulnerability level (0) to the highest vulnerability level (1) (**Source:** Yusuf and Francisco, 2009)*

2.2 Organic soils and natural peatlands

SE Asia, being one of the most vulnerable regions in the world for climate change has one of the major contributions from peatlands, while other tropical land-use change, rapid expansion of urbanization, and increasing economic output are other important sources. The Subsidiary Body for Scientific and Technological Advice (SBSTA) of the United Nations Framework Convention on Climate Change (UNFCCC) invited the IPCC to include lands with organic soils and with wet mineral soils, as another vulnerable source contributing to greenhouse gas estimates.

Formation of peats

The formation or genesis of peat is when the production of organic matter (through biochemical processes) is greater than its chemical breakdown. Not all organic materials are classified as peat, for example, litter is a special type of organic material and not peat. Peatlands are formed by the accumulation of partially decayed vegetation matter at rates of up to 5 millimeters per year over millennial timescales in low-lying areas that are frequently waterlogged, due to heavy rainfall and/or periodic inundation (Anderson, 1964). Peat can be classified as i) Natural Forest Peat, which is slightly aerated and ii) Peat Swamps, which is formed under swampy conditions with strongly anaerobic conditions (Kurbatov, 1968). In natural forest peat, lignin and carbohydrates appear to be decomposed so it generally has a low content of such organic compounds, whereas under

swamp conditions, peats are characterized by high contents of cutin and presence of unaltered lignin and cellulose (Table 2.1). The specific hydro-topography under anaerobic conditions, characterize peatlands, to be a marsh, swamp, bog or mire. To elaborate, (i) Mire are peatlands, where peat is currently forming and accumulating, (ii) Bogs are peatland, which receives water solely from rain and/or snow falling on its surface, (iii) Fen are peatlands, which receives water and nutrients from the soil, rock and groundwater as well as rain and/or snow (mostly found in temperate or boreal regions). Most peat in tropical region belongs to peat swamps in their natural state. As peat accumulates in the depression, beyond the level at which the water is drained from the basin, it no longer acts as an inert mass, but as an active reservoir holding a volume of water against drainage. Natural peat contains between 85 % and 95 % of water.

Table 2.1: *Peat classification (**Source:** Kurbatov, 1968)*

Fraction	Swamp Peat		Forest peat
	Carex-swamp 30% decomposed	**Reed-swamp 40% decomposed**	**Birchwood 55% decomposed**
Bitumen	3.3	1.1	8.8
Humic acids	32.2	33.6	52.2
Hemicellulose	15.0	8.6	1.0
Cellulose	3.5	3.7	0.0
Lignins	12.9	18.6	0.0
Cutin	11.9	5.2	16.0
Not determined	21.2	29.2	22.0

Organic soils are natural bodies occurring in bogs as a result of the accumulation of plant remains in a water-saturated environment. They vary in morphological features primarily because of varying degrees of alterations on different kinds of plants by microbiological activities as affected by certain local geological, chemical, topographical, and microclimatological influences. They are identified on the basis of criteria 1 and 2, or 1 and 3 listed below in Table 2.2 (IPCC 2013). Lands with organic and wet soils are crucial in maintaining the Earth's carbon balance as they contain soils with high organic carbon content (Mitra et al., 2005; Joosten and Couwenberg, 2008; Donato et al., 2011).

Table 2.2: *Classification of organic soil (FAO, 1988)*

Criteria 1	Organic horizon ≥ 10cm.
Criteria 2	≥ 20cm organic content
Criteria 3	Soils are exposed to water saturation episodes and has either: **a.** 20 % organic matter, if the soil has no clay; or, **b.** 30 % organic matter) if the soil has 60% or more clay; or, **c.** An intermediate proportional amount of organic carbon for intermediate amounts of clay.

Natural peatlands are highly vulnerable natural resource and cover 50-70% of global wetlands (Fig. 2.3a). In SE Asia, peatlands cover an area of nearly 25 million ha (Fig. 2.3b) and store approximately 69 Gt of carbon, which is 77% of the world's tropical peatland carbon pool (88.6 Gt), of which 65% (57.4 Gt of carbon) is in Indonesia itself, distributed within 23.4 million ha of peatland (Page et al., 2011). These pristine peatlands (Fig.

2.4a), sequester net-carbon, slowing the release of CO_2 and thereby, countering anthropogenic impacts on the Earth's atmosphere. Huge biodiversity (1.4- 1.8 million species) takes refuge in these peatlands forests. Posa et al. (2011) reviewed that around 11% of plants recorded from peat swamp forests are reported only from that habitat. The majority (63%) of these restricted species are trees, followed by epiphytes and climbers (16%). They found that 23 to 32% of all species of mammals and birds in Peninsular Malaysia and Borneo have been recorded from peat swamp habitats.

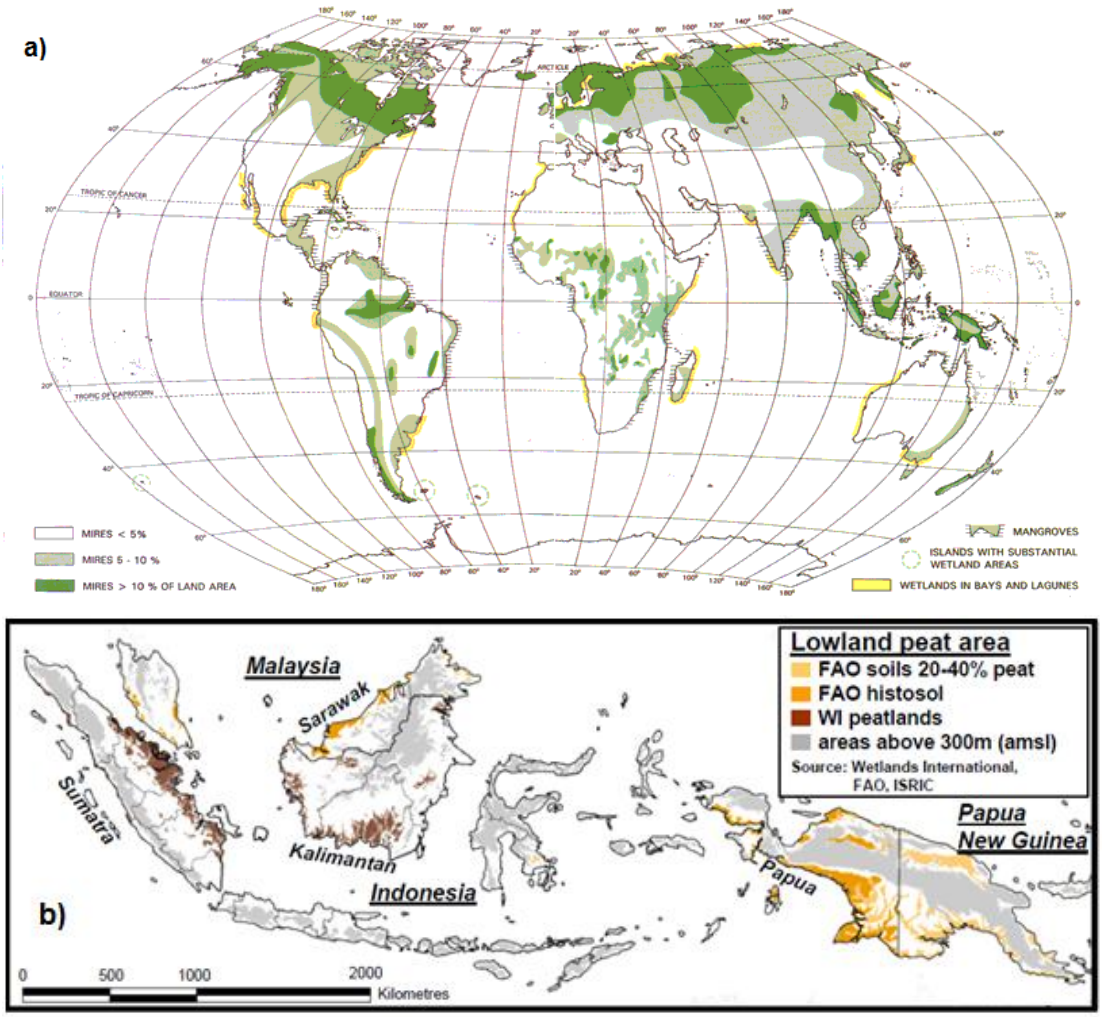


Fig 2.3: a) *Global peatland distribution (**Source**: FAO, 2008);* ***b)*** *Lowland peat extent in SE Asia (**Source**: Hooijer et al., 2006)*

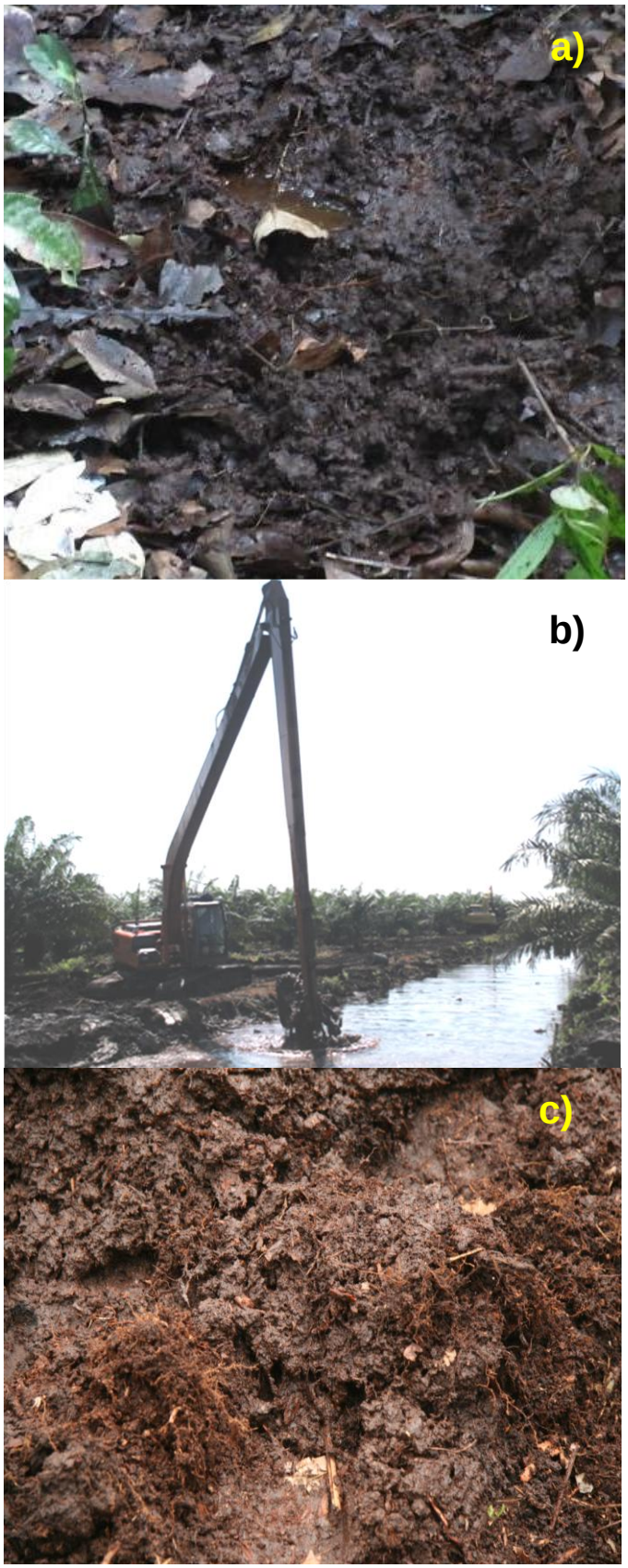


Fig.2.4: *Nature of peat.* ***a):*** *Nearly pristine peatland in Berbak National park depicting the waterlogged surface;* ***b):*** *Drainage canals used to lower water table in peat thereby creating aerobic conditions in top layers;* ***c):*** *Vertical cross-section area of degraded peat, showing fibric roots and partially decayed plant biomass.*
Photos courtesy: *Mishra S. and Swarup S., 2013*

2.3 Land use change: Deforestation and drainage in tropical peatlands

Southeast Asian peatlands are under threat from anthropogenic activities, predominantly drainage and deforestation for agriculture and human settlement purposes (Fig. 2.4b). Such land-use changes and hydrological interventions have resulted in drastic decrease in water table exposing the biomass sequestered in the peat, to air. This atmosphere-exposed peat, full of live/dead roots (Fig. 2.4c) and woody debris is highly prone to oxidation due to microbial activities. Industrial-scale plantations covered over 3.1 Mha, which is approximately 20% of Peninsular Malaysia, Sumatra and Borneo in 2010 (Miettinen et al., 2012a). A strong acceleration of plantation development has occurred since 2000, as only 4% of the current plantation area existed in 1990 (Fig. 2.5 a & b). The projections of future conversion rates, based on historical rates over the past two decades, indicate that 6–9 Mha of peatland in insular SE Asia may be converted to plantations by the year 2020 (Fig. 2.5c). The acceleration of plantation development since 2000 has been particularly fast in South Sumatra, Riau and Sarawak, which together accounted for 75% of all new plantations established since 2000 (Miettinen et al., 2012b). Interspersed within the plantations are small areas of temporary human settlements. In peninsular Malaysia and the islands of Sumatra and Borneo, some 60% of peat swamps had been partly or completely deforested by 2007, usually accompanied by drainage. Currently only 10%

of the peatlands remained in pristine condition (Miettinen and Liew, 2010). This gives an indication of land-use change occurring in SE Asia at an alarming rate, which results in many devastating outcomes discussed in next section.

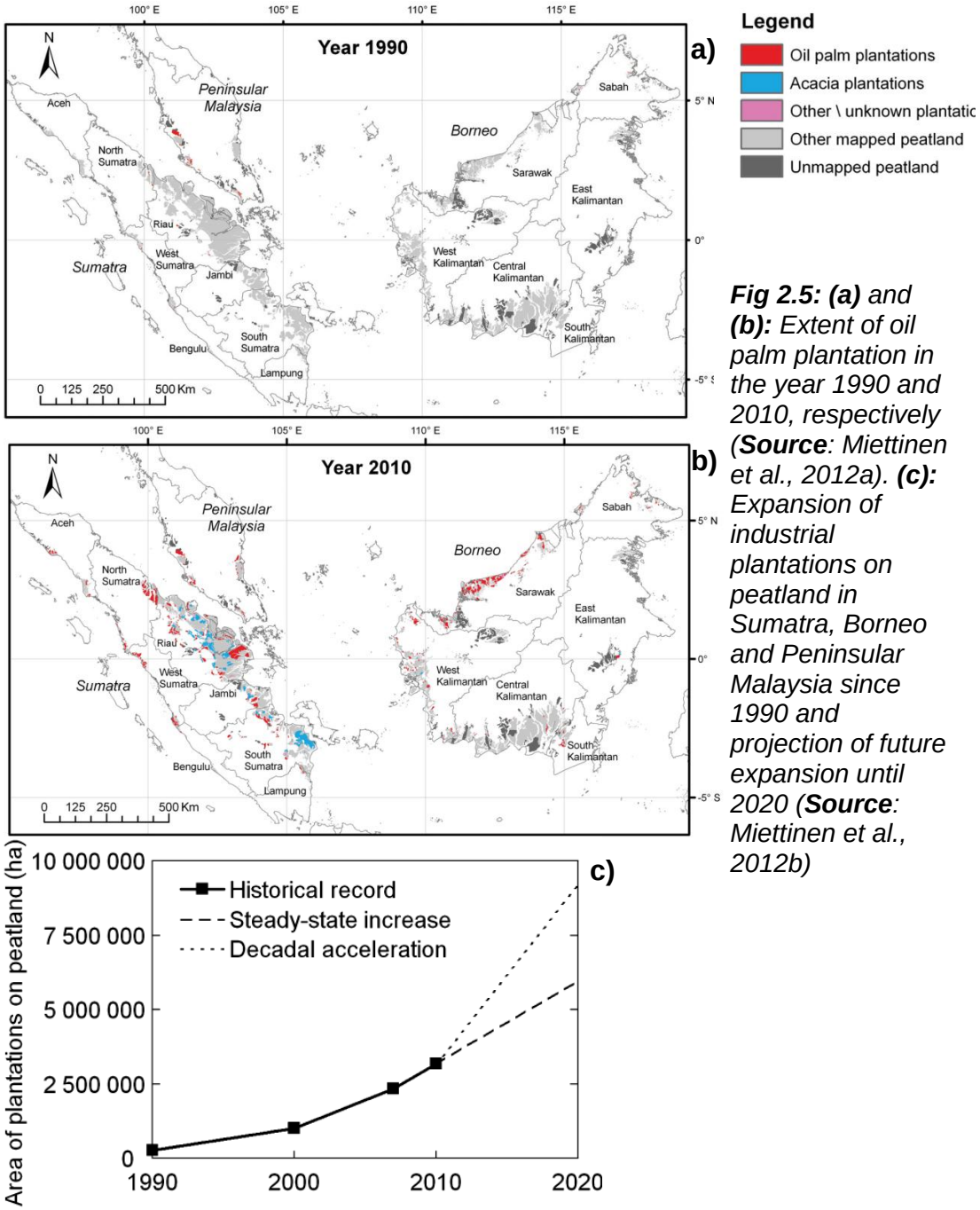


Fig 2.5: (a) *and* ***(b):*** *Extent of oil palm plantation in the year 1990 and 2010, respectively (****Source****: Miettinen et al., 2012a).* ***(c):*** *Expansion of industrial plantations on peatland in Sumatra, Borneo and Peninsular Malaysia since 1990 and projection of future expansion until 2020 (****Source****: Miettinen et al., 2012b)*

2.4 Greenhouse gas emissions and subsidence

Carbon dioxide emissions: *Land-use change has resulted in the highest levels of greenhouse gas (GHG) emissions from any single source in SE Asia, contributing nearly 230– 310 Mt CO_{2e} per year (excluding forest fires), which is equivalent to 8% of global carbon emissions from fossil fuels.* Recurrent biomass burning contributes aerosol, plant/peat-derived aromatics and between 0.81 Gt and 2.57Gt of carbon, equivalent to 13% to 40% of mean annual global carbon emissions from fossil fuels (based on estimates from peatland fires in Indonesia in 1997- Page et al., 2002). Estimates of net carbon losses and resultant CO_2 emissions from peatland drained for agriculture, range from 30–40 tCO_2 ha^{-1} yr^{-1} (Murdiyarso et al., 2010; Hergoualc'h and Verchot, 2011) to as high as 70 tCO_2 ha^{-1} yr^{-1} (Couwenberg et al., 2010; Jauhiainen et al., 2012), excluding forest biomass losses, fire losses and peat organic matter losses in the initial years after drainage. *Emissions from Indonesia alone are 516 Mt/yr (141 Mt CO_{2e}), which is 58% of global peatland emissions (Hooijer et al., 2006). The annual carbon emission from peat oxidation is expected to increase to somewhere between 380 and 920 Mt CO_{2e} by 2020, if there is no change in the rate of land-use change (Miettinen et al., 2012).* Carbon losses from such emissions and through fluvial processes have led to tropical peatlands, being transformed from carbon sinks to carbon sources (Fig. 2.6) (Moore et al., 2013). Based on study by Moore et al., (2013), carbon gain of intact peat swamp forest (PSF) estimated to be 94g C m^{-2} yr^{-1} (net

C sink). *However, the net ecosystem carbon balance of disturbed PSF is estimated to be 530g C $m^{-2}yr^{-1}$ (net C source)*. Oxidation of organic matter has been shown using mesocosms of boreal peat, to be due to stimulation of microbial growth, thereby causing the breakdown of organic matter and release of carbon dioxide in a biogeochemical cascade (Fenner and Freeman, 2011).

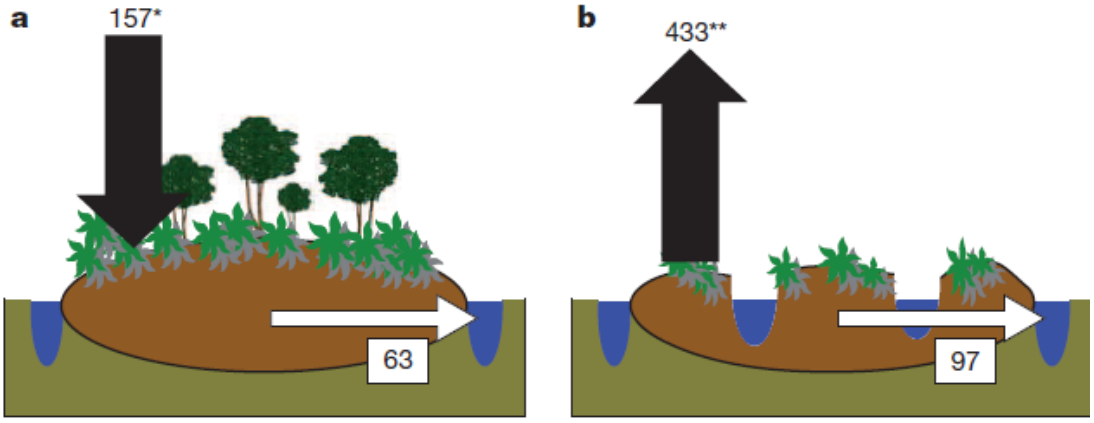


Fig. 2.6: *Carbon balance and DOC age attribution of intact (a) and disturbed (b) Peat Swamp Forest (PSF). Net ecosystem exchange (black arrows; in grams of carbon in CO_2 per m^2 per year) and fluvial TOC loss (white arrows; in grams of carbon per m^2 per year) estimates in the intact PSF (a) and degraded PSF (**Source**: Moore et al., 2013)*

Nitrous oxide emissions: Apart from CO_2, other peat-derived GHGs are also emitted to the atmosphere, thereby, disturbing the weather quality in the region. The peat soils are inherently nutrient-deficient, therefore, in order to increase the yield of plantations, there are high applications of nitrogenous fertilizers that lead to generation of N_2O, which has a global warming potential (GWP, cumulative radioactive forcing) 296 times greater than that of CO_2 on a 100-year time horizon. The significant warming

impacts of N_2O, synergized by its long atmospheric life time (>100 years or slow degradation) and increasing emissions are most evident in the agricultural sector. The emission data of N_2O from peatlands under agriculture in SE Asia, are scarce. Peatlands under commercial plantations, are expected to significantly affect N_2O emission budget. Temperate/boreal peatlands have shown substantial effects on N_2O emissions as shown in various studies (Danevcic et al. 2010, Shimizu et al. 2010, Ernfors et al, 2011*). In tropical peatlands, N_2O fluxes from sites, such as, non-drained forest, drained forest, deforested burned peat, and agricultural non-fertilized peat in Central Kalimantan, ranged from 0.6 – 9.2% of the total GWP (Jauhiainen et al., 2012); data on emissions from plantations have not been studied in detail but are anticipated to be higher.* It is, therefore, critical to estimate N_2O emissions from peatlands, as they are being heavily used for agricultural purposes.

Methane emissions: In addition, drainage canals are a source of methane emissions (with CH_4 having a GWP of 61% of the total on a 100 year time horizon) (Jauhiainen et al, 2012c). Northern peatlands are a net source of methane (CH_4) with an annual release of 46 Tg CH_4 to the atmosphere (Gorham, 1991), which is equivalent to 12.2% of the global total emission amount (Wuebbles and Hayhoe, 2002). This has significant implications to global warming. Methane is produced during the decomposition of organic matter by methanogenic bacteria under anaerobic conditions (Jauhiainen et al., 2005). *Methane fluxes are*

negligible at low water levels and amount to up to 3 mg $CH_4 m^{-2} h^{-1}$ at high water levels, which is low compared with emissions from boreal and temperate peatlands. CH_4 diffusing towards the atmosphere may also be oxidized to CO_2 by methanotrophic bacteria, at times when oxic conditions are present in the upper peat profile e.g., during the dry season when the water table falls below the surface (Couwenberg et al., 2010; Inubushi et al., 2003; Jauhiainen et al., 2005, 2008).

Peat subsidence from microbial-mediated peat oxidation

The oxidation of drained peat is causing rapid subsidence by disappearance of the surface layers in the peat (Kool et al., 2006; Couwenberg and Hooijer, 2013). In a recent study from the Sumatra region, peat was reported to subside at a rate of 5 cm yr^{-1}, of which 92% loss is due to oxidation and not compaction or plant respiration (Hooijer et al., 2012). A study site in Jambi, Sumatra demonstrates that low subsidence rates were associated with high and rising water tables, while highest rates were obtained when water tables dropped sharply in periods of low rainfall (Fig. 2.7). This indicates importance of understanding peat subsidence through microbial-mediated peat oxidation, as management of water table alone cannot stop peat subsidence completely. The implication of this trend is that already prevailing lowlands will subside more rapidly. Plantations life will be shortened from normal 20-25 years to shorter durations. The short term immediate impact of this degradation is evident from loss of biodiversity, fires and GHG emissions. Due to the increased

flood flow in downstream, coastal pollution with organic mud has been observed (Hooijer et al., 2012). On a longer term, the drastic impacts include drainability problems due to loss of gradient, affecting agricultural productivity. Due to this, livelihood of local people relying on these lands is expected to be affected. Further, there is a risk of increased flooding by rivers due to loss of height above sea level.

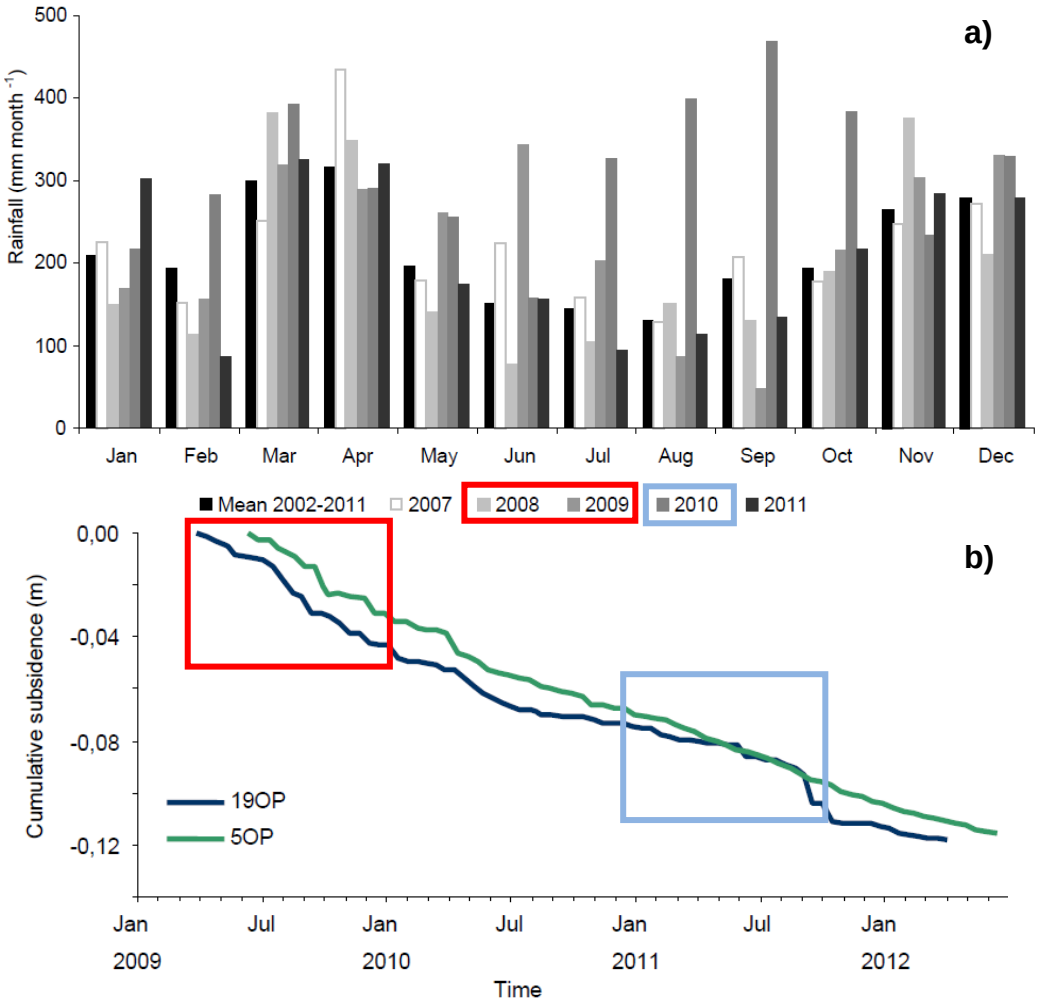


Fig. 2.7: (a) *– Monthly rainfall at the oil palm (OP) sites in Jambi (Sumatra) compared with average monthly rainfall (2002–2011), based on TRMM satellite data.* ***(b)*** *– Cummulative subsidence measured between March/June 2009 and March/June 2012 in the 5OP and 19OP sites, averaged over 17 and 34 monitoring locations, respectively (**Source**: Couwenberg and Hooijer, 2013).* ***Red box*** *indicate the low rainfall regimes (2008-09) leading to low water tables and thus, high subsidence. However,subsidence do not stop even in high rainfall times*

*(2010-2011)- **blue box**, leading to high water table season, indicating role of other factors (microbial mediated peat oxidation) resulting in peat subsidence*

2.5 Structure and functions of peat microbial community

While geochemical conditions affect microbial communities, they, in turn, affect their environment. In most ecosystems, changes in land-use patterns impact both microbial diversity and their activity (functions) (Wardle et al., 1998; Ollivier et al., 2011; Putten 2012). Water table depth also affects water stress, which has been shown to have direct and indirect influences on soil bacterial community composition (Fierer et al., 2003). In order to successfully manage the rapid change in land use from pristine conditions of peatlands and monitor the progress as well as effectiveness of ecosystem restoration interventions, it is important to study microbial community composition and functions in peatlands.

Microbial communities in temperate peatlands

Temperate peatlands are known to have a wide variety of microbes capable of growing and metabolizing over a wide range of pH and growth conditions. Williams and Crawford, (1984) noted that bacterial genera in peatlands of Minnesota include *Bacillus*, *Pseudomonas*, *Achromobacter*, *Cytophaga*, *Micrococcus*, *Chromobacterium*, *Clostridium*, *Streptomyces*, *Actinomyces*, *Mycobacterium*, *Micromonospora*, and *Nocardia*. Such a diverse group of bacteria suggests a complex ecological web, with microbes specialized for many niches. The microbial ecology of temperate peatlands and its relationship to GHG emissions is well studied (Opelt et

al., 2007; Ausec et al., 2009; Kim et al., 2012; Tveit et al., 2012 and Tveit et al., 2014). Sphagnum bogs in New England comprises of high diversity of bacterial communities and consistent difference between surface and sub-surface assemblages (Morales et al., 2006). The microbial activity and bacterial community structure investigated in two types of temperate marsh (a drained grassland fen soil having a neutral pH with 16% soil organic carbon and another bog soil – low pH 4.5 in a swampy forest with 45% soil organic carbon content that has occasional anoxic conditions) suggested that the soil pH affected the bacterial community structure (Ausec et al., 2009). In a controlled study on mesotrophic peatland in UK, it was shown that 3-degree warming effect caused a shift in the bacterial community structure in the oxic and partial oxic zones, rather than in anoxic zones, with an increased amount of CO_2 production upon warming. However, there was no significant difference in the methanogens (Kim et al., 2012).

The combined metagenomic and metatranscriptomic study on arctic permafrost peatlands identified a large and diverse set of genes encoding plant polymer-degrading enzymes that were comparable to microbiota from temperate and subtropical peat/soil (Tveit et al., 2012). The majority of these genes were assigned to three bacterial phyla, *Actinobacteria*, *Verrucomicrobia* and *Bacteroidetes*. Actinobacteria seemed to be particularly important, having a metabolic potential for carrying out several of the key steps in soil organic carbon (SOC) degradation. Dryer and more

oxygenated active layers likely lead to increased peat decomposition, owing to lowered concentrations of phenolic substances, caused by increased activity of aerobic microorganisms that synthesize phenol oxidases. Taxonomic annotation revealed that *Actinobacteria* and *Bacteroidetes* were the dominating polysaccharide decomposers (Tveit et al., 2014).

Microbial communities in tropical peatlands

In contrast, for tropical peatlands, we have a relatively poor understanding of the relationship of microbial diversity and factors influencing community structure for intact as well as tropical peatlands under land-use change. Two recent studies of intact-forested peatlands from Thailand (Kanokratana et al., 2011) and Malaysia (Jackson et al., 2009) used pyrosequencing and fingerprinting approaches to describe their microbial diversity and functional properties, respectively. They demonstrated the capability of such techniques to show broad phylogenetic diversity and genetic potential to degrade biomass, respectively.

In the intact peatland of Thailand, the community was dominated by aerobic microbes together with a significant number of facultative and anaerobic microbial taxa. *Acidobacteria* and diverse *Proteobacteria* (mainly *Alphaproteobacteria*) constituted the major phylogenetic groups, with minor representation of archaea and eukaryotic microbes. It can be inferred that the study has revealed only a fraction of the total microbial

diversity of the peat swamp forest, which demonstrate tropical peatlands as unique microbial niche with probable high biodiversity (Kanokratana et al., 2011). Based on the pyrosequencing data in this study (Kanokratana et al., 2011), *Acidobacteria* and *Proteobacteria* together with other bacterial phyla, e.g., *Firmicutes*, *Bacteroidetes*, and *Actinobacteria*, were potent plant polysaccharide degrading microbes, which play an important role in plant biomass degradation in the tropical peat swamp forest ecosystem. Many of the bacterial genera in these phyla are well-known plant polysaccharide degrading microbes producing a variety of lignocellulolytic and amylolytic enzymes and play a key role in plant biomass degradation in soil and various other environments (Béquin and Aubert, 1994; Pandey et al., 2000).

In the tropical Malaysian peat swamp forest, molecular and enzymatic techniques were used to examine patterns in prokaryotic community structure and overall microbial activity in the vertical profile at three depths. Archaeal assemblages in the peat swamp forest, sediments sampled were dominated by previously uncultured members of the *Crenarchaeota*, while bacterial communities were largely composed of members of the Acidobacteria. While archaeal assemblages were somewhat similar in the 20-cm and 50-cm samples, bacterial communities showed broad depth related patterns, including a steady decrease in diversity in clone libraries when depth increased. Extracellular enzyme activities, suggest that cellulolytic activity may be confined to the surface,

but oxidative processing of phenolics material (such as by peroxidase) could occur deeper in the sediments.

Given both the ecological and economic importance of these peatlands, it will be useful to understand the differences among various land-use patterns in degraded tropical peatland with respect to microbial ecology and its relationship with GHGs. Microbes play a critical role in geochemical cycling and thus return the elements to the nature for reuse. Because of their high capacity of growth and very high surface to volume ratio (Cho and Azam, 1988), they can produce high turnover rates of geochemical processes. Microbes have been the major drivers of C and N cycles in pristine environments, oceans, fresh water systems and soil. The tropical peatland carbon balance (shown by blue text and lines in Fig. 2.8) is determined largely by the net balance between carbon uptake in photosynthesis and carbon release through ecosystem respiration by: (a) autotrophic respiration (b) heterotrophic respiration, involving the loss of carbon as CO_2 and CH_4, by organisms involved in aerobic and anaerobic decomposition of organic matter. The cycling of nitrogen (shown by green text and lines in Fig. 2.8) makes some tropical peatlands a source of the potent greenhouse gas nitrous oxide (N_2O), especially when fertilizer has been added to promote agricultural productivity.

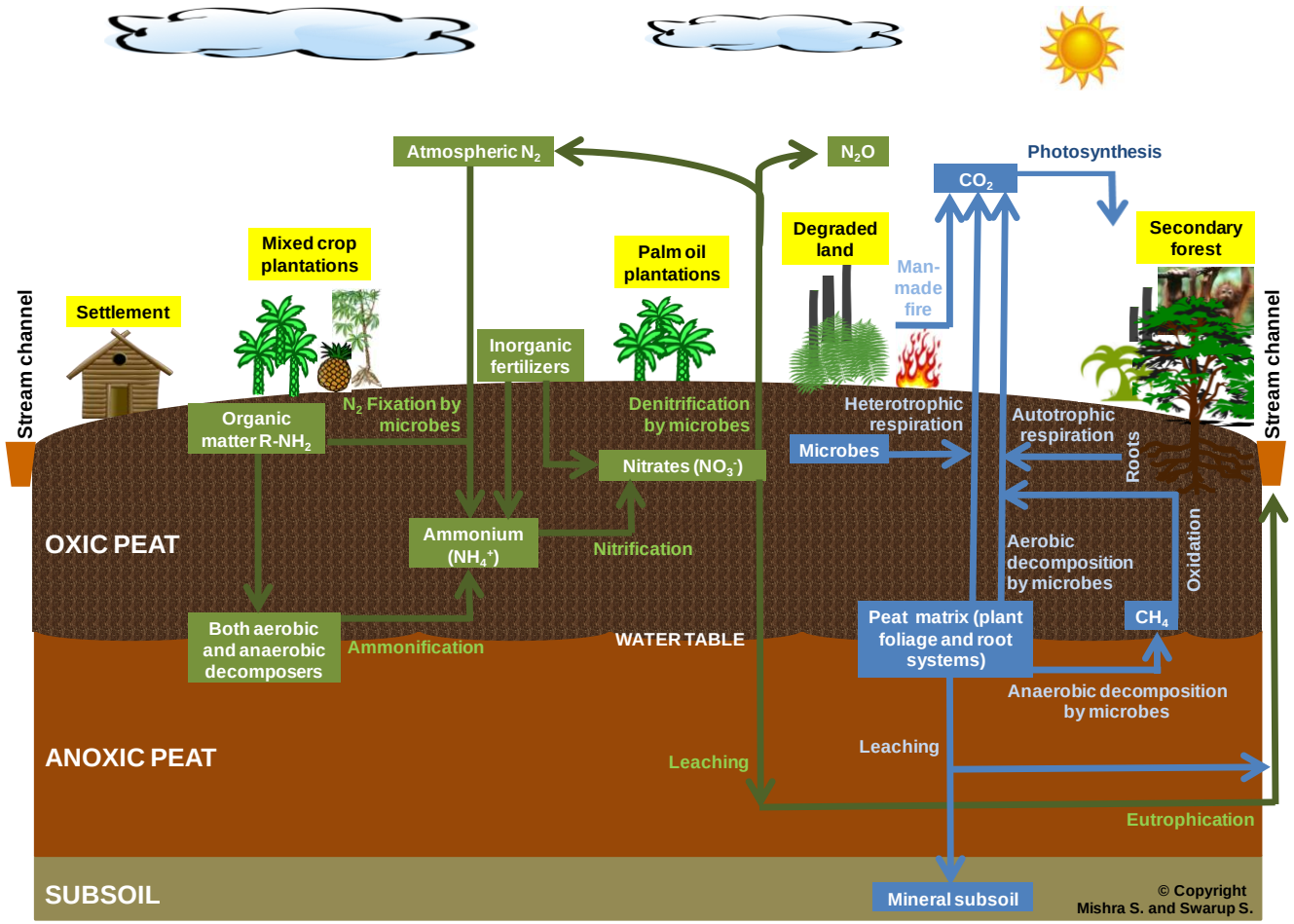


Fig. 2.8: *Schematic diagram of carbon (blue text and box) and nitrogen (green text and box) cycles – processes, flow-path and store in tropical peat (**Copyright:** Mishra S. and Swarup S., 2013)*

2.6 Experimental approaches in understanding microbial ecology

Microorganisms exist in their own natural environment and have learned to adapt to the environmental pressures / traits. Natural microbial communities are one among the most complex, diverse and important clusters of organisms in the biosphere. The biodiversity of these communities, which is dependent on the extraordinary variety of the metabolic pathways in which they are involved, play a key role in maintaining the ecosystem functioning. Based on research questions, the kind of techniques that needs to be adopted depends on the research question we address. Fig. 2.9 shows different methods that can be adopted in this field to understand assemblages and functional aspects of microbial ecology

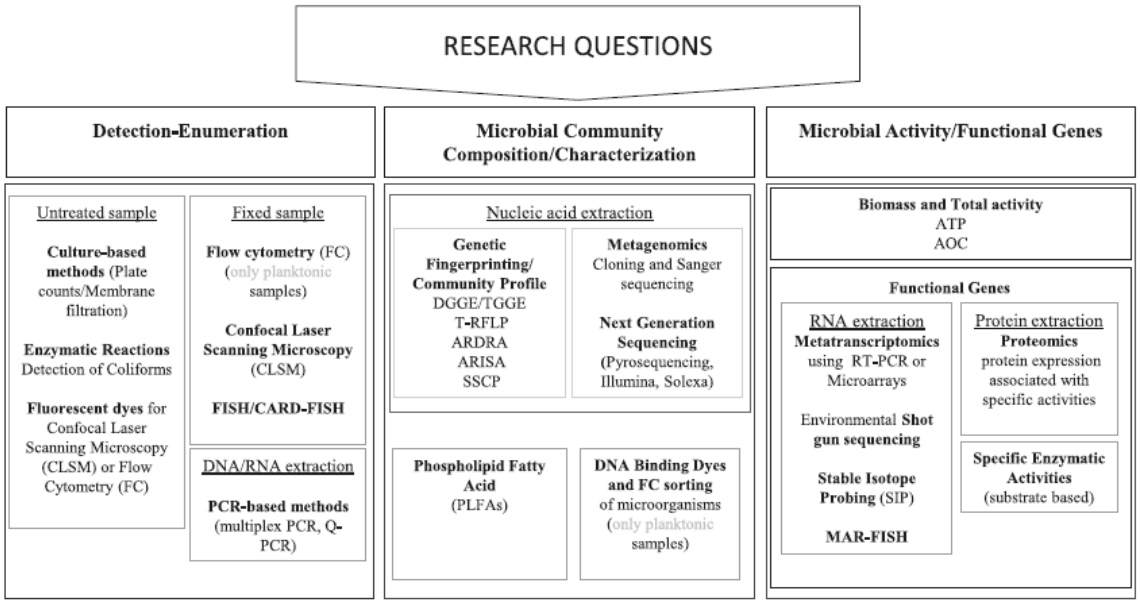


Fig. 2.9: *Scheme showing the different techniques available to characterize microbial communities (**Source:** Douterelo et al., 2014)*

DNA figure printing (T-RFLP)

Terminal –Restriction Fragment Length Polymorphism (T-RFLP) is widely used to detect the changes in the microbial community structure and composition. It relies on DNA polymorphisms existing between 16S rDNA sequences of bacteria belonging to different species. The technique relies on the assumption that different microbial species have polymorphism in the position of a restriction site of a restriction enzyme. Hence, 16S rDNA variable region (of choice) is amplified with fluorescent labeled primers and digested with a combination of restriction enzymes. The fluorescent labeled primer is a fluorescent dye, such as TET (4,7,2′,7′-tetrachloro-6-carboxyfluorescein) or 6- FAM (phosphoramidite fluorochrome 5-carboxyfluorescein). The resolving power of T-RFLP can be enhanced by using fluorescent labels on both forward and reverse primers. In addition, more number of restriction profiles can be generated by using higher number of restriction enzymes. The profile of the restriction fragments is recorded using capillary electrophoresis. The resulting profile of fragments is the signature of microbial community of interest. The banding pattern can be used to measure species richness and evenness as well as similarities between samples.

Next-generation sequencing (Metagenomics)

To circumvent the limitations of culture-dependent techniques in representing the actual microbial diversity, culture – independent methods

have been developed to detect and quantify microorganisms. In the Roche/454 approach, the library fragments are mixed with agarose beads that carry oligonucleotides complementary to the 454-specific adapter sequences on the fragment library. In this way, each bead is associated with a single fragment. Each of these fragment: bead complexes is isolated into individual oil: water micelles that also contain PCR reactants, and (emulsion PCR) of the micelles produces approximately one million copies DNA fragment. These amplified sequences are then sequenced. The sequence data is then assembled into larger contigs and annotated using database search.

Metabolomics

Metabolites are substrates and end-products of enzymatic reactions regulated through dynamic biochemical and gene expression changes in the microbes (Fiehn, 2002). Metabolomics attempts to study the role of metabolites in the physiological and developmental state of organisms and their responses to perturbations. Measurement technologies such as mass spectrometry (MS) are commonly used for profiling metabolite levels. Such measurements of metabolite concentrations, along with their metabolic pathway information are used for deriving biological interpretations. By providing a real-time measure of the metabolite signals in various metabolic pathways, metabolomics approaches provide an accurate snapshot of the specific biochemical phenotype.

MS-based metabolic profiling are either direct injection of the sample into MS or using chromatography techniques such as gas chromatography (GC), high-performance or ultra-performance liquid chromatography (LC) and capillary electrophoresis (CE) in conjunction with mass spectrometry to provide better separation and resolution of metabolite profiles.

Liquid chromatography

In this study, LC-MS is used for non-targeted metabolomics to detect both thermo-labile and thermo-stable metabolites. A number of columns based on reverse phase, ion exchange and hydrophobic interactions principles can be used to better separate the compounds to be eluted in the MS (Allwood and Goodacre, 2010). Ultra-high performance liquid chromatography (UHPLC) provides a fast and efficient way to increase chromatographic resolution and detection range. The components for the stationary and mobile phases are separated based on different affinities. An untargeted analysis is used to screen potential and putative metabolites of interest. These metabolites are then subjected to a targeted analysis for metabolite ID verification, quantitation, functional interpretation, and pathway analysis.

Chapter 3: Microbial and Metabolic Profiling

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3. 1 Background

The importance of understanding microbial ecology and their functions leading to peat oxidation, in the context of tropical peatlands have been discussed in earlier Chapters. In most ecosystems, changes in land-use patterns impact both microbial diversity and their activity (Wardle et al., 1998; Ollivier et al., 2011; Putten 2012). Water table depth, which directly affects the oxygen levels in the peat layer (Lahde, 1969), is an important factor in shaping bacterial community structure. Water table depth also affects water stress, which has been shown to have direct and indirect influences on soil bacterial community composition (Fierer et al., 2003).

As a first step to underpin the overall goal of this study, we aimed to understand the impacts of land-use change and peat management practices on microbial community structure and metabolic functions. Towards this direction, our approach is based on the ability of molecular profiling to capture shifts in community structure and metabolic profiling to reflect the functional outcome of metabolic activities of microbes, plant roots and their exudates, respectively.

In this Chapter, we report the effects of water table depth and oxygen availability, land-use patterns, age of drainage and peat thickness on

microbial diversity in degraded peatlands of Indonesia, using these two microbial profiling approaches. We will focus on five land-use patterns from a contiguous study site: (a) degraded forest, which includes drained and heavily deforested peat swamp forest; (b) degraded land, which includes deforested and drained peatlands that have yet to undergo conversion for agricultural use; (c) oil palm plantation, which includes peatland area under palm plantations; (d) settlements, which includes peatland area under palm plantations interspersed with human settlements; and (e) mixed crop plantation, which includes peatland area under palm plantations, pineapple and tapioca. In order to determine importance of various management practices of these peatlands on the structure and functioning of the microbial communities, we studied the influences of physicochemical parameters. Based on our findings reported in this Chapter, we make recommendations that will help in the classification, improved management and sustainability of tropical peatlands.

3.2 Materials and methods

3.2.1 Site description and sampling

The study area is located in peatlands of the eastern part of Jambi province, Sumatra, Indonesia (Fig. 3.1). Forested tropical peatlands are extensive in this area and a variety of land-use patterns are present due to land intensification activities. Land-cover classification was performed using visual image interpretation and manual on-screen delineation of land-cover polygons. The classification scheme was mainly based on variation in physical vegetation characteristics (e.g., height, sparseness, etc.) and included the main phases of the tropical peatland conversion and degradation processes (Miettinen et al., 2012b). The Landsat image and base maps of field sites were obtained from the University of Jambi, Jambi, Indonesia. The coordinates of monitoring sites were recorded using handheld global positioning system (GPS) devices. The coordinates of these sites were inserted onto the base maps using ArcView and ArcMap programs (ArcGIS-Esri, CA, USA).

The overall mapped area in the eastern part of Jambi comprised a total of 3390 km^2 (Fig. 3.1), out of which water/seasonal water comprised 11 km^2, or 0.3% of total mapped area. The land-cover classes used in Miettinen et al. (2012b) were regrouped and reclassified for this study. The land cover comprising (1) slightly and moderately degraded peat swamp forest (PSF) (1656 km^2, or 49% of total mapped area) was classified as “intact PSF”;

(2) heavily degraded PSF and secondary forest (543 km^2, or 16 %) was classified as “degraded forest”; (3) shrubs, fern/grass and clearance area (712 km^2, or 21 %) was classified as “degraded land”; (4) commercial plantations (279 km^2, or 8 %) was classified as “oil palm plantation” or “mixed crop plantation”, depending on land cover in that area; and (5) small holder mosaic and built-up areas (188 km^2, or 6 %) was classified as “settlements”. For this study, the contiguous land-use types were chosen that were present in Site A and Site B. Intact PSF was not included in this study because of being non-contiguous.

The coordinates of sampling locations distributed across two broad areas, referred to as Site A and Site B, were 103°53'52.58" E, 1°43'12.47" S and 103°49' 32.23" E, 1°40'58.24" S, respectively (Fig. 3.1). The total areas covered by the sites were 42 and 6 km^2, respectively. Out of five land-use patterns, degraded land with similar peat physical, geological and hydrological conditions was present in both Site A and Site B (Fig. 3.1). The acronyms of sites contain four letters and the explanations are provided in Table 3.1. All study sites (Fig. 3.1) have been affected by fire in the past. The only exception is the degraded forest (DHFN: **D**eep peat depth, **H**igh water table, Degraded **F**orest and **N**ew drainage <10 years; please refer to legends of Fig. 3.1 for acronyms of site description). The fire events have occurred in the past in degraded land (DHAN) in Site A and B in 2004 and 2005, respectively. Burning occurred in oil palm and mixed crop plantation sites in Site B (MHPN, DHPN, MHXN and SHXN) in

2004, sites with oil palm plantations in Site A in low water table depth (MLPO) in 2001, and sites in oil palm plantations (DHPO) and in settlements (DHTO) in 2000. As part of routine management practices, fertilizers are applied to the sites that fall within oil palm and mixed crop plantations. The main categories of fertilizer are nitrogen–phosphorus–potassium (NPK 16:16:16) and urea, which are applied three times a year, and potassium chloride (KCl), which is applied once a year.

Table 3.1: *Nomenclature of sampling locations*

Alphabet no.	Characteristics	Abbreviations[#]	Description
1	Peat depth	D	Deep peat depth (7-10 m)
		M	Medium peat depth (3-7m)
		S	Shallow peat depth (0-3m)
2	Water table	H	High water table (0-45 cm)
		L	Low water table (>45 cm)
3	Land use	F	Degraded forest
		A	Degraded land
		P	Palm oil plantation
		T	Settlement area
		X	Mixed crop plantation
4	Age of drainage	N	New (<10 years- drainage)
		O	Old (>10 years- drainage)

[#]*Example,* ***DHFN→D****eep peat depth,* ***H****igh water table, Degraded* ***F****orest,* ***N****ew (<10 years- drainage)*

In order to monitor the hydrological parameters, both rainfall and water table depths were measured periodically using rain gauges at strategic locations and dipwells in each transect. The dipwells consisted of perforated PVC pipes anchored into peat, reaching the mineral subsoil. These dipwells were used to monitor water table depth changes every two

weeks, since 2009. Rainfall measurements were monitored on a daily basis. Average water table depths and total rainfall were calculated for every month. Data from August 2009 to August 2010 are being reported in this study. Sampling was performed in March 2010, preceding which the highest monthly rainfall (370±25 mm) in the period studied was recorded in February 2010. At each sampling location, a $1m^3$ pit was dug. Three equidistant pits were used for sampling in each transect. These transects ranged between 120 and 550m at different sampling locations. We took samples at a predetermined distance from the water table along the wall of the pit. At this distance, we reached horizontally 10 cm into the peat to collect least disturbed samples. This process was repeated for each of the four walls of the pit at the same distance from the water table. A composite was then prepared using these four samples. The number of specified distances in sampling varied according to the water table depths in different sites. Samples using the above mentioned strategy were collected at a distance of 20–30 cm above water table (AWT) and from 20–30 cm below water table (BWT) in sterile 50mL tubes from all sites, with exceptions at 4 sites. At three oil palm plantation sites in the MLPO transect (Fig. 3.1), the water table was extremely low (80 cm below peat surface level); hence samples were collected from 20–30 cm and 50 cm AWT, respectively. One location within a mixed crop plantation was flooded; hence, only one sample was collected BWT and none from the AWT position. Peat water samples for metabolic profiling were collected

from dipwells adjacent to each pit. All samples were shipped on ice to the laboratory and processed immediately. In order to analyze the oxygen availability at each sampling point, an OX-N Clark-type oxygen sensor (Unisense, Aarhus, Denmark) was used and data were recorded manually.

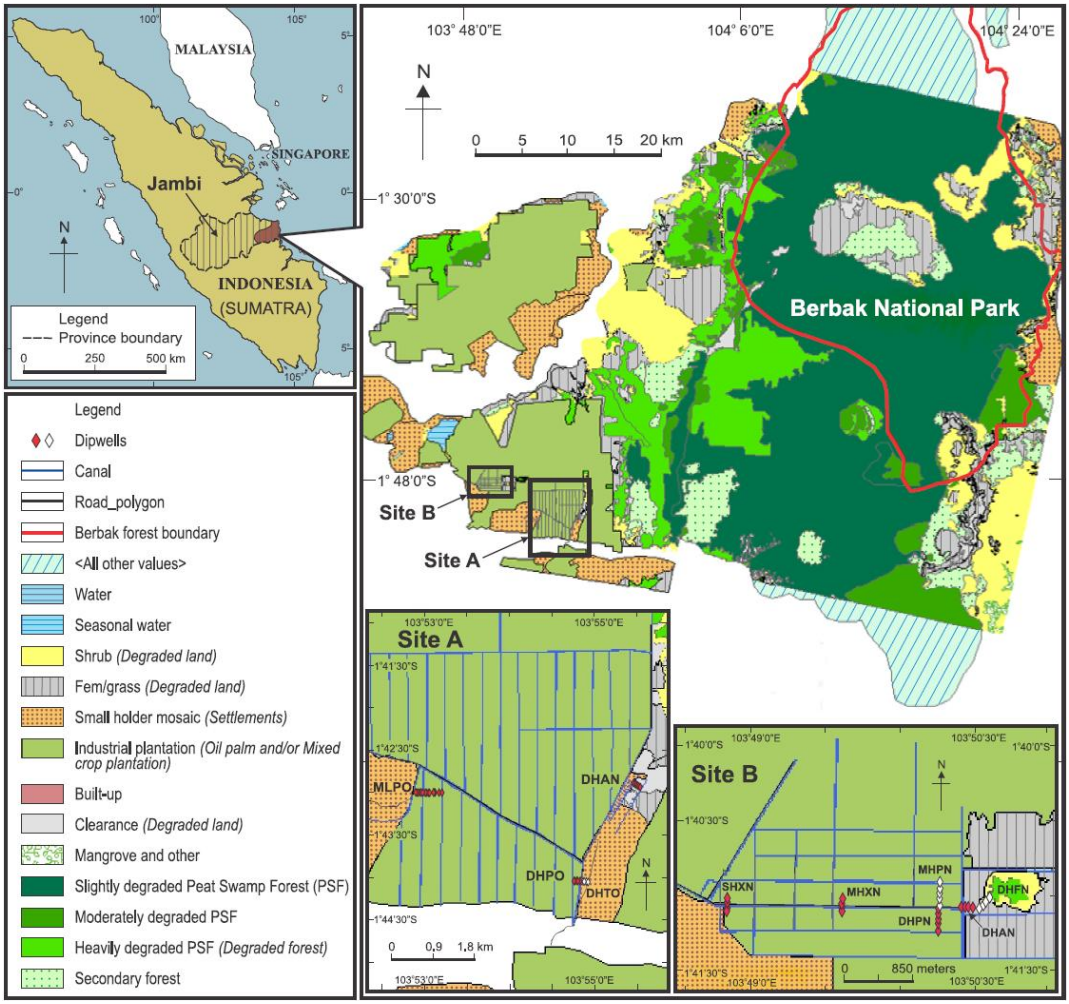


Fig. 3.1: *Map showing the Sumatra Island in Indonesia (top left) and land cover present in eastern part of Jambi province (right). Study sites in this region of Jambi, Site A and Site B are located at geographical locations: 103^{o} 53' 52.58"E, 1^{o} 43' 12.47"S and 103^{o} 49' 32.23"E, 1^{o} 40' 58.24"S, respectively. Abbreviations are given in Table 3.1.Land-use patterns described in this study are italicized in the legends of land cover.*

3.2.2 Microbial community structure (Terminal restriction fragment length polymorphism (T-RFLP) analysis)

Bulk peat gDNA was extracted using a ZR Soil Microbe DNA MidiPrep™ extraction kit (Zymo Research Corporation, Irvine, USA) based on the manufacturer's protocol, with minor modifications. The extracts were quantified spectrophotometrically (Nanodrop ND-1000, Nanodrop Technologies, Wilmington, DE, USA). Microbial 16S rRNA genes were amplified using universal primer, BSF517-GCCAGCAGCCGCGGTAA and BSR1541/20- AAGGAGGTGATCCAGCCGCA (Wilmotte et al., 1993). For T-RFLP analysis, forward primer was labeled with 6-carboxyfluorescein (FAM) at the 5' end and reverse primer was labeled with photo-induced electron transfer (PET) at the 3' end. PCR was performed in triplicate (50µL reaction) using 50ng of template DNA and the following parameters: initial denaturation at 95°C for 10 min, followed by denaturation 95°C for 1 min, annealing at 58°C for 30 s, extension at 72°C for 1 min and final extension at 72°C for 7 min. Agarose gel electrophoresis, followed by staining of products with SYBR Green (Invitrogen, USA) was performed to check amplified product size and concentration. Amplicons from three replicates of PCR were pooled and cleaned up using NucleoSpin® Extract II according to the manufacturer's instructions. 500ng of each amplicon were digested with restriction enzymes *Alu I* and *Bsu RI* (Fermantas) at 37°C for 16h. Digests were then purified using the NucleoSpin® Extract II kit, and an aliquot of 1µL was mixed with 8.5µL HiDi formamide (Applied

Biosystems, Foster City, CA, USA) and 0.5µL of internal size standard (Applied Biosystems) for T-RFLP reactions. The labeled terminal-restriction fragments (TRFs) were detected on an ABI 3730XL automatic DNA sequencing machine (Applied Biosystems) in the GeneScan mode. For data collection from the DNA sequencing machine, GeneMapper software (Applied Biosystems) was used to compare relative lengths of TRFs with the internal size standard. For profile comparison, minimal and maximal cut-offs of 50 and 600bp, respectively, were set and fragments with peak height below 75 were removed as filter noise.

3.2.3 Chemical analysis

Five grams of peat were mixed with 40mL of analytical grade water. The mixture was shaken at 200 rpm overnight to obtain a bioavailable extract for microorganisms from peat (Reynolds and Clarke, 2008). These extracts were used for analysis of total dissolved organic carbon (DOC) and inorganic carbon using a total organic carbon analyzer (TOC-V CPH E200V 220V, Shimadzu). Aliquots from the same remaining extracts were also analyzed for anions such as fluoride, chloride, nitrite, nitrate, phosphate, sulfate and cations such as sodium, ammonium, potassium, magnesium, and calcium using an ion chromatography analyzer (ICS-5000, Dionex).

Peat water samples were run through a Solid Phase Extraction cartridge using an Oasis® HLB cartridge (1 cc/30mg; 30µm, Waters, USA) in order

to extract, concentrate and clean up the metabolites (Parab et al., 2009). The samples were analyzed using ultra-performance liquid chromatography (UPLC) in a Waters ACQUITY UPLC™ system (Waters Corp., MA, USA), equipped with a binary solvent delivery system and an autosampler. The chromatography was performed on a Waters ACQUITY C_{18} 1.7µm column (100 X 2.1 mm). Mass spectrometry was performed based on MS conditions using a mass spectrometer equipped with an electro spray ionization source (UPLC-TOF-Bruker Daltonics). Data were extracted using Bruker Daltonics profile analysis software.

3.2.4 Data analysis

To analyze the variation in microbial community structure as well as difference in metabolic functions, due to influence from analyzed parameters (namely, water table, land-use patterns, age of drainage and peat thickness), multivariate statistical techniques (PRIMER 6, PRIMER-E, Ltd., Plymouth, United Kingdom) were used to calculate distance matrices using Bray–Curtis similarity indices and one-way ANOSIM (Analysis of Similarity) coefficients (Legendre and Legendre, 1998). Unconstrained ordination plots with 100 iterations using non-metric multidimensional scaling (nMDS) based on Bray–Curtis similarity were used to represent the outcome (Kruskal 1964, Shepard 1962). To analyze the relative influence of different parameters over microbial communities, two-way ANOSIM (Clarke, 1993) was used. The global R statistic value (generated using one-way or two-way ANOSIM) indicates the degree of separation

between the two communities, with values close to unity indicating more separation and a zero value indicating no difference between the groups.

To analyze influences of physicochemical traits on the microbial community structure, CCA (Canonical Correspondence Analysis) was performed using Canoco (version 4.5 for Windows, PRI Wageningen, the Netherlands) (Lepš and Šmilauer, 2003). Presence/absence of TRFs was used as “species” data. Physicochemical data (anions, cations, DOC and inorganic carbon) were included in the analysis as “environmental” variables. Ordination biplots approximating the weighted differences between the individual communities (T-RFLP patterns) with respect to each of the geochemical factors (represented as arrows) were drawn. The relative importance of geochemical factors in explaining variation in the microbial T-RFLP profiles was explained by the length of the corresponding arrows, and the angle between arrows indicated the degree to which they were correlated. The impact of physicochemical variables over microbial community structure was calculated using a Monte Carlo permutation test based on 1000 random permutations (Rasche et al., 2011).

To predict the phylogeny of the microbial species at the taxa level from the TRFs of 16 S rDNA, Fragment Sorter Suite (FRAGSORT) (ver. 5.0; Agricultural Research and Development Center, Ohio State University) and Phylogenetic Assignment Tool (PAT) (Kent et al. 2003) were used, adopting the methodology described in Lefebvre et al. (2010). Microbial

Community Analysis (MiCA) – a virtual digest program (Shyu et al., 2007) – was used to construct a reference database for each set of primers.

3.2.5 Clone library sequencing of 16 S rDNA genes

In order to validate the presence of predicted species evaluated from FRAGSORT analysis, a clone library using 16 S rDNA from two randomly chosen sites that differed in water table, land-use pattern and oxic conditions was prepared. The sites chosen belong to settlements with high water table and oil palm plantations with low water table. The samples were taken from both oxic and anoxic zones of settlements and only from oxic zones of oil palm plantation sites.

The cleaned PCR product (using same non-labeled primer sequences as described earlier) of 16 S rDNA from settlements and oil palm plantation sites was cloned into 3956 bp pCR® 4-TOPO® vector using TOPO TA Cloning kit for sequencing (Invitrogen, USA) according to the manufacturer's protocol. One Shot® TOP 10 Competent Cells (Invitrogen, USA) was used in order to transform the recombinant plasmid. DNA Sequencing was performed on a DNA sequencer (ABI 3130 Genetic Analyzer) using forward or reverse M13 primers, on plasmid DNA extracted using Wizard® Plus SV MiniPrep DNA Purification System (Promega, USA) from individual clones. DNA sequence data were analyzed as described previously (Reuben et al., 2012). Briefly, sequences were trimmed and edited using MEGA5 (Tamura et al., 2011).

MAFTT (Katoh et al., 2009) was used for aligning the sequences and identifying reverse orientation. Sequences were then reverse-complemented using MEGA5. Vector contamination was checked using the vector screening tool in Sequin (http://www.ncbi.nlm.nih.gov/Sequin/sequin.hlp.html), and a chimera check was performed using Bellerophon (Katoh et al., 2009) followed by Mallard 1.2. (Ashelford et al., 2006). Sequences of 302 clones were submitted to GenBank, and the nucleotide sequence data reported in this paper are published in the GenBank nucleotide database under accession numbers JF739556–JF739857.

3.3 Results

3.3.1 Influences of peat characteristics on microbial community structure

Oxygen availability and water table

Oxygen availability was lower in the below water table (BWT) samples compared to above water table (AWT) samples by a factor of three or more (Fig. 3.2a). The BWT oxygen levels were similar across all land-use types. Based on these, henceforth, we use the terms “oxic” and “anoxic” conditions to refer to oxygen availability in AWT and BWT zones, respectively. Effects of oxygen levels on microbial DNA profiles were analyzed (Fig. 3.2b). In the ordination plot, samples from the low water table sites (across all oil palm plantations) tended to cluster together, regardless of oxic and anoxic zones. The remaining samples clustered roughly into oxic and anoxic zones, although samples from degraded land from anoxic zones were mixed within oxic zones. BWT points found within AWT group are of two types. One of these BWT group is in a cluster that is highly influenced by low water table (as shown in the Fig. 3.2b). This explains why this subgroup has both AWT and BWT points all coming from low water table sites. Of the remaining 8 BWT points within the AWT group, 3 belonging to oil palm plantation sites are right at the periphery of the BWT specific group. Another one (marked by arrow) is an outlier which was due to flooding. This leaves 4 BWT points belonging to degraded

land, which we believe have high water table fluctuations as the water table is not controlled by hydrological interventions. While forested areas are also not managed, they are unlikely to have large fluctuations due to closed canopy. Thus, the degraded forest BWT point falls in the BWT specific group. Anoxic zones supported more complex microbial communities than oxic zones, although this was not the case for samples from degraded land (Table 3.2). The pH values measured from all sites did not show any significant correlation with Shannon diversity indices from different land-use types at both oxic and anoxic zones

Table 3.2: *Shannon diversity indices for different land use patterns based on 16S rDNA and metabolic diversity*

Land-use patterns	Above water table (Oxic zones)	Below water table (Anoxic zones)	Metabolic diversity
Degraded forest	3.14	3.53	4.07
Degraded land	3.44	3.27	4.28
Settlements	3.19	3.38	4.24
Oil palm plantations	2.62	2.68	4.33
Mixed crop plantations	3.48	3.62	4.47

Average diversity indices for different land use patterns based on 16S rDNA were within ±0.17 and ±0.08 in the oxic and anoxic zones, respectively

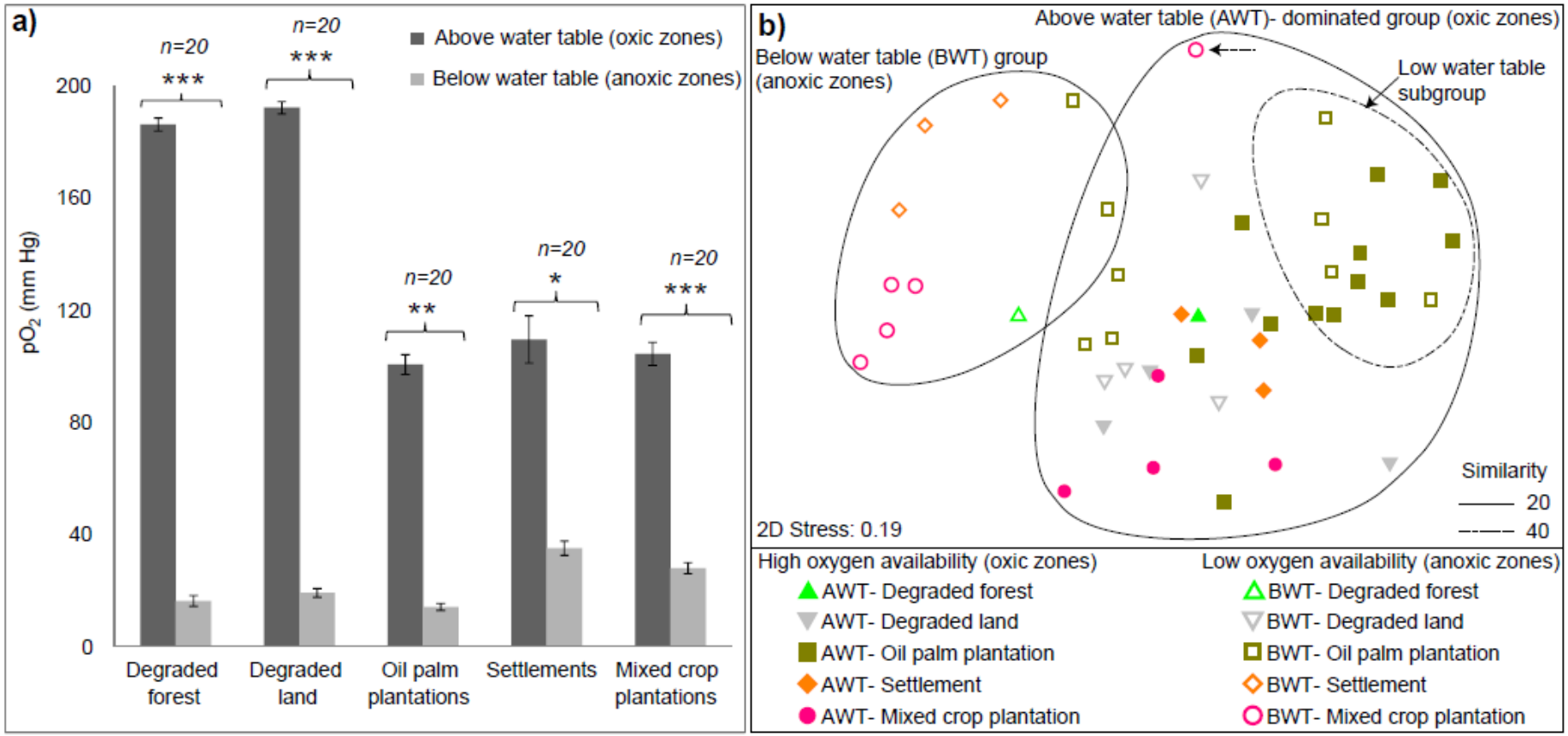
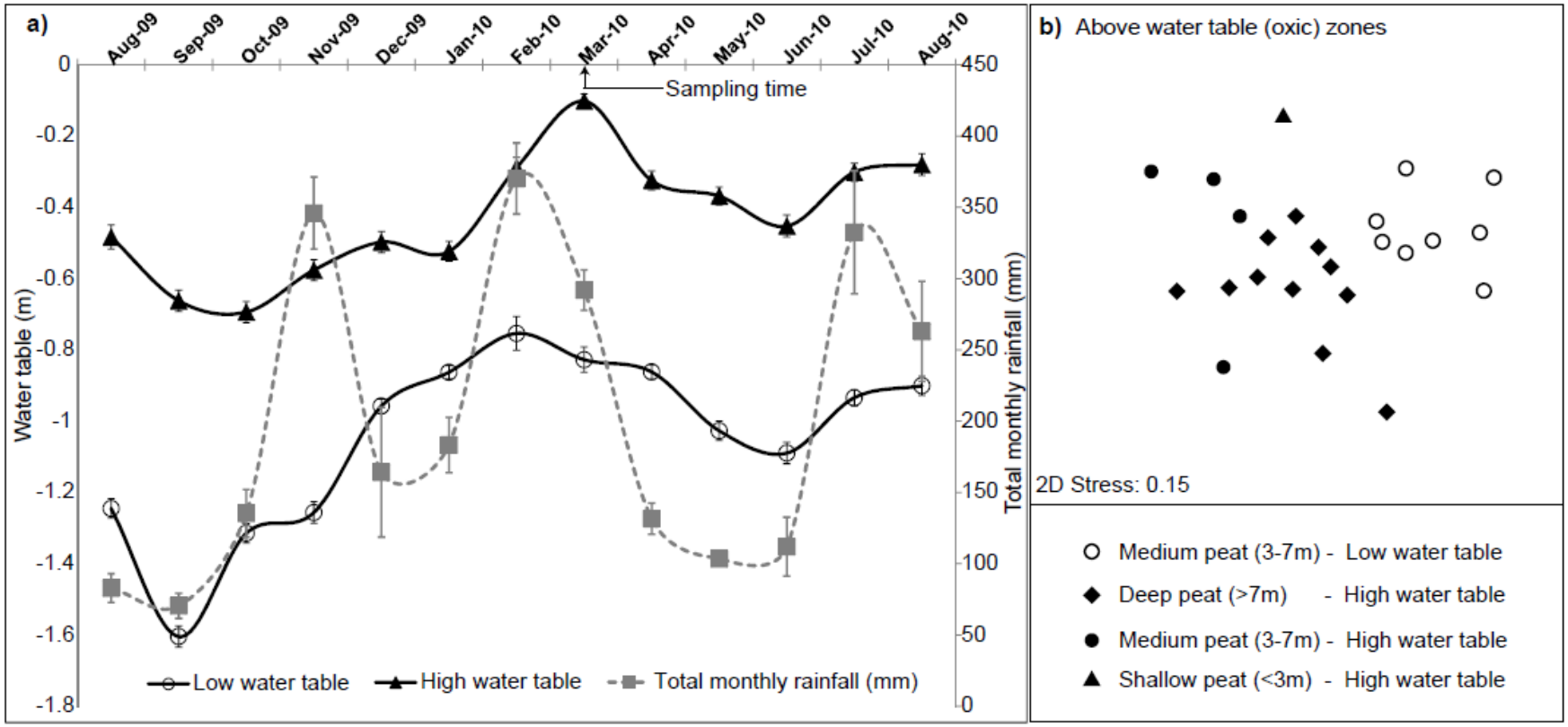


Fig. 3.2. *Oxygen levels (pO_2), mm Hg* ***(a)*** *and nonmetric multidimensional scaling (nMDS) ordination plot based on Bray-Curtis similarities calculated from presence/absence data of 16S rDNA TRFs abundances* ***(b)*** *at above (oxic zones) and below (anoxic zones) water table positions in different land-use types. Level of significance in (a) is: *p< 0.05, **p< 0.01, ***p< 0.001 and 'n'= total number of independent measurements. The error bars in (a) denotes standard error.*

Continuous monitoring of the water table and rainfall revealed that variation in water table from Aug 2009-Aug 2010 was influenced by rainfall in that period, with maximal rainfall in Feb 2010 of 370±25 mm, averaged over all sites (Fig. 3.3a). Sampling was performed during the time when water table was highest. Variation in the water table pattern was similar for all sites except for five oil palm plantation sites (MLPO) that had low water table (>50 cm). The water table in sites with high water table depths fluctuated from -0.1m to -0.7m in the period under study. However, these fluctuations ranged from -0.75 and -1.6m in the sites with low water table depths in the same period. Hence, exposing the low water table sites to different durations of oxic and anoxic regimes compared to high water table sites. Between-site comparisons of microbial community composition showed statistically significant clustering based on water table level in oxic zones (Fig. 3.3b; Table 3.3- One-way ANOSIM values).

Water table depth greatly influenced microbial diversity in both oxic and anoxic zones, with the influence being greater in the oxic zone. We analyzed pair-wise combinations of peat characteristics using two-way ANOSIM analysis. It revealed major influence of water table and land-use pattern over other two characteristics (Table 3.3: Two-way ANOSIM values). The variations in microbial communities due to water table differences for both oxic and anoxic zones were significant across land-use, peat thickness and age of drainage (Table 3.3: Two-way ANOSIM values).


a)
Aug-09
Sep-09
Oct-09
Nov-09
Dec-09
Jan-10
Feb-10
Mar-10
Apr-10
May-10
Jun-10
Jul-10
Aug-10
Sampling time
Water table (m)
Total monthly rainfall (mm)
0
-0.2
-0.4
-0.6
-0.8
-1
-1.2
-1.4
-1.6
-1.8
450
400
350
300
250
200
150
100
50
0
Low water table
High water table
Total monthly rainfall (mm)
b) Above water table (oxic) zones
2D Stress: 0.15
Medium peat (3-7m) - Low water table
Deep peat (>7m) - High water table
Medium peat (3-7m) - High water table
Shallow peat (<3m) - High water table

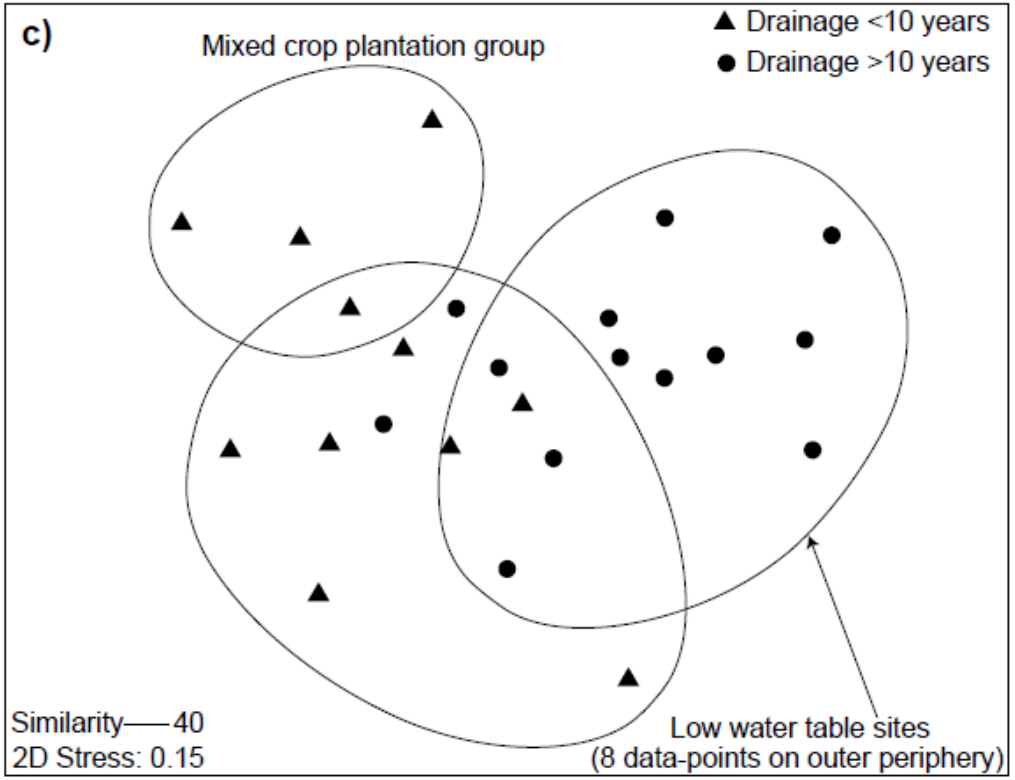


Fig. 3.3: (a) *Rainfall and water table data from Aug 2009 to Aug 2010 at all sampling locations from Site A and Site B. The locations are shown in Fig. 1. Water table levels were averaged across locations with high water table (between 0-45 cm) and low water table (>45 cm), respectively. The averaged values are represented as "high water table" and "low water table", respectively.* ***(b)*** *Nonmetric multidimensional scaling (nMDS) ordination plot, based on Bray-Curtis similarities calculated from presence/absence data of 16S rDNA TRFs abundances, showing variation between microbial community across different water table depth and peat thickness, from above (oxic zones) water table positions. Low water table (LWT), high water table (HWT) and different peat thickness samples are represented by open symbols, closed symbols and different shapes, respectively.* ***(c)*** *Nonmetric multidimensional scaling (nMDS) ordination plot, based on Bray-Curtis similarity indices calculated from presence/absence data of 16S rDNA TRFs abundance from above water table (oxic) zones showing variation based on age of drainage. The error bars in (a) denotes standard error.*

Table 3.3: *One-way (bold letters in diagonal cells) and Two-way (non-bold letters in off-diagonal cells) ANOSIM showing variation in microbial community structure due to an individual parameter across other parameters tested in oxic and anoxic zones. The top value in each non-bold cell refers to influence by the individual parameter across others (left to right), whereas the bottom value in the same cell shows the converse (right to left). Values in cells can be directly compared within same cells or between cells of oxic and anoxic zones. As an example of within- cell comparison: in oxic zones, the influence of water table was much higher across different land-use patterns (global R: 0.702), peat depth (0.946) and age of drainage (0.609) than vice versa (0.189, 0.344, NS: −0.009), respectively. However, in anoxic zones, land-use pattern had high influence across water table (0.468), peat depth (0.636) and age of drainage (0.532), respectively*

Global R statistics									
Above water table (oxic) zones					**Below water table (anoxic) zones**				
	Water table	Land use	Peat depth	Age of drainage		Water table	Land use	Peat depth	Age of drainage
Water table	**0.527**^{c}	0.702^{b} 0.189^{a}	0.946^{b} 0.344^{a}	0.609^{c} -0.009	Water table	**0.359**^{b}	0.541^{a} 0.468^{b}	0.878^{a} 0.266^{a}	0.66^{b} 0.247^{a}
Land use		**0.267**^{a}	0.485^{b} 0.358	0.361^{b} 0.76^{b}	Land use		**0.413**^{b}	0.636^{c} 0.268	0.532^{c} 0.101
Peat depth			**0.214**^{b}	0.433^{c} 0.574^{b}	Peat depth			**0.165**^{a}	0.563^{b} 0.643^{b}
Age of drainage				**0.389**^{c}	Age of drainage				**0.192**^{a}

Level of significance is: $^{a}p< 0.05$, $^{b}p< 0.01$, $^{c}p\leq 0.001$

Land-use pattern, age of drainage and peat thickness

Unlike the oxic zone, where water table was the predominant factor influencing microbial community structure, in the anoxic zone, land-use pattern had an equally strong influence as water table on microbial community structure (Table 3.3). In such anoxic zones, the microbial diversity decreased in different land-use types in the following order: mixed crop plantations> degraded forest> settlements> degraded land> oil palm plantations (Table 3.2). In both oxic and anoxic zones, highest diversity of microbial communities was found in mixed crop plantations, whereas, least diversity was present in oil palm plantations. Age of drainage and peat thickness had a weaker yet statistically significant affect as compared to water table and land-use patterns in shaping the microbial community profiles in both oxic and anoxic zones (Table 3.3: One-way ANOSIM values). In the ordination plot of the microbial communities based on age of drainage, two sub-groups dominated by low water table sites and mixed crop plantations, respectively, revealed that these two parameters had additional influences (Fig. 3.3c). As in the case of age of drainage, in the ordination plot based on peat thickness, there was a sub-group of low water table sites in oxic zones (Fig. 3.3b).

3.3.2 Distribution of microbial taxa

In order to identify the dominant members of the microbial communities, we predicted the taxa using the TRFs. To validate the taxa group

identified, a clone library was created that revealed differences in abundance of taxa based on water table, land-use pattern and oxygen availability (Table 3.4), which was consistent with our previous findings (Fig. 3.2, Fig. 3.3b). *Acidobacteria* had 100% similarity with the predicted taxa as mentioned above for all the sites sampled for clone library. The coverage between the predicted taxa and clones identified for Gammaproteobacteria ranged from 56.7% to 89.3% between the sites sampled. The highest coverage between the predicted taxa and clones identified was found in the site with settlements in oxic zones. Based on sequence database searches with the clone sequences, two species were identified based on identities to known entries. *Brevundimonas* sp., reported initially from saline soils (Wang et al., 2012) was found abundant in settlements.

The relative abundance of microbial taxa in high and low water table depths (Fig. 3.4) showed that the distribution of microbial species in the anoxic zones are almost identical across high and low water table sites. Among the five most abundant taxa (*α-, β-* and γ- *proteobacteria*, *Actinobacteria* and *Firmicutes Bacillales*), *Actinobacteria*, had the largest difference in relative distribution abundance between different water table depth sites for oxic zones.

Table 3.4: *Correspondence of clone data in comparison to predicted species. 'AWT': above water table samples and 'BWT': below water table samples*

Species distribution (taxonomic level)	**MLPO- AWT (from oxic zones)** (**M**edium peat-**L**ow water table-Oil **P**alm plantations- Drainage >10 years)			**DHTO-AWT (from oxic zones)** (**D**eep peat-**H**igh water table-**S**ettlements- Drainage >10 years)			**DHTO- BWT (from anoxic zones)** (**D**eep peat-**H**igh water table-**S**ettlements- Drainage >10 years)		
	No. of Clones (102)	No. of predicted taxa by FRAGSORT	% Coverage	No. of Clones (104)	No. of predicted taxa by FRAGSORT	% Coverage	No. of Clones (96)	No. of predicted taxa by FRAGSORT	% Coverage
Environmental (Unclassified)	*71*	*313*	*22.7*	*60*	*278*	*21.6*	*74*	*281*	*26.3*
Gammaproteobacteria	*19*	*34*	*56.7*	*25*	*28*	*89.3*	*15*	*23*	*65.2*
Alphaproteobacteria	*5*	*45*	*11.2*	*10*	*41*	*24.4*	*3*	*41*	*7.3*
Acidobacteria	*5*	*5*	*100.0*	*3*	*3*	*100.0*	*2*	*2*	*100.0*
Actinobacteria	*0*	*53*	*0.0*	*1*	*49*	*2.0*	*0*	*22*	*0.0*
Betaproteobacteria	*2*	*44*	*4.5*	*2*	*37*	*5.4*	*2*	*41*	*4.9*
Planctomycetes	*0*	*12*	*0.0*	*1*	*9*	*11.1*	*0*	*14*	*0.0*
Proteobacteria	*0*	*4*	*0.0*	*2*	*3*	*66.7*	*0*	*1*	*0.0*

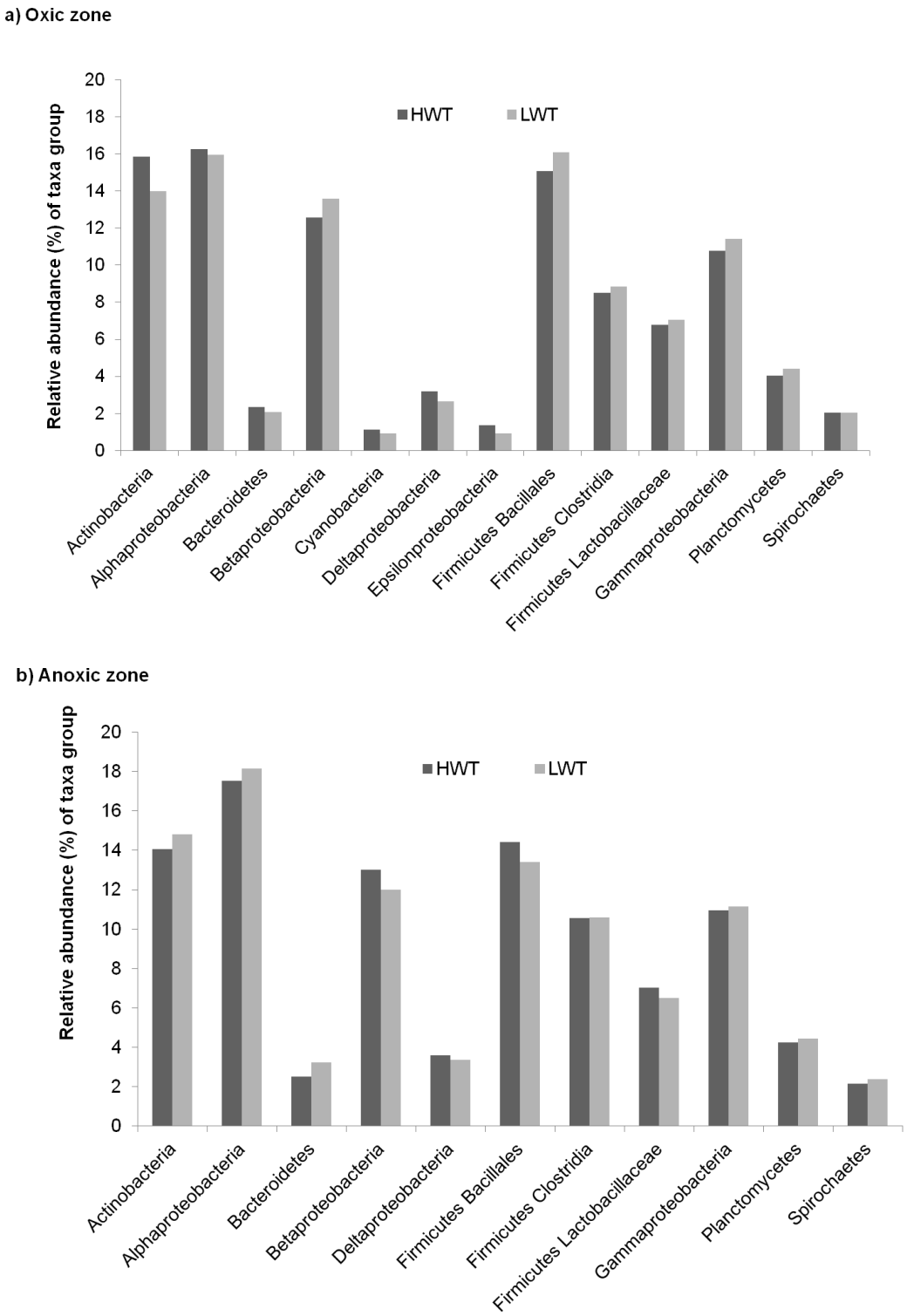


Fig. 3.4: *Relative abundance (%) distribution of microbial population from all sites with high water table (HWT) and low watertable (LWT), based on phylogenetic assignation using FRAGSORT from a) above water table (oxic) zones and b) below water table (anoxic) zones. For oxic zones of LWT sites, average of predicted taxa from depths, 20-30 cm and 50 cm from water table, were taken*

3.3.3 Relationship of environmental and geochemical parameters with microbial community structure

Canonical correspondence analysis was used to identify the association of environmental and geochemical traits with microbial communities from different land-use patterns (Fig. 3.5a, 3.5b and Table 3.5). Microbial communities from the oil palm plantations were associated with nitrate levels in both oxic (Fig. 3.5a) and anoxic (Fig. 3.5b) zones. Nitrate levels were lower in the anoxic zones compared to oxic zones by 20-30 folds, which is likely to drive differences in their community structures. Microbial communities in the mixed crop plantations, on the other hand, were mainly associated with DOC, ammonium and phosphates. Salinity had some influence on microbial communities from sites with mixed crop plantations and settlements; latter corroborated the identified salinity associated species from settlements.

Metabolic profiling of peat water from different land-use patterns was performed (Fig. 3.6) and was compared with microbial profiling (Fig. 3.2b) to directly evaluate the effects of bioavailable organics that influence the microbial communities. Microbial communities from anoxic zones were marginally separated based on land-use patterns (Fig. 3.2b). The communities were separated based on habitat, as revealed by significant separation of the flooded site (indicated by arrow) from the non-flooded sites of mixed crop plantations (Fig. 3.2b). When comparing the functional data from metabolic profiling (Fig. 3.6), distinct clusters of different land-

use types were formed. Metabolic profiling not only differentiates the land-use patterns but also clearly distinguishes samples based on geographical sampling position. For example, two sites from degraded land in Site B formed a distinct cluster from the other two sites from degraded land in Site A (DHAN in Fig. 3.1). Similarly, two oil palm plantation samples (extreme left of Fig. 3.6) though belonging to different peat thickness (MHPN and DHPN in Fig. 3.1) were clustered very close as they were from the same transect.

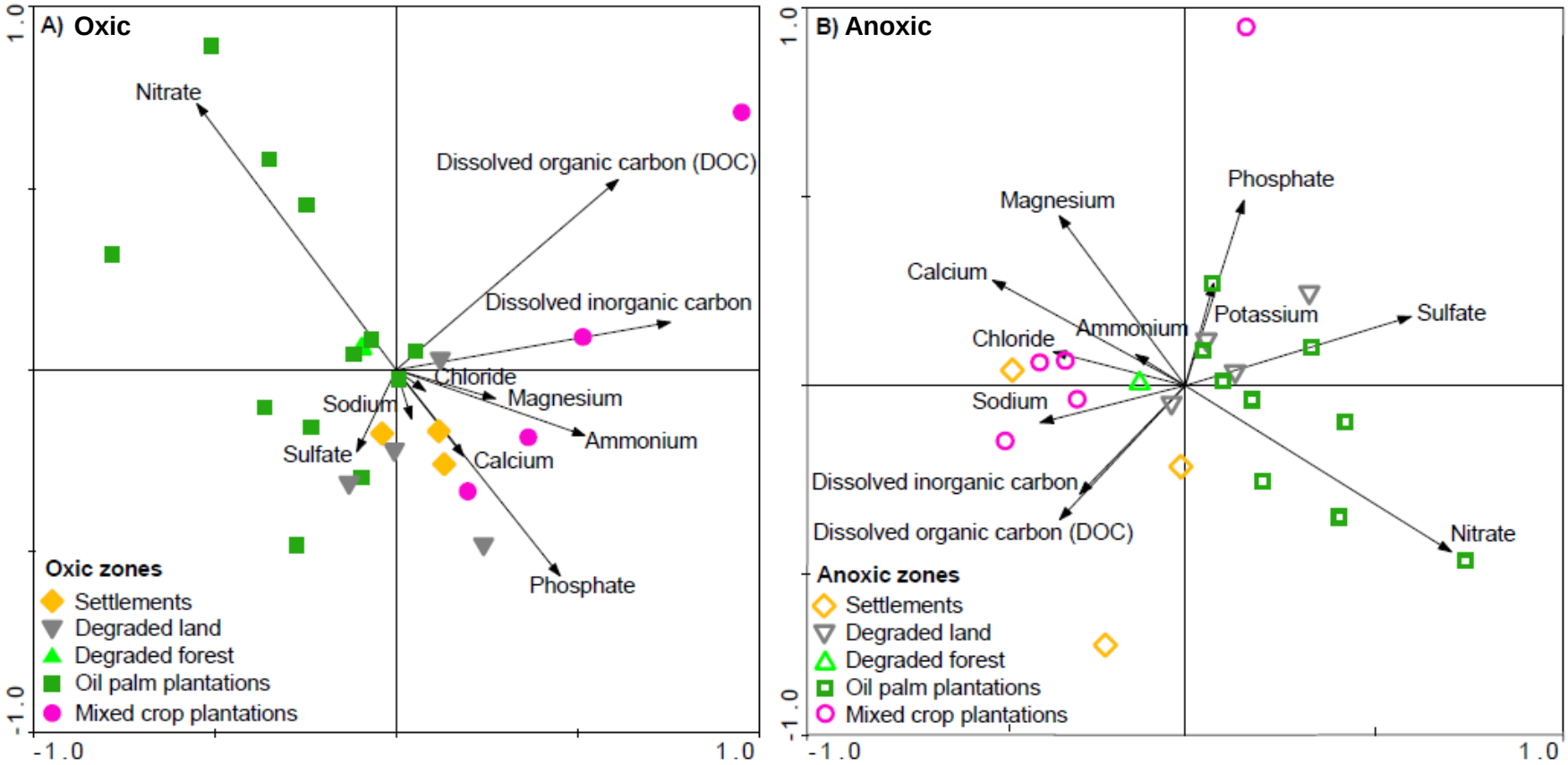


Fig. 3.5: *Canonical correspondence analysis of 16S rDNA gene based T-RFLP datasets and environmental data in different land-use patterns from oxic (a) and anoxic (b) zones. Geochemical data, represented with arrows are: nitrates, dissolved organic carbon (DOC), dissolved inorganic carbon, chloride, magnesium, ammonium, sodium, calcium, sulfate and phosphate. Test of significance (p-value) of all canonical axes is 0.05 and 0.009 in oxic (a) and anoxic (b) zones, respectively.*

Table 3.5: *Relationship between the microbial community structure and geochemical parameters as revealed by canonical correspondence analyses and Monte Carlo permutation tests performed separately for 16S rDNA gene-based T-RFLP data sets and geochemical data*

Sampling zones	Monte Carlo permutation test (p-value) of first / all canonical axes	Percentage variance of bacterial community-environment relation		Bacterial community-environment correlation / Eigen values	
		Canonical axis 1	Canonical axis 2	Canonical axis 1	Canonical axis 2
Above water table (oxic) zones	0.03/0.05	21.1	14.7	0.978 / 0.366	0.945 / 0.256
Below water table (anoxic) zones	0.004/0.009	17.6	14	0.980 / 0.362	0.979 / 0.289

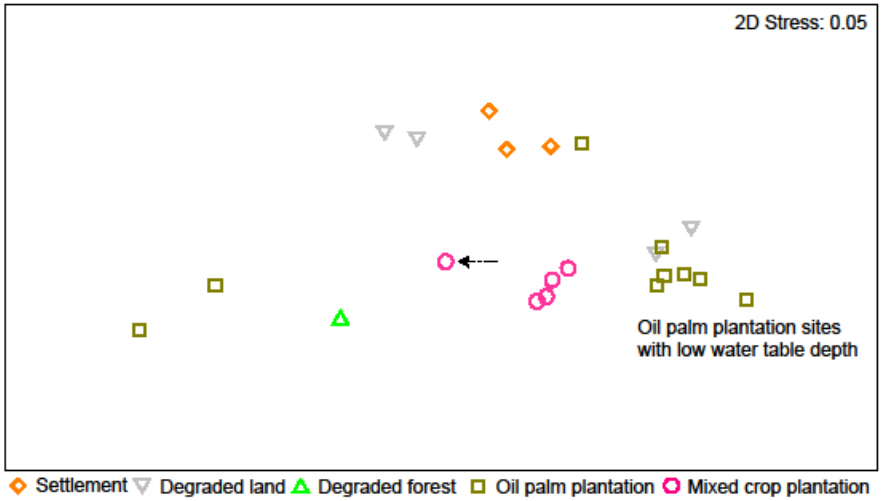


Fig. 3.6: *Nonmetric multidimensional scaling (nMDS) ordination plot, based on Euclidean distance calculated from intensity of metabolites extracted from peat water of different land-use patterns. The arrow represents the flooded site with mixed crop plantations*

3.4 Discussion

Microbial and metabolic markers that represent the complex nature of microbial communities and metabolic processes of diverse biota, respectively, provided the resolving power to distinguish different habitats. This resolution ranged from centimeter scale in depth measurements to kilometer scale, where sites were distributed within the 48 km^2 of the study area. Thus, the same set of molecular markers provided a dynamic range of resolution at four orders of magnitude. Microbial markers have been extensively used to study alteration in community structures due to changes in land-use patterns at large scales of spatial distribution, such as, in Pacific Northwest marine sediment communities (Braker et al., 2000), in high levels of nuclear waste-contaminated vadose sediments at the Hanford Site in the US (Fredrickson et al., 2004), in Western Amazon soils (Jesus et al., 2009) and in Antarctic dry valley (Chan et al., 2013), among other biogeographic locations. In comparison, there are relatively few studies that have used metabolites as function-based markers for understanding variation at large scale of spatial distribution. Both sets of molecular markers distinguished different land-use types, but with different levels of resolution. Compared with microbial profiles, those of metabolites were additionally able to differentiate land-use types from locations that are separated by nearly 8 km distance. Our findings about microbial profiling have led us to identify geochemical factors that influence the state of degraded peatlands. In addition, metabolic profiling, which relies on

markers derived from functions of diverse biotic communities and not just bacteria, provide a finer classification of peatland sites. Metabolic profiling can, therefore, be used in developing better practices for mapping peatlands, which can be a tool for both management and policy development.

While there have been reports of effects of land-use change and hydrology on CO_2, N_2O and CH_4 emissions (Jauhiainen et al., 2008; 2012a; 2012b; 2012c) and hydrology on subsidence (Hooijer et. al., 2010 and 2012), our approach allows multiple parameters to be evaluated simultaneously using a single molecular profiling approach. Our findings show that microbial profiles from peatland sites are most influenced by variations in water table and land-use patterns. These two are followed by age of drainage and peat thickness in influencing the microbial community structure. Across degraded peatland that are under hydrological managements, water table fluctuates due to drainage, rainfall and other physical parameters (Jauhiainen et al., 2008; Hooijer et al., 2010). The ability of microbial markers to distinguish the low and high water table sites show their robustness to capture differences in community structures despite the differences in the range of fluctuations in water level at these sites. Fluctuations in water table are likely to influence microbial community structure through oxygen and nutrient availability on one hand and selection of bacteria that can withstand drying-wetting cycles on the other hand. Such changes in microbial community structure along a

hydrological gradient have been reported in other natural ecosystems, such as in forested, temperate pine wetlands (Yu and Ehrenfeld, 2010). However, temporal fluctuations in water table in high and low water table sites leading to rapid drying and wetting, as seen here, were not present in the pine wetland study. Hence, microbial profiling presents a practical approach to monitor peat responses to both rapid short-term and long-term hydrological changes.

Mechanisms by which water table influences microbial communities are likely to be different in a depth profile, depending on oxygen availability. In the oxic zones, low water table sites undergo more pronounced cycles of drying and wetting compared to high water table sites. Drying and wetting of peat leads to alternating aerobic and anaerobic physiological responses of the microbes. Thus, drying-wetting process selectively enriches those resilient members of microbial communities that can tolerate these changes both in physical environment and physiological functions. Examples of such successful resilient microbial taxa are *Actinobacteria* and *Firmicutes* that have very thick peptidoglycan layer to withstand changes in physical environment and adapt to broad range of oxygen availability. Hence, they thrive well in diverse and extreme environments from deep sea to dry deserts (Cowan and Tow, 2004; Bull A., 2011). Both *Actinobacteria* and *Firmicutes* were the most represented taxa groups in all our study sites, irrespective of water table depth, land-use pattern or oxygen availability. Physiologically, aerobic-anaerobic cycling can affect

both nitrogen and carbon metabolism. Denitrifying assemblages are favored under the wetter and low oxygen conditions of the drying-wetting cycles, as shown in clay loam of wetland mesocosms (Peralta et al., 2013). Likewise, in carbon metabolism, this cycling is known to activate different pathways in oxic and anoxic conditions. Enzyme systems, such as phenol oxidase and β-glucosidase that are involved in the degradation of recalcitrant phenolic (lignin and its derivative) and cellulosic materials, respectively, are active in high oxygen conditions (McLatchey and Reddy, 1998). On the other hand, activities of many hydrolases that degrade complex carbon polymers are high under low oxygen conditions, such as in boreal river system (Sinsabaugh et al., 1991), in anaerobic sludge digestor (Nybroe et al., 1992) and in the rumen of cattle (Lee et al., 1999). Such activation of different groups of enzymes in drying-wetting has been reported from mesocosms of peat in boreal region (Fenner and Freeman, 2011). Combined oxidation of recalcitrant and labile carbon, accompanied with outgassing of carbon dioxide can lead to direct loss of peat. This extrapolation directly predicts that peat loss will be higher in low water sites, where these pronounced cycles of drying-wetting are prevalent, compared to high water table sites. From sites in the Sumatran region, peat subsidence that is considered as proxy for carbon loss is indeed higher in low water sites (water table depth: -0.7 ± 0.2 m and subsidence rate: 5 ± 2 .2 cm y^{-1}), when compared to high water table sites (water table

depth: -0.56 ± 0.06 m and subsidence rate: 3.9 ± 0.5 cm y^{-1}) (Couwenberg and Hooijer, 2013).

In contrast to the oxic zones in low water table sites that experience fluctuations, the anoxic conditions present in the water saturated zones of low and high water table sites, affect microbial communities through possibly different mechanisms. First is through exposure of microbial communities to prolonged stable anoxic conditions. Such conditions can support both high microbial diversity and abundance, as shown in this study. Consistent with our findings, similar increase in microbial diversity with decreasing oxygen availability along a depth profile has been reported in a stratified lake (Garcia et al., 2013). The second mechanism is through direct effects of water saturation in the anoxic zones, where dissolved organic matter becomes freely available for microbial communities. Availability of dissolved organic matter in the water saturated anoxic zone plays a major role in shaping microbial communities, through its the quantity and composition. Both, root exudation from plant communities and degradation products of lignocellulosic materials, contribute to the dissolved organic matter that drives microbial assemblages (Farrar et al., 2003). Hence, dissolved organic matter provides a critical link between the above and below ground communities (Wardle et al., 2004). Microbial and metabolic profiling approaches in our study, therefore, capture the outcomes of these plant-microbial-peat interactions. Such interactions are heavily

impacted by changes in land-use patterns, where different plant communities support their land-cover specific microbial populations (Bardgett et al., 2005).

The nutrient pool for microbes is influenced not only by root exudates and lignocellulosic degradation products, but also, by the carbon products resulting from fire events. Both aliphatic and aromatic carbons are added to the pool of dissolved organic matter present in the ecosystems that have experienced fire events, as shown for pine forest (Czimczik et al. 2003) and boreal ecosystems (Neff et al., 2005). All our sites, with the exception of degraded forest, also have fire histories of 5-10 years, respectively, which likely contributed fire-related carbon forms to the nutrient pools. While such carbon joins the carbon pool after fire events, as expected, there is a concomitant decrease in abundance and activity of microbial communities immediately after the fire events in forest soils (Certini G., 2005). The present state of microbial assemblages at our sites, therefore, reflects the recovered and adapted communities over a period of time. Southeast Asian peatlands have a unique history of experiencing repeated fires, which can be both spatially extensive and can affect specific sites at multiple occasions. Our findings that the microbial communities and metabolic profiles could not separate the sites based on fire history, indicates resilience of the microbial communities to recover over 5-10 year period, since the sites first experienced a fire event. This duration of recovery is consistent with that reported for boreal forest fires

(Dooley and Treseder, 2012). Whether functionally important, yet non-resilient bacteria are lost in this process cannot be ascertained from our current approach and will require metagenomics approaches.

Anthropogenic chemical inputs, such as by fertilization, in these managed peatlands had a great influence on microbial community structure. Nitrates and phosphates, contributed by fertilizer applications, were among the top three factors that influenced overall microbial diversity in oil palm plantations. Similar influence of organic manure and mineral fertilizer treatment occurs on the abundance and diversity of gram-negative bacteria, *Actinobacteria* and fungal communities in the red soil of China (Zhong et al., 2010). Since nitrogenous fertilizers are heavily used in the management of plantations on tropical peatlands, N_2O is likely to be released, as demonstrated from other agricultural lands, such as, from agricultural soils in Australia (Dalal et al., 2003), India (Aggarwal P., 2008) and Africa (Hickman et al., 2011). One of the most abundant taxa in our study, *Actinobacteria* have been shown to actively reduce N_2O to N_2, not only in the anoxic zone of palsa peat (Palmer and Horn, 2012), but also in other habitats, such as, agricultural soils (Philippot et al., 2002) and in Uranium contaminated sediments (Akob et al., 2008). Hence, it will be important to estimate N_2O emissions from tropical peat plantations that use mineral fertilizers and also the utility of N_2O reducing bacteria in such plantations. In contrast to plantations, salinity is a major influencing factor in settlements and mixed crop plantations. Both these land-cover types

have most intensive human activities. Salinity is likely to be due to anthropogenic contributions. Nearly 2.5 km^2 out of the total study area of 48 km^2 belongs to settlements and mixed crop plantations, thus representing a significant influence on the microbial communities in this region. Hence, microbial profiling can help reveal influences of both management-related and anthropogenic activities on peat.

One of the major findings of our study is that monoculture of oil palm plantations supported the least diverse microbial communities and consisted of lowest levels of dissolved carbon content. On the other hand, mixed crop plantations consisting of upto five plant species only, supported most diverse microbial communities and had the highest levels of dissolved carbon content. In tropical peatlands of Kalimantan, land conversion from secondary forest to paddy field (monoculture plantations) led to the decrease in carbon content, together with a decrease in microbial abundance (Hadi et al., 2001), which is consistent with our findings. Carbon levels increased, when paddy-soybean rotation cropping was followed with a further decrease in microbial abundance. This possibly underlines the importance of adopting simultaneous mixed plantations rather than sequential crop-rotations, as evident in our study. Similar decrease in microbial diversity from mixed crop plantations to monoculture of crops has been reported for many cases such as *Lolium*, *Trifolium spp.*, *Plathymenia*, Sudan grass and tall fescue (Marilley et al., 1998; Meimei et al., 2008; Pagano et al., 2009; Zarea et al., 2009).

Similarly, microbial diversity is lower in fallow and woodland, when compared to grassland with multiple plant communities on temperate peatlands (Brake et al., 1999). Low microbial diversity in oil palm plantations, as seen in our study, can be sensitive to environmental pressures thereby leading to reduction in their productive period.

3.5 Conclusions

We conclude that rapid drying-wetting of peatlands is associated with high peat oxidation rates and that this process selectively enriches certain resilient microbial species which can rapidly switch between aerobic and anaerobic conditions. Sites that are characterized as ‘low water table sites’ undergo more rapid fluctuations in water level than ‘high water table sites’. We would test this hypothesis about these confounding parameters in Chapter 5 of this thesis and understand how peat oxidation occurs upon rewetting. A strong association of nitrates with microbial community structure was found in oil palm plantation sites. Mixed crop plantations, which have a more diverse plant cover than monoculture oil palm plantations, contain more DOC, have a high diverse metabolic profile, support a more diverse microbial community and importantly experience a lower rate of peat subsidence, a proxy for oxidation-led peat loss.

Chapter 4: Relationships of microbiome and environmental traits with peat oxidation (subsidence)

4. 1 Background

Peat oxidation resulting in its subsidence, leads to high rates of carbon loss that occur during conversion of forested tropical peatlands to other land-use types. This subsidence serves as a surrogate measure of CO_2 emissions to the atmosphere. In SE Asia, large-scale subsidence monitoring studies in *Acacia* and oil palm plantations on peatland, showed that peat oxidation is occurring at an alarming rate of ~5cm/yr at average water table depths of 0.7m from those sites (Hooijer et al., 2012). With this high subsidence rate and a carbon density of 0.043 g cm^{-3}, a net carbon loss of ~18 t ha^{-1} y^{-1} (~66 t CO_2-eq ha^{-1} y^{-1}) is estimated from these plantations more than five years after drainage (Couwenberg and Hooijer, 2013). Peat oxidative degradation of soil organic carbon (SOC) linked with microbial activities has been well understood in temperate peatlands (Tveit et al., 2012, 2014). In two Arctic peat soils, majority of the large and diverse set of genes encoding plant polymer-degrading enzymes, were assigned to three bacterial phyla, *Actinobacteria, Verrucomicrobia* and *Bacteroidetes*. In these peat soils, anaerobic metabolic pathways and the fraction of methanogenic archaea increased with peat depth, demonstrating a gradual transition from aerobic to anaerobic zones. The relative abundance of transcripts associated with cellulose degradation

decreased with depth, while the transcripts for hemicellulose debranching increased, which indicates the difference in the polysaccharide composition of the peat in the deeper and older layers.

The advent of metagenomic sequencing has provided a powerful new tool for investigating the structure and functions of microbial communities. Such investigations can help generate hypothesis on metabolic potential for shaping biogeochemical cycles and linkages of taxonomic and functional assemblages. Structure and functional potential of microbial communities has been studied using metagenomics in different ecosystems, such as, agricultural farms (Rampelotto et al., 2013; Carbonetto et al., 2014), permafrost (Guan et al., 2013), grasslands (Delmont et al., 2012), among many others. This technology has been proved to be an important tool to understand the microbial and functional diversity. Metagenomic sequencing diverse ecosystems, such as, from cold deserts, hot deserts, forests, grasslands, and tundra have revealed that the communities found in plant-free cold desert soils typically had the lowest levels of functional diversity (diversity of protein-coding gene categories) and the lowest levels of phylogenetic and taxonomic diversity, compared to those with abundant plant cover (Fierer et al., 2012).

In contrast, for tropical peatlands, we have a relatively poor understanding of the microbiome and their functional potential. Only one study of intact forested peatlands from Thailand (Kanokratana et al., 2011), describe the microbial diversity and functional potential using pyrosequencing

approach. However, no reports are available on the nature of community structure after land-use change. This study aimed to determine the functional potential of peat microbiome that is associated with peat oxidation and its linkages with the environmental traits that play an important role in peat functioning.

4.2 Materials and method

4.2.1 Study site description and sample collection

The location of study was within Sites A and B (Fig. 3.1). The samples were collected from (i) degraded forest (DHFN – Site B); (ii) oil palm plantations (DHPN – Site B) and (iii) degraded land (DHAN – Site A) in Oct 2010. The details of the site and transect have been described in Section 3.2.1 of the previous Chapter 3. Sampling was performed in October 2010. The total monthly rainfall recorded in the months of September and October 2010 were 214±13 mm and 313±19 mm, respectively. At each sampling location, a $1m^3$ pit was dug. Three equidistant pits were used for sampling in each transect. Peat samples were collected at a distance of 20–30 cm above water table (AWT) with the same sampling strategy as mentioned earlier in Chapter 3 (Section 3.2.1).

4.2.2 Measurement of peat characteristics

Monitoring subsidence, water table and peat depth

In order to monitor the hydrological parameters, both rainfall (daily) and water table depths (fortnightly), were measured periodically using rain gauges at strategic locations and 5 dipwells in each transect (Section 3.2.1). The monitoring poles, consisting of perforated PVC tubes that were anchored into the mineral subsoil below the peat, that were used for water

table measurements and peat thickness, were also used as subsidence monitoring poles (Fig. 4.1). Thick wooden block were placed around the poles for measurement accuracy. Care was taken not to disturb the immediate surroundings of the subsidence poles. Locations where disturbance was evident were excluded from analysis. The subsidence rate was monitored fortnightly, since July 2009, from all land-use patterns mentioned in Fig. 3.1. Subsidence data reported in this Chapter is from July 2009 to Oct 2010 from three land-use patterns (degraded forest, degraded land and oil palm plantations) as mentioned in Section 4.2.1.

Fig. 4.1: *Monitoring pole for water table depth and peat subsidence measurements*

Insitu peat characteristics

In order to measure the *insitu* peat physicochemical properties (one time-point CO_2 emission during peat sampling, peat surface humidity), a portable infrared gas analyzer EGM-4 (PP Systems, Hitchen, UK) was

used. An OX-N Clark-type oxygen sensor (Unisense, Aarhus, Denmark) was used and data were recorded manually, in order to analyze the oxygen availability, pH, redox, peat temperature at each sampling point (respective depths from AWT, where peat samples were collected). In order to measure air temperature and humidity, thermohygrometer was used.

Chemical analysis for anions and cations such as chloride, nitrate, ammonium etc was also performed based on the methodology described in Section 3.2.3 (Chapter 3).

4.2.3 DNA extraction, 454 sequencing and data processing

Bulk peat gDNA was extracted using a ZR Soil Microbe DNA MidiPrep™ extraction kit (Zymo Research Corporation, Irvine, USA) based on the manufacturer's protocol, with minor modifications. The extracts were quantified spectrophotometrically (Nanodrop ND-1000, Nanodrop Technologies, Wilmington, DE, USA).

Whole-community metagenomic DNA was extracted from AWT samples collected from three pits within each land-use type. Extracted genomic DNA was pooled within respective land-use pattern and sent for 454 sequencing to Prof. Stephan Schuster lab at Pennsylvania State University, USA. The sequencing was performed on a Genome Sequencer (GS) XLR70+ FLX system (Roche Applied Science, Mannheim, Germany) using Titanium chemistry based on methods

described earlier (Poinar et al., 2006; Allentoft et al., 2009). The Fastq files in the raw format from the 3 samples were quality-trimmed using “Cutadapt” (Martin M., 2011). The details of sequencing read statistics are shown in Table 4.1a.

Taxonomic and functional annotation

The paired read sequences were imported and the paired-end protocol of MEGAN5 was used (Huson et al., 2011) to obtain taxonomic profiles of the three land-use patterns. Annotated taxonomic assignments were linked to functional roles with SEED 3.8.1 (Overbeek et al., 2005) and KEGG-Release 72.1 (Kanehisa and Goto, 2000) classifications using MEGAN5. KEGG pathway memberships were used to assign putative pathways. The abundance of read counts at different levels of NCBI taxonomy (‘class’, ‘order’, ‘family’, ‘genus’, ‘species’), and functional categories (SEED and KEGG) were exported as DSV format in .txt files for statistical analyses.

4.2.4 Statistical analyses

In order to analyze taxonomic and functional potential profiles of the peat microbiome, comparative abundance matrices with taxa or orthologous genes indexed by rows, and samples indexed by columns, with elements containing the corresponding normalised read counts, were exported from MEGAN earlier. The taxonomic profile at the Genus level for bacteria and Archaea or all taxa combined, as well as functional potential (KEGG and

SEED, levels 2 and 3) were exported and further analyzed using Excel 2007 and R-packages version 3.1.0. The exported taxonomic and functional potential profiles (SEED and KEGG) were uploaded in the online available Krona Excel template (Excel Macro-Enabled Workbook 2007) and the charts were viewed in Mozilla Firefox version 34 (Ondov et al., 2011).

In order to understand the correlation of physicochemical/environmental parameters with peat taxonomic and functional potential, we used Pearson correlation to calculate the correlation coefficient. *p*-value was calculated and adjusted using Benjamini-Hochberg method (Benjamini and Hochberg, 1995). The correlations were plotted using program in R version 3.1.0. To understand the relatedness of most significant physicochemical and in-situ environmental parameters, respectively, with taxonomic and functional (SEED and KEGG) profile, features that showed significant correlation ($R^2 > 0.3$ and False Discovery Rate (FDR) adjusted *p*-value <0.05) with the environmental parameters were visualized using the 'pheatmap' function in R. For heatmap, the data were row-scaled to highlight the distribution of abundance for each feature. The R-scripting was performed with Mr. Shivshankar Umashankar, PhD student in Metabolite Biology lab (Prof. Sanjay Swarup's group).

4. 3 Results

4.3.1 Taxonomic distribution of peat microbiome in different land–use pattern

Metagenome sequence abundance based annotation indicated that majority (77 – 81%) of the sequences belonged to bacteria (Table 4.1a). Though the unclassified sequences and unassigned sequences in MEGAN accounted between 0.4 – 3% of the total sequences, the unclassified sequences at 'Class' level within abundant kingdom was quite high (Table 4.1b). A rarefaction curve of the three samples in Fig. 4.2d shows the sequencing depth of the samples. A total of 3,485,832 reads were successfully mapped and annotated with MEGAN using the parameters detailed in the Section 4.2.3. The full details of the read statistics for sequencing are provided in Table 4.1a.

Within microbial communities, *Proteobacteria* was the abundant phyla, followed by *Actinobacteria* and *Firmicutes* (Fig. 4.2). *Actinobacteria* was found to be higher (21% of total microbial communities) in abundance in degraded forest compared to oil palm and degraded land (18% and 15%, respectively). Within phylum, *Actinobacteria*, the most abundant order was *Actinomycetales* (97.4, 96.4, and 95 of total *Actinobacteria* present in degraded forest, oil palm plantations and degraded land, respectively). The distribution of Archaea was comparable in the land-use patterns studied. There was no clear difference in species abundance in rest of the

community composition (Fig. 4.2).The overall microbial diversity of degraded forest, oil palm plantations and degraded land was 5.078, 5.172 and 5.221, respectively, based on Shannon Diversity Index.

***Table 4.1a**: General info for peat microbial metagenome Bp: means base pairs; % non-ACGT = Percentage of bp that are non- ACGT; Transl. reads = Translated reads (in silico); aa = amino acids*

	Degraded Forest	Oil palm Plantations	Degraded Land
Reads	1,070,200	1,419,551	996,081
Megabyte (Mb)	425.8 Mb	641.2 Mb	402.3 Mb
Longest assembled contigs	10392 bp	5648 bp	25547 bp
Average read length (bp)	397.9	451.7	403.9
Average GC%	61.6%	61.2%	60.7%
% non-ACGT	0.0175%	0.0162%	0.0249%
% distribution at Kingdom level			
Bacteria	81.1	81.4	76.9
Archaea	10.1	9.6	11.8
Eukaryota	5.0	5.8	7.5
Viruses	0.5	0.5	0.6
Unclassified sequences	0.5	0.4	0.5
Sequences unassigned in MEGAN	2.7	2.2	2.7

Table 4.1b: *Percent distribution of unclassified at 'class' level within abundant 'Kingdom'*

Within each kingdom (Unclassified at 'class' level)	Degraded Forest	Oil palm Plantations	Degraded Land
Bacteria	8.1	8.5	8.2
Archaea	14.3	14.7	14.1
Virus	47.9	51	50

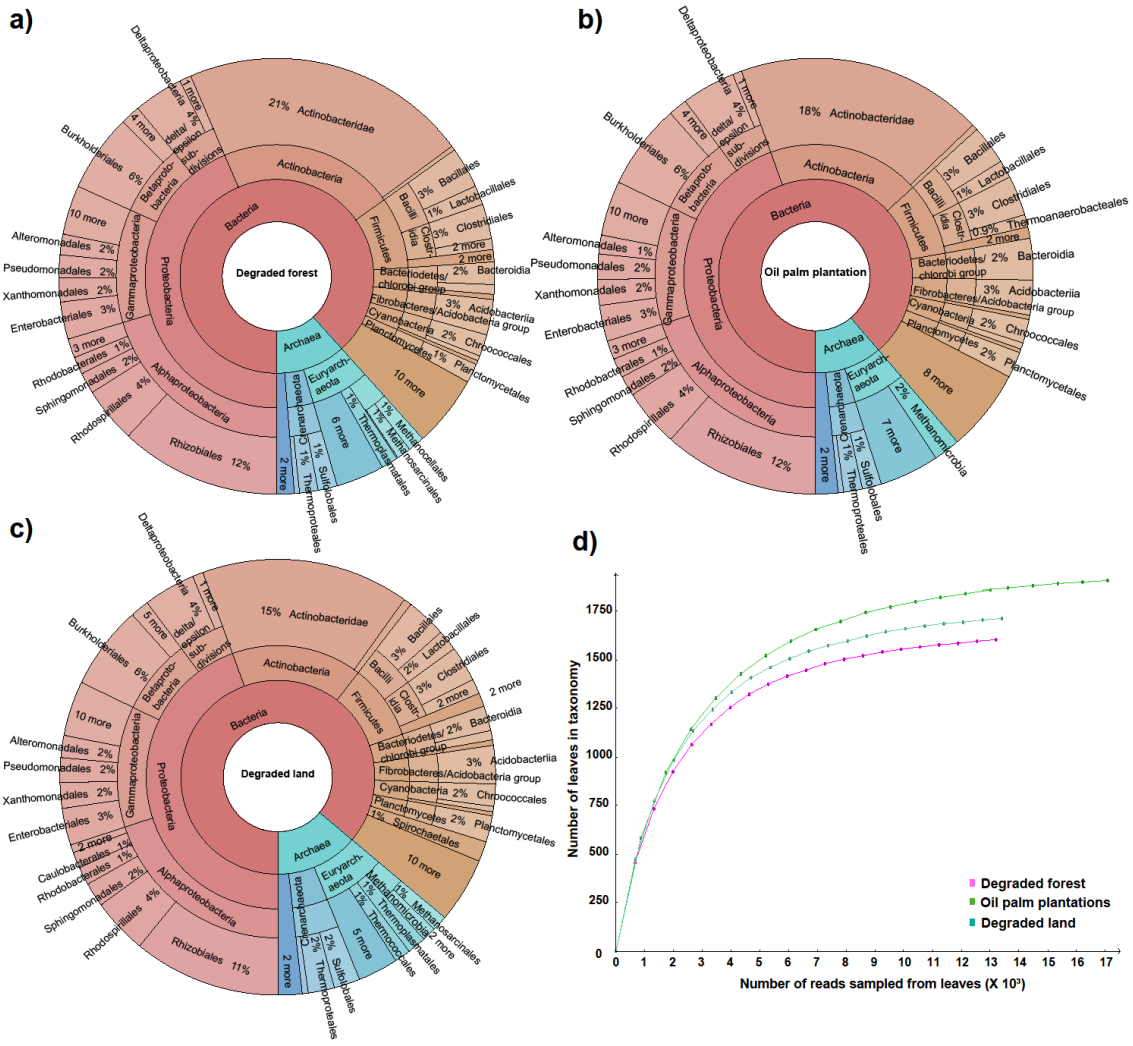


Fig 4.2: *Taxonomic distribution of microbial communities in* ***a)*** *degraded forest,* ***b)*** *Oil palm plantations and* ***c)*** *Degraded land. The rarefaction curve of the microbial communities' profiles/ metagenome data for these three land-use types is shown in* ***d)****.*

4.3.2 Functional potential of peat microbiome in different land–use pattern

Functional potential of peat microbial communities showed that approximately a quarter (≈26%) had unclassified functions (based on KEGG annotation), demonstrating tropical peat community to be a novel microbial niche (Fig. 4.3). The microbiome of degraded land had more diverse functional categories (KEGG annotation) compared to degraded forest and oil palm plantations, based on the diversity indices, which was highest in degraded land, followed by oil palm plantations and degraded forest in that order (Diversity indices as 4.114, 4.085 and 4.075, respectively). The top 25 abundant functional categories based on KEGG annotation revealed that metabolism-related profiles followed a marginal increasing trend starting from degraded land to oil palm plantations and degraded forest, except for nucleotide metabolism (Fig. 4.4a). The top 5 most abundant within metabolism-related functional annotations reveals that there was no substantial difference between the land-use types. Almost one-fourth of total energy, lipid, cofactors–vitamins, other amino acids metabolism and biosynthesis of other secondary metabolites belongs to methane, fatty acid, porphyrin–chlorophyll, seleno compound metabolism and streptomycin biosynthesis, respectively (Table 4.2). Other pathways relating to nitrogen metabolism (*energy metabolism*), CO_2 fixation (*energy metabolism*), benzoate (*xenobiotics biodegradation*), toluene degradation (*xenobiotics biodegradation*), phenylpropanoid

biosynthesis (b*iosynthesis of other secondary metabolites*) are among the top 5 abundant pathways within the metabolism categories reported in Table 4.2.

The functional potential based on SEED annotations revealed higher diverse set of functional categories as 5.756, 5.764 and 5.751, respectively, for degraded forest, oil palm plantations and degraded land, when compared to KEGG (described in previous paragraph). The top 25 abundant categories revealed that oil palm plantations had lower abundance compared to degraded forest and degraded land for almost all categories within carbohydrate and amino acid metabolism (Fig. 4.4b). However, this trend was opposite in the DNA metabolism profiles, where oil palm plantations had the highest abundance among the three land-use patterns. *One-carbon metabolism was the most abundant pathway, among all others that was annotated in the SEED database* (Fig. 4.4b).

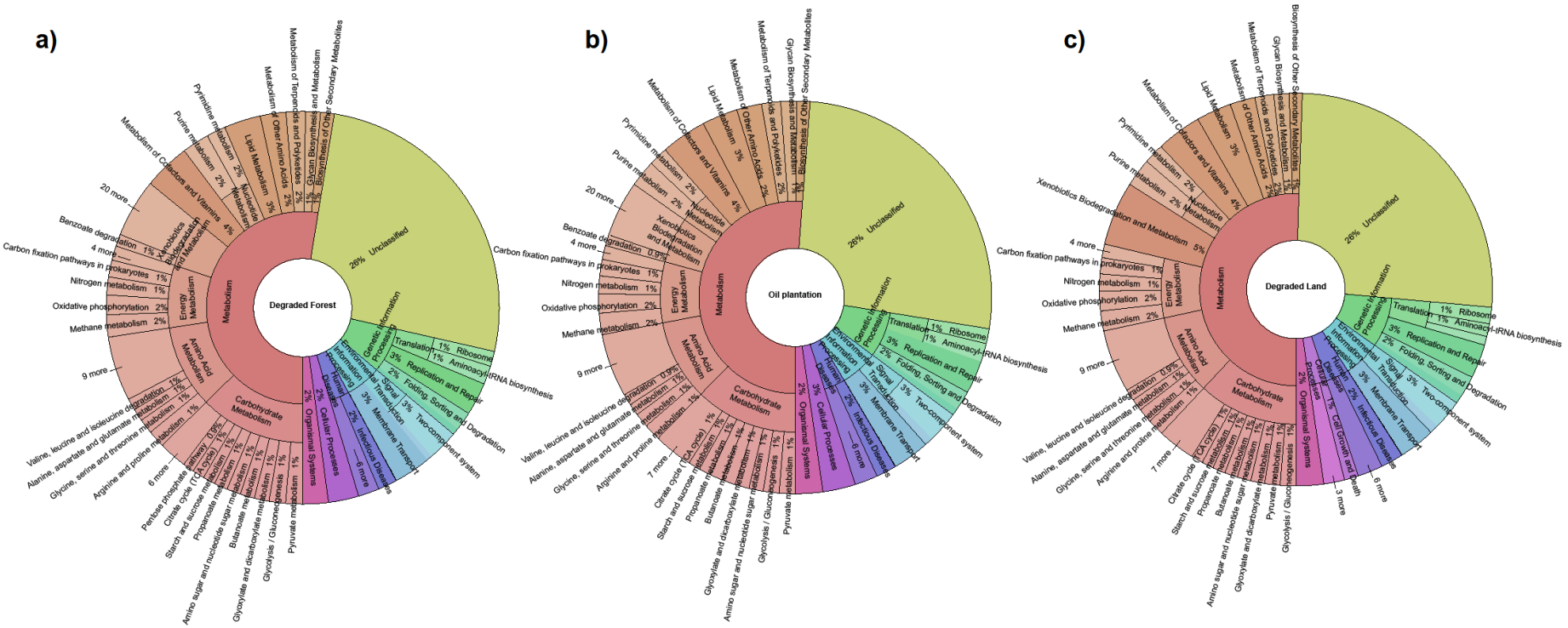


***Fig 4.3**: Functional potential of peat microbiome – **a)** Degraded forest, **b)** Oil palm plantations, **c)** Degraded land*

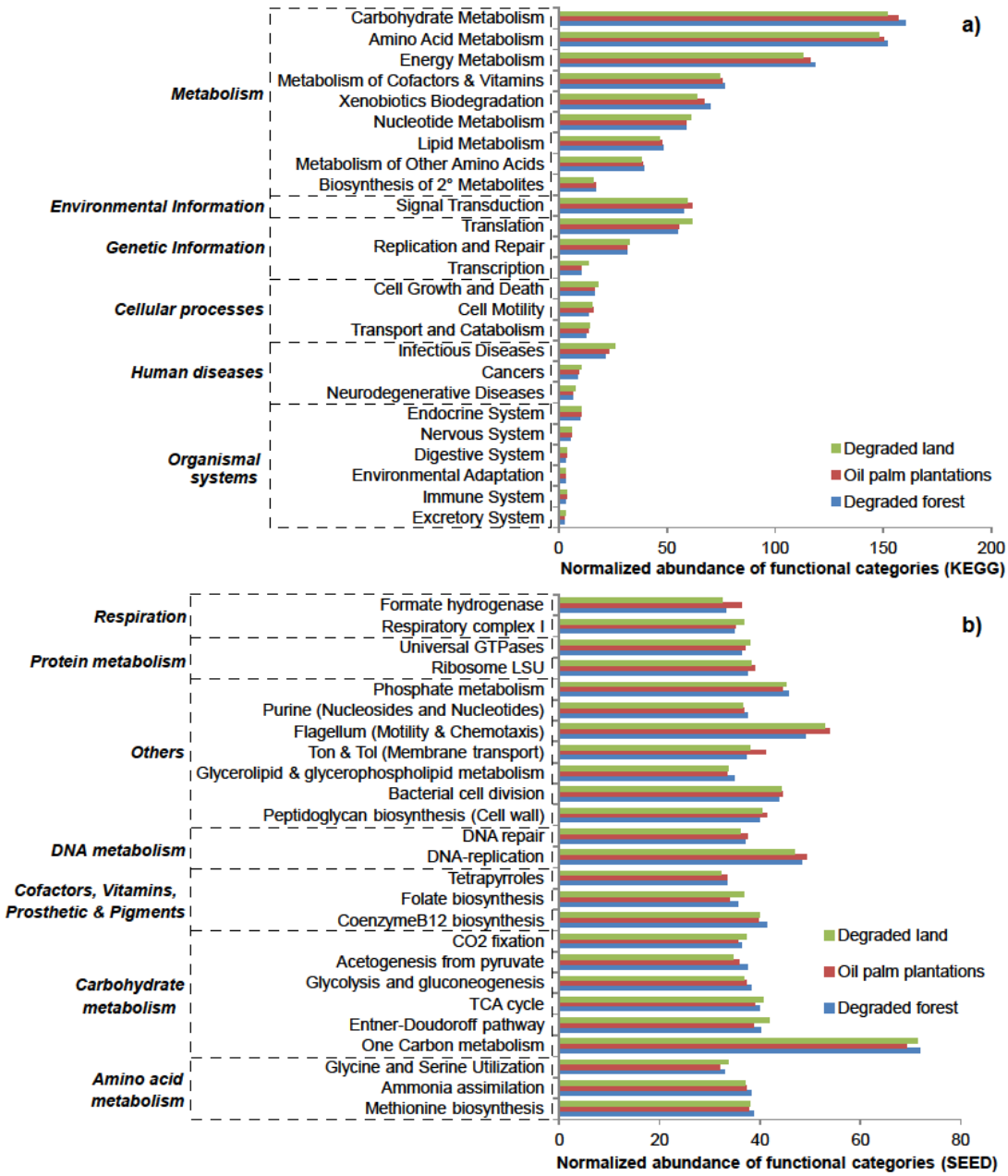


Fig. 4.4*: Functional potential of top 25 mostabundant KEGG (a) and SEED (b) categories.*

Metabolism related KEGG pathways	Degraded forest	Oil palm plantations	Degraded land	
Pyruvate metabolism	11.0	10.6	10.6	**Carbohydrate Metabolism**
Glycolysis / Gluconeogenesis	10.7	10.6	10.7	
Glyoxylate and dicarboxylate metabolism	9.5	9.3	9.5	
Butanoate metabolism	9.3	9.2	8.9	
Amino sugar and nucleotide sugar metabolism	9.1	9.6	9.5	
Methane metabolism	24.4	24.1	23.1	**Energy metabolism**
Oxidative phosphorylation	21.6	21.7	23.1	
Nitrogen metabolism	19.7	19.8	19.4	
Carbon fixation pathways in prokaryotes	18.6	18.6	18.7	
Carbon fixation in photosynthetic organisms	7.6	7.4	7.4	
Fatty acid metabolism	22.9	22.5	22.5	**Lipid Metabolism**
Glycerophospholipid metabolism	16.0	16.8	16.3	
Glycerolipid metabolism	13.0	12.8	12.7	
Fatty acid biosynthesis	12.6	12.9	12.8	
Sphingolipid metabolism	6.7	6.2	5.7	
Arginine and proline metabolism	14.2	14.3	14.2	**Amino Acid Metabolism**
Glycine, serine and threonine metabolism	12.3	12.1	12.3	
Alanine, aspartate and glutamate metabolism	10.3	10.1	10.3	
Valine, leucine and isoleucine degradation	9.7	9.7	9.7	
Cysteine and methionine metabolism	8.7	9.0	8.8	
Selenocompound metabolism	22.9	22.9	23.0	**Metabolism of Other Amino Acids**
beta-Alanine metabolism	22.2	22.2	22.1	
Glutathione metabolism	20.3	20.8	20.9	
Taurine and hypotaurine metabolism	12.3	11.7	11.4	
Cyanoamino acid metabolism	9.4	9.5	9.2	
Porphyrin and chlorophyll metabolism	23.7	23.5	22.6	**Metabolism of Cofactors and Vitamins**
Pantothenate and CoA biosynthesis	11.6	12.4	12.3	
Nicotinate and nicotinamide metabolism	11.4	11.6	12.1	
One carbon pool by folate	10.4	10.3	10.8	
Ubiquinone and other terpenoid-quinone biosynthesis	9.0	8.5	9.0	
Streptomycin biosynthesis	24.1	24.4	25.0	**Biosynthesis of Other Secondary Metabolites**
Tropane, piperidine and pyridine alkaloid biosynthesis	15.0	14.1	14.4	
Phenylpropanoid biosynthesis	11.4	11.7	10.7	
Isoquinoline alkaloid biosynthesis	11.4	10.9	11.1	
Novobiocin biosynthesis	9.6	9.9	10.3	
Benzoate degradation	17.2	17.2	17.0	**Xenobiotics Biodegradation and Metabolism**
Aminobenzoate degradation	13.1	13.1	13.2	
Chloroalkane and chloroalkene degradation	7.5	7.4	7.5	
Toluene degradation	6.7	6.9	6.7	
Chlorocyclohexane and chlorobenzene degradation	5.8	5.6	5.7	

Table 4.2: *KEGG pathways within each metabolism categories. Top 5 within each category is reported here. The numeric value in each cell indicates percent abundance of those pathways in each metabolism categories.*

For example, the first cell (11%) in degraded forest indicates that pathways related to pyruvate metabolism were 11% of the total carbohydrate metabolism pathways that were annotated.

4.3.3 Peat characteristics and their correlation with environmental and physicochemical traits

The year 2010 was extreme wet period throughout the year, including the month of sampling (Oct 2010) based on water table and rainfall patterns (Fig. 3.3a and Table 4.3). The water table depth at the time of sampling ranged between 40 and 50 cm among the three land-use pattern monitored in this study. The total monthly rainfall recorded in the months of September and October 2010 were 214±13 mm and 313±19 mm, respectively.

The redox potential was highest in oil palm plantations, followed by degraded forest and land, in that order and was positively correlated with ammonium, which followed the similar trend (Fig. 4.5 and Table 4.3). On the other hand, redox potential was negatively correlated with potassium, which was found highest in degraded land.

Salinity was found lowest in degraded forest and was positively correlated with other environmental traits such as, peat surface humidity, peat moisture content, pH, and air/peat temperature (Fig. 4.5 and Table 4.3). Nitrates were found two-fold and 30 times higher in oil palm plantations, when compared with degraded forest and degraded land, respectively. They were significantly correlated with sulfates that were highest in oil palm plantations, followed by degraded forest and land, in that order (Fig. 4.5 and Table 4.3). Nitrites were not detected in any of the samples.

The average rate of subsidence in the three land-use patterns has been reported for a quarter more than a year (July 2009 – Oct 2010) in this Chapter. It was lowest in degraded land for the reported year. The subsidence rate followed the similar trend as observed for carbon dioxide emissions during sampling time (Fig. 4.5 and Table 4.3). The subsidence rate was positively correlated with the single time-point CO_2 emissions performed during peat sampling (Pearson's correlation: 0.78 – Fig. 4.5). The subsidence rate was positively correlated with nitrates and sulfates.

Table 4.3: *Environmental traits (Insitu and physicochemical analysis) '±'denotes standard error*

Environmental traits	Degraded forest	Oil palm plantations	Degraded land
Site information: hydrological and peat characteristics			
Transect length (m)	300	240	200
Peat depth (m) *(n=12)*	9.8±0.08	8.7±0.15	9.9±0.14
Annual subsidence rate (cm/yr) (from July 2009 – Oct 2010) *(n=6)*	7±0.3	6.2±1.2	5.3±0.6
Insitu peat characteristic/environmental parameters (Sampling time – Oct 2010)			
Carbon dioxide (ppm) *(n= 9)*	616±22	506±9.5	435±12
Peat surface humidity (%) *(n= 9)*	38±0.4	40±0.5	52±0.8
Peat temperature (°C) *(n= 9)*	26±0.1	27±0.4	27±0.17
Air temperature (°C)*(n= 9)*	30±0.3	30±0.8	38±0.4
Air humidity (%) *(n= 9)*	80±1	77±3.3	49±2.2
Water table depth (cm) at the time of sampling *(n= 3)*	40+3.3	52±2.5	41±1.3
pH *(n= 12)*	3±0.1	3±0.1	4±0.1
ORP- Redox (mV) *(n= 12)*	437±5.3	449±1.8	407±6
Dissolved oxygen (pO_2 – mmHg) *(n= 12)*	232±4	211±3	224±4
Physicochemical analysis (µg/gm of peat) (n=3)			
Peat moisture content (%)	79±1.65	80±1.4	83±0.32
Chloride	13±2.3	23±2.6	23±6.5
Nitrite	*n.d.*	*n.d.*	*n.d.*
Nitrate	116±8.6	233±7.4	8±0.5
Phosphate	141±4.2	113±6.1	95±4.4
Sulfate	38±1.7	45±1.6	31±0.4
Sodium	9±0.7	10±0.3	16±2
Ammonium	41±3.2	46±0.1	27±1
Potassium	28±1.7	19±0.9	56±3.3
Magnesium	6±1.5	4±1.5	20±0.4
Calcium	6±0.8	10±0.6	15±0.6

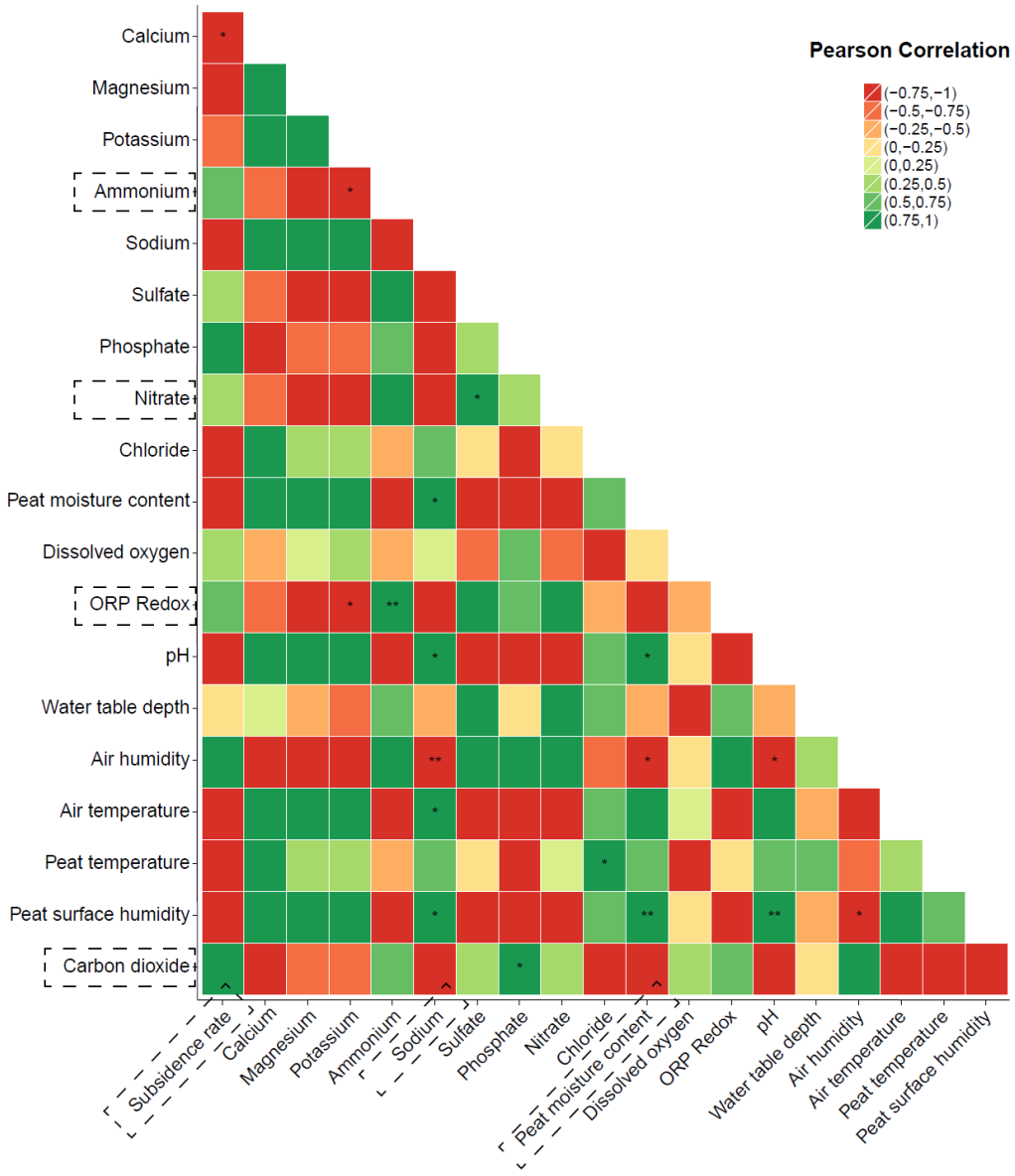


Fig 4.5: *Correlation matrix of the environmental traits including physicochemical parameters. The boxed parameters were picked up for evaluating the relationships with the taxonomic and potential functional profiles*

4.3.4 Linkages between environmental traits with taxonomic profiles and functional potential of peat microbiome

Correlations of taxonomic profiles with environmental traits

While evaluating the correlations of the taxonomic profiles with environmental traits, it was revealed that *Actinobacteria* (abundant in degraded forest – 21% (Fig. 4.2)) were negatively correlated with ammonium (Fig. 4.6a), sodium (Fig. 4.6d) and oxidative reduction potential (Fig. 4.6c). *Euryarchaeota* that were abundant in degraded land were found positively correlated with peat moisture content (Fig. 4.6b), subsidence rates (Fig. 4.7b) and ORP (Fig. 4.7c), while being negatively associated with ammonium (Fig. 4.6a) and nitrates (Fig. 4.6c) On the other hand, Firmicutes abundant in oil palm planatations sites were positively correlated with nitrates (Fig. 4.6c). The *Brachyspiraceae* species (genus *Spirochaetes*) abundant in degraded land were positively correlated with moisture content (Fig. 4.6b) and were negatively with ammonium (Fig. 4.6 a).It can be elucidated that microbial-mediated peat oxidation leading to CO_2 emissions were associated with diverse taxa group (Fig. 4.7b) dominated with *Proteobacteria* and *Firmicutes*. On the other hand, subsidence rates only showed positive correlations with *α-, γ-proteobacteria* and *Euryarchaeota*. The species belonging to *Synergistetes* and *Acidobacteria* genus were abundant in oil palm plantations were negatively correlated with subsidence rates (Fig. 4.7b).

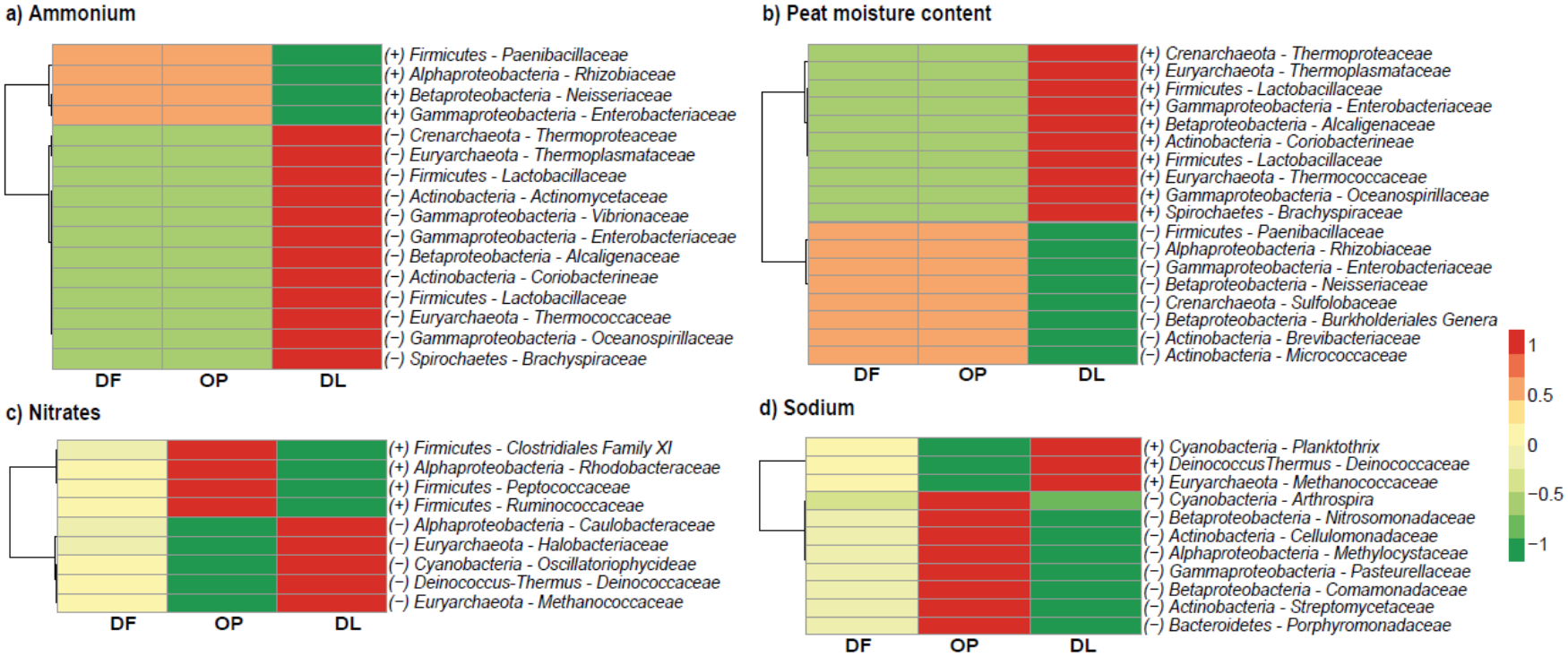


Fig 4.6: *Correlation of selected physicochemical parameters with the taxonomic profiles found in the tropical peatlands. The color (red to green) indicates the range from high to low abundance of the species belonging to specific taxa. The (+) and (-) indicates positive and negative correlations, respectively, of physicochemical variable with the respective taxa. (**DF:** Degraded forest; **OP:** Oil palm plantations; **DL:** Degraded land)*

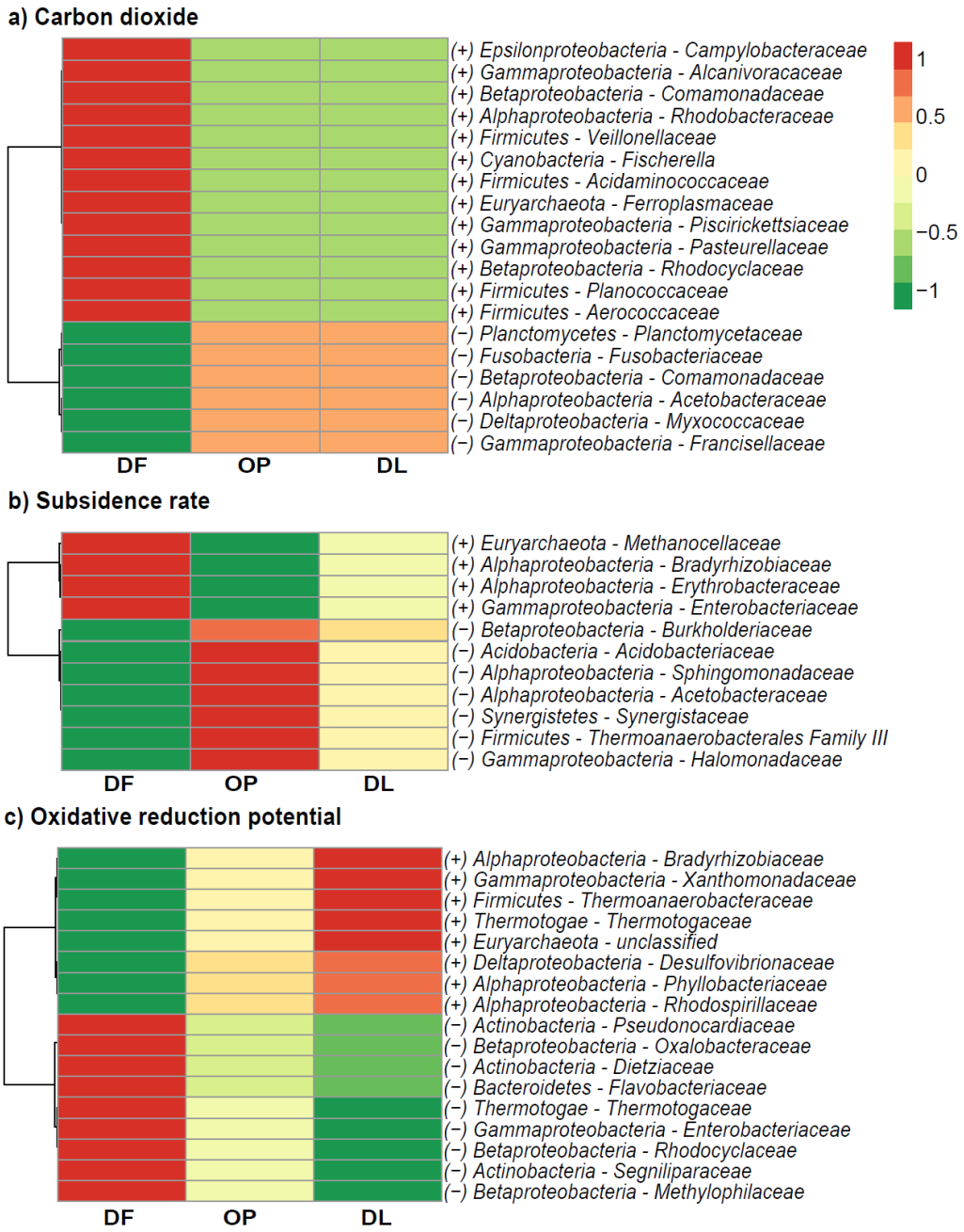


Fig 4.7: *Correlation of selected insitu environmental parameters with the taxonomic profiles found in the tropical peatlands. The color (red to green) indicates the range from high to low abundance of the species belonging to specific taxa. The (+) and (-) indicates positive and negative correlations, respectively, of insitu variable with the respective taxa. (**DF:** Degraded forest; **OP:** Oil palm plantations; **DL:** Degraded land)*

Correlations of microbial functional potential with environmental traits

Based on KEGG annotations, the pathways that were correlated with environmental traits are reported in Fig. 4.8 and 4.9. The metabolism-related pathways that were correlated with CO_2 emissions (Fig. 4.9a) and subsidence rates (Fig. 4.9b), comprised mainly of carbohydrate (glyoxylate, glycolysis – pentose phosphate) and amino acid (phenylalanine, gutamine, serine – threonine and seleno compound) metabolism, that were in abundant in degraded forest. These correlated pathways that are correlated with peat oxidation are among the top five abundant metabolism-related pathways (Table 4.2). However, none of these pathways could found annotated in correlation with ORP (Fig. 4.9c). The pathways related to phenolpropanoid, flavone and flavonol biosynthesis were positively correlated with ammonium, while being negatively associated with peat moisture content (Fig. 4.8a, b). Metabolic pathway related to folate metabolism abundant in degraded land had inverse correlations with nitrates (-) and sodium (+) (Fig. 4.8c, d).

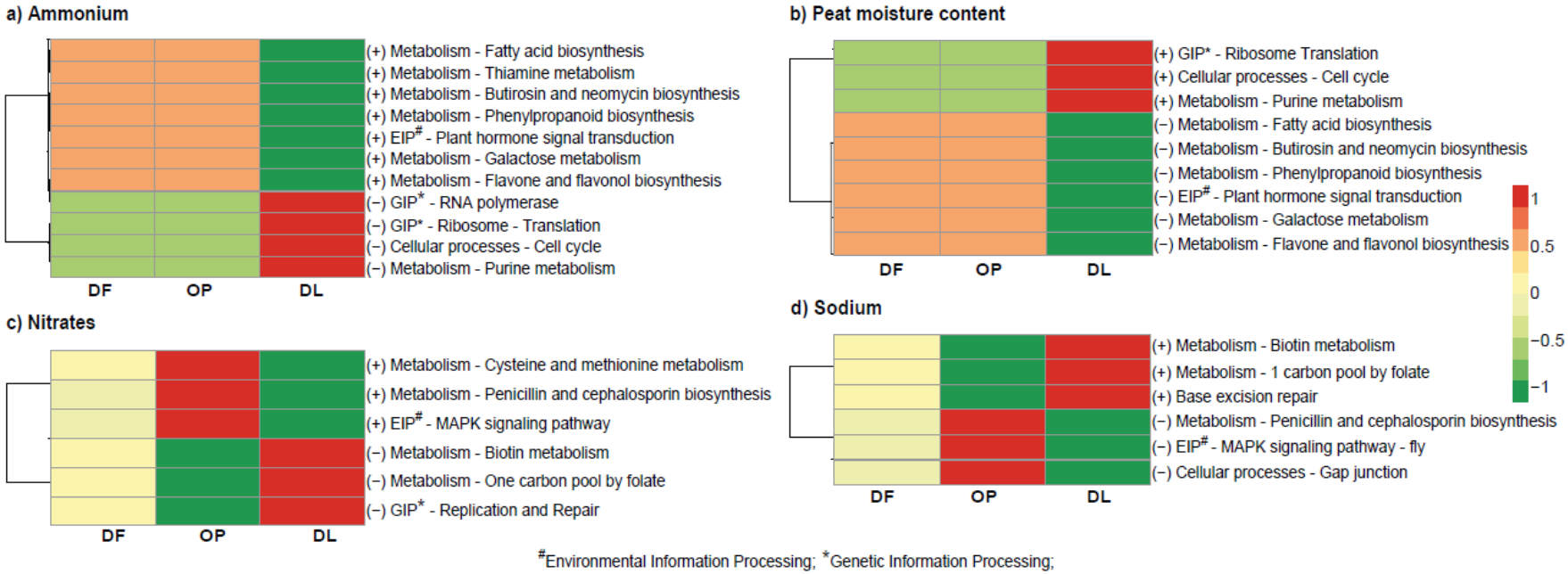


Fig. 4.8: *Correlation of selected physicochemical parameters with the KEGG functional potential found in the tropical peatlands. The color (red to green) indicates the range from high to low abundance of the species belonging to specific taxa. The (+) and (-) indicates positive and negative correlations, respectively, of physicochemical variable with the respective taxa. (**DF:** Degraded forest; **OP:** Oil palm plantations; **DL:** Degraded land)*

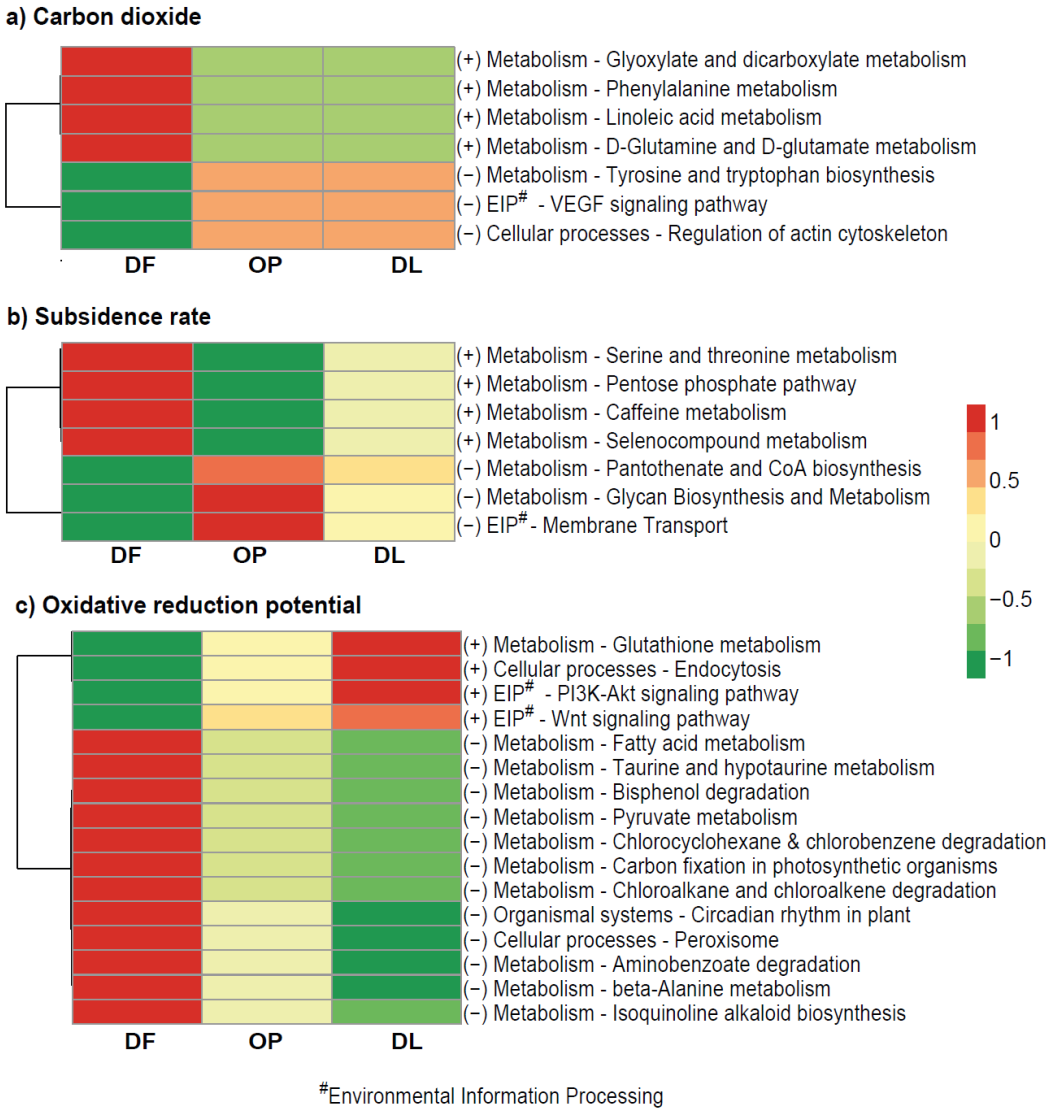


Fig. 4.9: *Correlation of selected insitu environmental parameters with the KEGG functional potential found in the tropical peatlands. The color (red to green) indicates the range from high to low abundance of the species belonging to specific taxa. The (+) and (-) indicates positive and negative correlations, respectively, of physicochemical variable with the respective taxa. (**DF:** Degraded forest; **OP:** Oil palm plantations; **DL:** Degraded land)*

The diversity of categories of functional pathways that got annotated in SEED (Fig. 4.10, 4.11) was higher compared to KEGG (Fig. 4.8, 4.9), which corroborated with the diversity indices reported earlier in Section 4.3.2. Amino acid (Lysine, cyanophycin – urea cycle) degradation and nitrogen metabolism (ammonia assimilation) that was higher in degraded forest and oil palm plantations was positively correlated with ammonium (Fig. 4.10a). Pathways linked with nitrogen (nitrate ammonification), carbohydrate (glycogen, sorbitol, raffinose) DNA (DNA replication, repair) and iron metabolism that were abundant in oil palm plantations, were positively correlated with nitrates (Fig. 4.10c). DNA topisomerases and replication pathways that were abundant in degraded land were inversely related between ammonium and peat moisture content (Fig. 4.10a, b). *Actinobacteria* that were abundant in degraded forest (Fig. 4.2a) was positively correlated with carbon dioxide emissions, under the category of membrane transport (Fig. 4.11a). Carbohydrate (pyruvate, one-carbon), phosphorus and amino acid (leucin) metabolism was highest in degraded forest and was positively correlated with subsidence rate. Isoprenoid synthesis that was higher in degraded land and oil palm plantations compared to degrade forest was positively related to oxidative reduction potential and sodium (Fig. 4.10d, 4.11c).

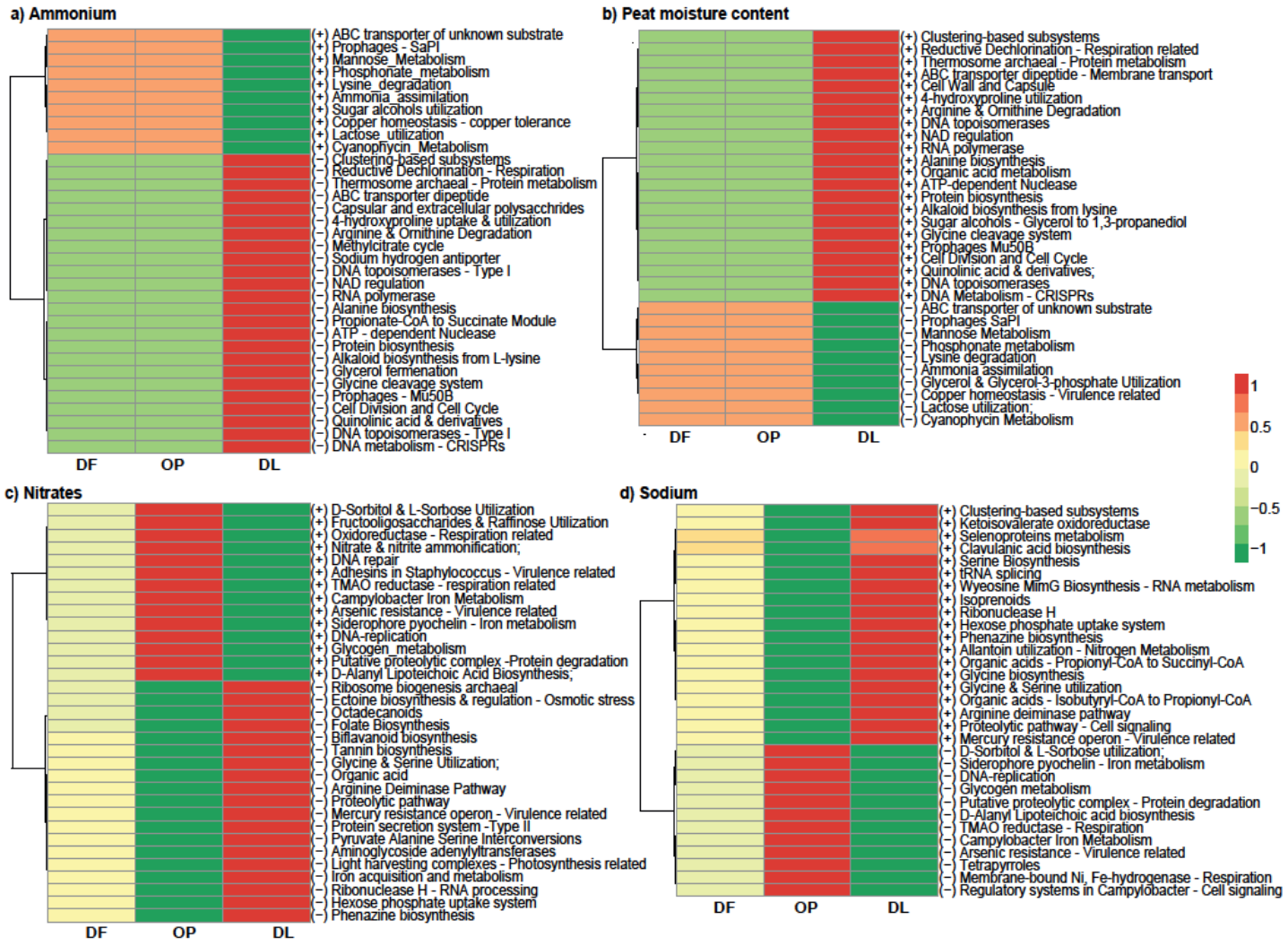


Fig. 4.10: *Correlation of selected physicochemical parameters with the SEED functional potential found in the tropical peatlands. The color (red to green) indicates the range from high to low abundance of the species belonging to specific taxa. The (+) and (-) indicates positive and negative correlations, respectively, of physicochemical variable with the respective taxa. (**DF:** Degraded forest; **OP:** Oil palm plantations; **DL:** Degraded land)*

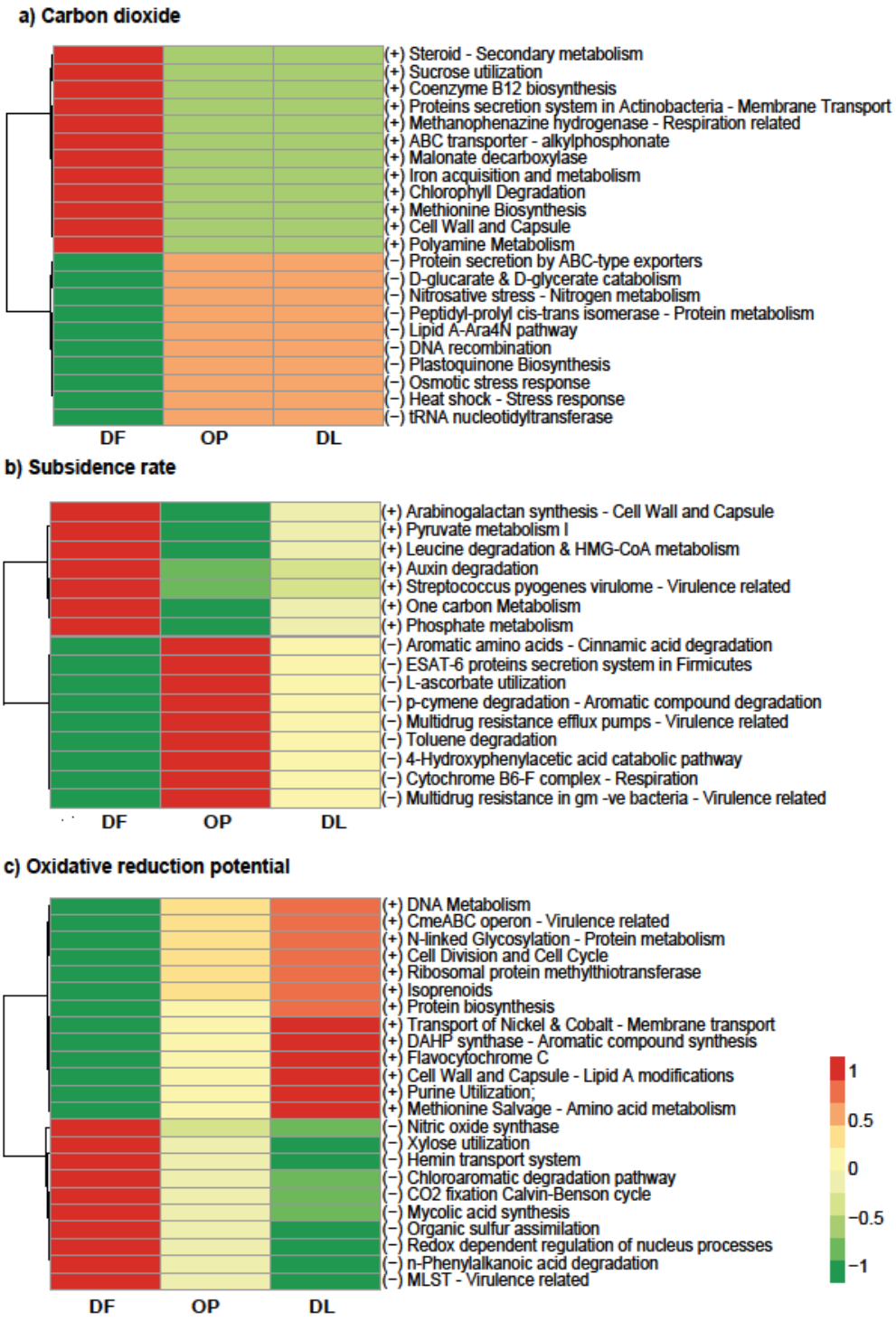


Fig. 4.11: *Correlation of selected insitu environmental parameters with the SEED functional potential found in the tropical peatlands. The color (red to green) indicates the range from high to low abundance of the species belonging to specific taxa. The (+) and (-) indicates positive and negative correlations, respectively, of physicochemical variable with the respective taxa. (**DF:** Degraded forest; **OP:** Oil palm plantations; **DL:** Degraded land)*

4. 4 Discussions

Peat is mostly composed of organic matter, which comprises of (i) carbohydrates, such as, celluloses, hemicelluloses, pectin, chitin and some of the glycosides; (ii) bitumins, (iii) lignin and lignin – like compounds, (iv) humic substances and (v) other nitrogenous compounds (Delicato D., 1996). The discussion of this Chapter is aimed to interpret the data in terms of degradation /metabolism of (i) carbohydrates, (ii) lignin and phenolics, (iii) nitrogenous compounds, based on the functional potential, related taxonomic profiles and linkages with environmental traits.

Deep sequencing-based approach (reported in this Chapter) reveals most accurate prediction of abundance of microbial communities and their functional potential. In temperate peatlands, the C-degradation is reported to be linked with the abundance of certain taxa, such as *Actinobacteria,* among others (Pankratov et al., 2006; Mackelprang et al., 2011; Tveit et al., 2012; Tveit et al., 2014). In *Sphagnum* peat bog of Russia, *Actinobacteria* has also been reported to play an important role in aerobic cellulose degradation (Pankratov et al., 2006). A combined metagenomic and metatranscriptomic study on arctic permafrost peatlands (Tveit et al., 2012 and 2014) revealed that *Actinobacteria* seemed to be particularly important, having a metabolic potential for carrying out several of the key steps in soil organic carbon (SOC) degradation. In those sites, the taxonomic annotation also revealed that *Actinobacteria* and *Bacteroidetes*

were the dominating polysaccharide decomposers. Another study of permafrost peat showed that the active layer communities were dominated by *Actinobacteria, Proteobacteria* and *Chloroflexi*, while the permafrost microbiota also contained large populations of *Bacteroidetes* and *Firmicutes* (Mackelprang et al., 2011). In tropical peat, as reported in this study, similar abundance of these taxa was found. *Actinobacteria* and *Firmicutes* were the most abundant taxa after *Proteobacteria* (Fig. 4.2) in the metagenome data reported here, suggesting its potential role in the C-degradation of tropical peat. This observation is consistent, as the abundant species predicated using marker-based approach (Chapter 3) were also *Actinobacteria* and *Firmicutes*.

Peat oxidation with respect to degradation of lignin and lignin-like compounds involve understanding of flavone/flavonol and phenylpropanoid biosynthesis pathways that were abundant in degraded forest and oil palm plantations and were positively correlated with ammonium (Fig. 4.8a) but negatively associated with peat moisture content (Fig. 4.8b). Metabolism of phenylalanine through enzyme, phenylalanine ammonia lyase (PAL) in the phenylpropanoid pathway, leads to biosynthesis of flavonols, anthocyanins, tannins, coumarins, glycitein and glyceollin through variety of other enzymes. It is well documented that the biosynthesis of flavonols, flavonoids and phenylpropanoid was reduced when the PAL gene was suppressed in a transgenic tobacco plant (Elkind et al., 1990), in strawberry leaf disks

(Creasy, 1968), among others. Drought induces water migration from cells resulting in dehydration and eventual plasmolysis. However, in plant cells it is shown that (reviewed in – Chalker-Scott, 1999) water stress (low moisture content) induces high biosynthesis of anthocyanins (end products of phenylpropanoid pathway), which is consistent with our findings (Fig. 4.8b) about negative correlations of peat moisture content with biosynthesis of these compounds. Also, it is reported in a study on red wine grapes that water deficits have promoted higher concentrations of anthocyanins and other flavonols (Castellarin et al., 2007). Phenylalanine metabolism leading to the biosysnthesis of aromatic compounds is positively correlated with CO_2 emissions in our study (Fig. 4.9a). Certain products, such as, soluble phenolics and condensed tannins in the phenylalanine metabolism pathway has been reported to be increased with elevated CO_2 (Penuelas and Estiarte, 1998), which is consistent with our study. These findings suggest that tropical peat microbes have adopted well to break-down such recalcitrant phenolics.

One aspect to interpret peat oxidation is through estimating CO_2 emissions, which were monitored during the sampling time in our study sites (Table 4.3). Degraded land, which had no plantations and was drained for more 10 years, had the least CO_2 emissions in the land-use patterns studied. This could be attributed to low amounts of easily decomposable fresh litter and low root respiration. Similar reports has been documented from that show low CO_2 emission rates in drained

unvegetated agricultural peatland in Central Kalimantan that was drained about 20 years ago, when compared to vegetated land in the same region (Jauhiainen et al., 2004; Jauhiainen et al., 2008). CO_2 emissions were associated with diverse taxonomic groups (Fig.4.7a) that are linked with carbon oxidation. *Rhodocyclaceae* have been known to be involved in carbon oxidation in citrate cycle. *Planococcaceae* is reported to be involved in oxidation of variety of carbon substrates, including pyruvic, succinic acid and maltose (Shivaji et al., 2014). *Pasteurellaceae* is known for associated functioning in respiration, central metabolism, metabolism of carbohydrates and fatty acids, transcriptional regulation and transport (Ravcheev et al., 2007). *Comamonadaceae* is known to utilize cis-dichloroethene (cDCE) as a sole carbon and energy source (Coleman et al., 2002). *Acidaminococcaceae* are able to grow with glutamate as the sole source of energy. The fermentation of glutamate proceeds via the hydroxyglutarate pathway yielding, carbon dioxide, acetate, butyrate, and hydrogen as products (Buckel and Barker, 1974). *Firmicutes* are involved in C-degradation as discussed in previous paragraph, was also one of the abundant taxa that were correlated with CO_2 emissions. These evidences indicate that the tropical peat oxidation leading to CO_2 emissions is linked with functional potential from diverse taxa groups.

Another aspect to interpret peat oxidation is through field survey of continuous peat subsidence rates (1.25 years data reported in this study – Table 4.3). The positive correlation of peat subsidence rates, ammonium

and nitrates with taxonomic profiles, respectively, shows linkages of C and N coupling reaction enhancing peat oxidation in degraded tropical peatlands. *Bradyrhizobiaceae* (*Nitrobacter*) and *Rhizobiaceae* (*Sinorhizobium*), both belonging to order – *Rhizobiales* were, respectively, correlated with subsidence rates (Fig. 4.7b) and ammonium (Fig. 4.6a). The former order was found abundant in degraded forest and the later was moderately abundant in both degraded forest and oil palm plantations. *Nitrobacter* is known to oxidize nitrite into nitrate in soil at pH close to neutral; however, at low pH (as in tropical peatlands), nitrification is inhibited and ammonia starts to build up. There was no nitrite detected in our study sites. The nitrates that were found excessively abundant in oil palm plantations was due to external sources (i.e.) fertilizers. *Bradyrhizobiaceae* species that were correlated with subsidence rates (C-oxidation) has been reported in soil C cycling (Fan et al., 2014). *Rhizobiales* are generally known for fixing nitrogen in symbiosis with leguminous plants; however, in this study (Fan et al., 2014) potential microbial coupling of carbon and nitrogen cycling during decomposition of maize residue has been reported using ^{13}C-DNA-SIP, suggesting that residue decomposition may promote *Rhizobiales* as well as non-*Rhizobiales* N-fixation through C-feeding. Another class of taxa, *Enterobacteriaceae* (Fig. 4.7b) that were positively correlated with subsidence rates, are known for degrading pectin and reducing nitrate to nitrite (Abbott and Boraston, 2008). Also *Rhodobacteraceae* that was

positively correlated with nitrates (Fig. 4.6c) and C-oxidation (Fig. 4.7a) has been reported to possess genes for assimilatory nitrate and nitrite reduction pathways (Vollmers et al., 2013) in strains isolated from Arctic and Antarctic regions. *Rhodobacteraceae* (from marine ecosystem) uses TMA (trimethylamine) as a sole carbon and nitrogen source and is known to play an important role in the carbon and nitrogen cycling (Chen et al., 2011). *Firmicutes clostridia* found abundant in oil palm and positive correlated with nitrate (Fig. 4.6c), are known to produce energy via anaerobic respiration using compounds other than oxygen, such as nitrate (that is abundant in oil palm plantations sites in our study, due to fertilizer inputs), as its final electron acceptor (Hasan and Hall, 1975). In peat sediments of Florida Everglades (temperate region), *Firmicutes* are also known to be associated with denitrification (Gordon et al., 1986). An important role of denitrification, which is supported by the high nitrate concentrations, has also been suggested by metagenomic studies of permafrost affected soils (Mackelprang et al., 2011). *Firmicutes* are involved in C-degradation as discussed earlier (in the Discussion section of this Chapter 4), indicates that the role of C and N coupling is likely to enhance peat oxidation from sites that are heavily fertilized, as in our study sites. A field study from tropical peatland in Kalimantan has demonstrated gaseous losses of soil C and N from the study area to the atmosphere, elucidating oxic conditions after drainage to promote microbial C–decomposition and nitrogen mineralization in tropical

peat (Hadi et al., 2005). Our study sites that have high fertilizer inputs (i.e.) high nitrate concentrations is estimated to be involved with higher rates of C – degradation and N – metabolism. Therefore, to test this observation about C and N coupling, we propose a field survey estimating C and N dynamics in future directions of this study (Chapter 6).

Peat oxidation interpreted through CO_2 emissions elucidates the possibility of coupling of carbon oxidation with iron metabolism based on SEED annotations (Fig. 4.11a). *Ferroplasmaceae* species, which is an acidophilic iron-oxidizing member of *Euryarchaeota*, was positively correlated with CO_2 emissions (Fig. 4.7a). Relationships can be drawn from these observations, as these species were abundant in the same sites where iron related metabolism was annotated. It has been reported earlier that microorganisms can completely oxidize organic compounds with iron as the sole electron acceptor and that oxidation of organic matter coupled to dissimilatory iron reduction can yield energy for microbial growth (Lovley and Phillips, 1988).

Another aspect which is noteworthy is that, one carbon metabolism (Fig 4.4) was one of the highest abundant functional potential and methane metabolism (Table 4.2) was the highest within energy metabolism. We have discussed earlier various species that are involved in carbon metabolism. Organic carbon oxidation and suppression of methane production in vegetated and unvegetated freshwater wetland sediments has been coupled with iron metabolism (Roden and Wetzel, 1996). It is

also reported that methane oxidation in freshwater sediments was stimulated with ammonium concentration (Roy and Knowles, 1994). In our study sites where subsidence rates were elevated (degraded forest and oil palm plantation sites) higher ammonium concentration compared to degraded land was shown, (Table 4.3). In temperate swamps, nitrates and sulfates have been known to be potential inhibitors of methanogenesis (Amaral and Knowles, 1994). In our study sites, we had similar trend of CO_2 emissions, subsidence rate, nitrates and sulfates for different land-use types studied (high in degraded forest and oil palm plantation sites, when compared to degraded land – Table 4.3). These sites have huge application of fertilizers, increasing the concentration of nitrates, and, thus possibilities of methane oxidation in tropical peatland sites become high. It is well reported that methane fluxes to be extremely low from tropical peatlands (amounting to 3 mg $CH_4 m^{-2} h^{-1}$), compared to emissions from boreal and temperate peatlands. It has been demonstrated earlier that CH_4 diffusing towards the atmosphere may also be oxidized to CO_2 by methanotrophic bacteria at times when oxic conditions are present in the upper peat profile (e.g., during the dry season when the water table falls below the surface) (Couwenberg et al., 2010; Inubushi et al., 2003; Jauhiainen et al., 2005 and Jauhiainen et al., 2008). The metagenome analysis reported in this study is from the oxic zones. These findings give us a clear indication about the possibility of methane oxidation and its

linkages with iron metabolism, nitrates, ammonium and sulfates in the aerobic zones of the tropical peatlands.

4.5 Conclusions

Phenylalanine metabolism leading to the biosysnthesis of aromatic compounds is positively correlated with CO_2 emissions in our study. Flavone/flavonol and phenylpropanoid biosynthesis that were abundant in degraded forest and oil palm plantations were positively correlated with ammonium but negatively associated with peat moisture content. Tropical peat oxidation leading to CO_2 emissions is linked with functional potential from diverse taxa group. In degraded forest and oil palm plantations, *Rhizobiales* were abundant and were correlated with subsidence rates and ammonium concentrations, indicating linkages of these species with peat oxidation. Also, *Actinobacteria* and *Firmicutes* were among the most abundant taxonomic groups, demonstrating their linkages in oxidation of tropical peatlands. The positive correlation with subsidence rates suggests, coupling of C- and N- cycling leading to overall peat oxidation evident through high subsidence rates. Our sites followed the similar pattern for subsidence rates, nitrates, sulfates and ammonium and showed possibility of linkages with methane oxidation in the oxic zones of tropical peatlands, as one-carbon metabolism correlated positively with subsidence rate and CO_2 emissions data. Also, there is possibility of coupling of carbon oxidation with iron metabolism.

Chapter 5: Eco–physiological responses of peat microbial communities during switching to rewetting

5.1 Background

Peat oxidation in tropical peatlands as reported from field conditions elucidates high amount of CO_2 (Couwenberg et al., 2010; Murdiyarso et al., 2010; Page et al., 2011) and to a lesser extent of CH_4 emissions, from tropical regions (Jauhiainen et al., 2005; Jauhiainen et al., 2010; Verwer et al., 2008). In contrast, higher hot spots of CH_4 emission have been observed in temperate and boreal peatlands based on field conditions (Hendricks et al., 2007; Teh et al., 2011).

Tropical regions encounters high amount of rainfall and, thus, are prone to frequent drying-wetting. Drying–rewetting events can induce significant changes in microbial C and N dynamics and these effects can last much longer. A study from temperate peatlands showed that drought stimulates bacterial growth and phenol oxidase activity, resulting in reduction in the concentration of phenolic compounds in peat and stimulating microbial growth, which causes breakdown of organic matter and release of carbon dioxide (Fenner and Freeman, 2011). Upon re-wetting, the peat carbon loss to the atmosphere, did not stop, because rewetting increases the labile carbon levels and stimulates anaerobic decomposition (Fenner and Freeman, 2011). Similarly, a field study in tropical peatlands of Kalimantan, Indonesia, also showed that hydrological restoration did not

lead to any significant difference in the hummock/high surface or depression surface GHG budgets in either of the sites studied, (i) drainage-affected selectively logged peat swamp forest and (ii) deforested, drained, burned peatland (Jauhiainen et al., 2008). Another study conducted in lab-based conditions, that focused on studying the impacts of drying – rewetting from wetland soil (meadow in southern Sweden), showed that soil drying stimulated N mineralization and reduced denitrification (Venterink et al., 2002). However, upon rewetting, denitrification measured in the re-wetted cores was much higher compared to field conditions.

Hence, it is important to understand the fate of microbial physiological response upon rewetting in a controlled environment and to explore the influence of rewetting on CO_2 emission rates, leading to microbial-mediated peat oxidation (subsidence), from this region. Hence, the objective of this study was to understand the physiological responses of peat microbes and their associated changes in microbial community.

5.2 Materials and methods

5.2.1 Study site description and sample collection

The study location was DHPN within Site B (Fig. 3.1) as described in Section 3.2.1 of Chapter 3. Sampling was performed in first week of February 2014. The total monthly rainfall recorded in the months of December 2013 and January 2014 were 159±37 mm and 45±18 mm, respectively. At each sampling location, a $1m^3$ pit was dug (Fig. 5.1). Sample was collected in a random sampling design from four locations within oil palm plantation with an approximate distance of 250m between each sampling location. Peat samples were collected from three depths, (i) 20-30 cm below peat surface; (ii) 20-30 cm above water table and (iii) 20-30 cm below water table (Fig.5.1). Based on the dissolved oxygen data, we would refer these depths (in relative terms to each other) as (i) oxic zone; (ii) partial oxic zone and (iii) anoxic zone, respectively. The detailed methodology about the sample collection from the pit at different depths has been described in Section 3.2.1 of Chapter 3. Peat water samples were collected from each pit in triplicates in sterile falcon tubes from the water table that separates the peat in submerged anoxic conditions from rest of the peat vertical profiles (as shown in Fig. 5.1). These samples were then shipped to Lawrence Berkeley National Laboratory (LBNL), USA on ice within 36hrs after sampling with due regulatory approvals from Ecology Department, Earth Science Division, LBNL, USA. Part of this study (microcosm set-up, measurements of CO_2

and CH_4 emissions using respirometer) was performed in Ecology Department, Earth Science Division (ESD), LBNL in collaboration with Dr. Romy Chakraborty, Research Scientist at Ecology department, ESD, LBNL.

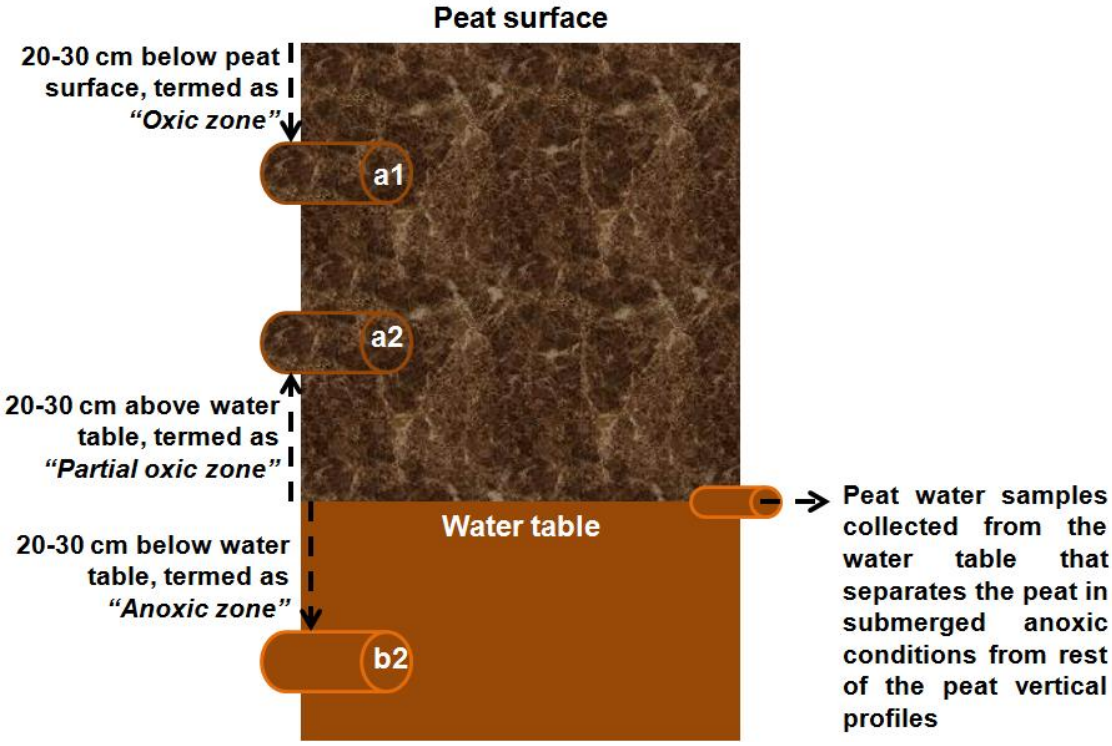


Fig. 5.1: *Sampling pit from where samples at respective depths were collected.*

5.2.2 Microcosm set-up, sampling design and monitoring

In order to understand the microbial physiological responses leading to gas emissions (CO_2 and CH_4) before and after rewetting, a 30 day microcosm experimental set-up was designed with 6 time-points, equally distributed before and after rewetting. The experiment was randomized at each level to remove any batch effects, unless otherwise stated: starting

from sample collection from pits, bottling samples in the serum bottles for microcosm set-up, any extraction from the peat samples.

Composite samples from two pits (randomly chosen) were made by mixing samples from each zone with respective zones of the other pit (please refer to Fig. 5.2a for details of the experimental design). Microcosm was set-up using 40 ml cleaned and autoclaved serum glass bottles. Three replicates of each composited sample from respective zone were bottled-up in those serum bottles (10gm wet weight in each bottle). Samples from anoxic zones were always bottled-up and opened during sampling time-points in anaerobic chamber only. In order to prepare samples as a control, another set of samples (in triplicates) from all the three zones (oxic, partial oxic and anoxic zones), was autoclaved three times with overnight growth, in between the three autoclaving procedure, for complete spores' fatal in the peat samples. All these bottles with samples (3 technical reps X 3 depths X 2 biological reps) and controls (2 technical reps X 3 depths X 2 biological reps) were then installed in Micro-Oxymax respirometer (Columbus Instruments) (Fig. 5.2b). The samples were maintained at room temperature (around 25-26°C), which is approximately equivalent to air temperature in those sites. Sacrificial samples in triplicates were also bottled-up for each time-point in order to monitor the microbial, metabolic and physicochemical changes before and after rewetting (Fig. 5.2a, c). Micro-Oxymax respirometer system represents a fully automated approach with automatic controls. The

principle of measurement involves air sampling from the head space of the sample chamber. It can measure both consumption and production rates and the net emissions are recorded as “accumulated emissions in µL”, as reported in this study.

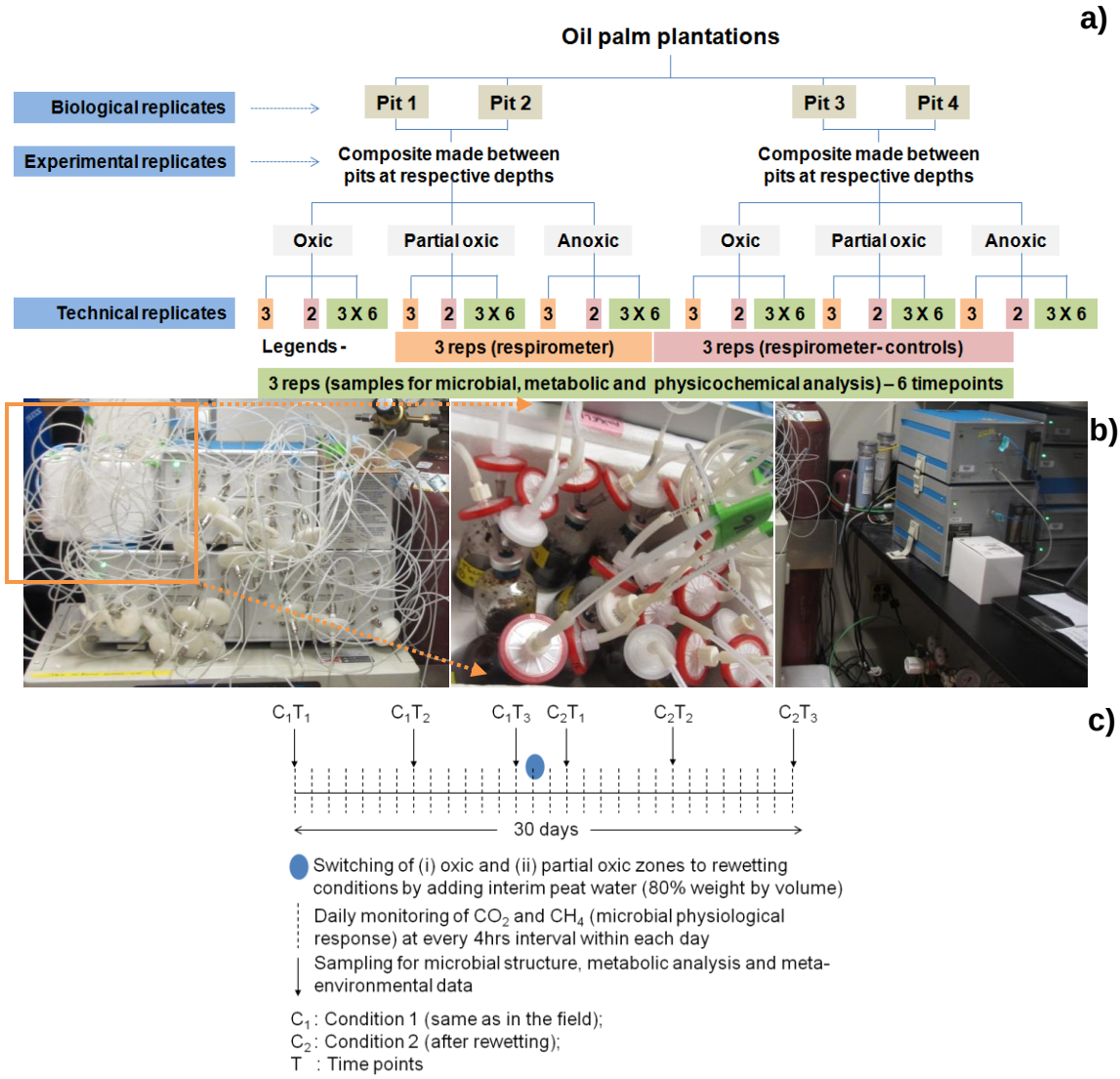


Fig. 5.2: a) *The experimental design to affect of rewetting on the microbial communities (structure and metabolic functional potential) and their physiological responses, in the oil palm plantation sites.* ***b)*** *Pictorial representation of microcosm set-up in the Micro-Oxymax respirometer. Left image depicts the front view; the middle image zoom-out the box, where the serum bottles with the samples were kept for 30 days and the right image shows the sensor detecting CO_2 and CH_4 and recording data at 4 hrs interval to the laptop attached.* ***c)*** *Time-line for the sampling schedule is shown. Please refer to Section 5.2.3 and 5.2.4 for the details of the parameters measured.*

Monitoring and analysis

The samples from each zone were monitored for 15 days with the environmental conditions that were set in the same range as in the field conditions. The data for gas analysis was recorded every 4 hrs per day in the Micro-Oxymax respirometer. The sacrificial bottles in triplicates from each zone were opened for sampling, during each time points (for before and after rewetting) as described in Fig. 5.2c.

In order to mimic the rewetting condition in the field scenario, a perturbation of rewetting was conducted on the 15th day of the experiment to the oxic (a1) and partial oxic (a2) zones, as these zones undergo rewetting cycles during water table fluctuations in the field. Composite of peat water from pits 1 and 2 were made by adopting similar strategy as described in Fig. 5.2a. Accordingly, composite of peat water from pits 3 and 4 was prepared. The composite water (80% weight by volume – 8 ml in 10 gm of peat) from respective pits was added using syringe to all the serum bottles with samples (a1 and a2 zones) that were hooked to the respirometer (including controls from those zones) and remaining sacrificial bottles (triplicates from C_2T_1, C_2T_2, and C_2T_3 – Fig. 5.2c). The 80% saturation was made based on the data reported for peat moisture content in oil palm plantations (Table 4.3 in Chapter 4).

5.2.3 Microbial community structure and environmental traits

The genomic DNA from each zone (in triplicates) and from each time-points (as described in Fig. 5.2c) was extracted using FastDNA™ SPIN Kit for Soil (MP Biomedicals) following manufacture's protocol. The methodology adopted for T-RFLP analysis is described in Section 3.2.2 (Chapter 3). In order to identify the variations in microbial community structure in different conditions and time-points (Fig. 5.2c), multivariate statistical techniques (PRIMER 6, PRIMER-E, Ltd., Plymouth, United Kingdom) were used to calculate distance matrices using Bray–Curtis similarity indices and one-way ANOSIM (for details, please refer to Section 3.2.4 of Chapter 3).

Water table measurement, rainfall and subsidence rates were monitored based on methodology described in Section 4.2.2 (Chapter 4). Subsidence data, as a proxy of peat oxidation reported in this Chapter is from July 2009 to October 2012. In-situ dissolved oxygen, from three zones (described in Fig. 5.1), air humidity and temperature was recorded using the methodology and equipments mentioned in Section 4.2.2 (Chapter 4).

Physicochemical analysis for anions and cations such as chloride, nitrate, ammonium etc was also performed based on the methodology described in Section 3.2.3 (Chapter 3).

5.2.4 Peat microbial metabolomics

In order to understand the microbial metabolic profiling and predictive functional potential before and after rewetting, samples from each time points were run through in-house developed metabolomics workflow. Briefly, cell disruption was achieved through bead beating, using a Tomy Micro Smash MS 100 bead beater (Tomy Seiko Co., Tokyo, Japan) and a Lysing Matrix E from the MP Biomedical lysing kit was used for this purpose, which constituted 0.5 mm diameter Yttria-Stabilized Zirconium oxide beads in screw-top micro centrifuge tubes. Peat samples, weighing 500 mg, were added to the lysing matrix along with 1 mL cold 80% methanol. The sample was subjected to bead beating at 4000 rpm for 20 s, and then thawed on ice for a minute. The procedure was repeated five times, and the extract was then centrifuged twice at 8000 x g for 10 min at 4 °C. The supernatant was filtered through a 3kDa MWCO cut-off filters. This final extract (~1mL) were pre-concentrated by lyophylization (Free-Zone −105 °C 4.5 L Freeze Dry system, Labconco, Kansas City, MO USA) to dryness, then dissolved in 100ul 50% methanol prior to LC-MS analysis.

Non-targeted metabolite profiling was carried out using a Zorbax SB-C18 Rapid Resolution HD column (2.1x50 mm, 1.8-micron) reverse phase column, at a column temperature of 50 °C, on an Agilent Infinite 1290 UPLC system (Agilent Technologies, Santa Clara, CA). The mobile phases were 0.1% formic acid in water (Eluent A) and acetonitrile with 0.1% formic acid (Eluent B). Chromatographic separation was carried

using the following gradient program: 0-2 min, 3% B; 2-12 min, 3-100% B; 12-13.5 min, 100% B; 14-16 min, 3% B. The flow rate was 0.3 mL/min. The injection volume was 5 µL for each individual analysis. Mass spectrometric analyses were carried out in both positive and negative ESI modes, using the following instrumental conditions: Capillary voltage, 3600V; Fragmentor voltage, 115V; Source gas temperature, 250 °C; Sheath gas temperature, 350 °C; Sheath gas flow, 12L/min; Nebulizer gas flow, 40 psi and Drying gas flow, 10 L/min. Mass Hunter B.05.01 software package (Agilent Technologies, Santa Clara, CA) was used for both acquisition and data processing.

UPLC-MS raw data files (exported as mzXml files by Proteowizard v3.0.6995 software) were first preprocessed by the open-source software MZmine v2.11 for feature detection. The molecular features (retention time and m/z pairs) that existed in less than 10% of the samples in either group were filtered out. The preprocessed data were then imported to SIMCA-P v13.0 software (Umetrics AB, Umea, Sweden). The data matrix was first Log-transformed, then Pareto scaled before multivariate statistical analyses, such as principal component analysis (PCA) and orthogonal partial least squares discriminant analysis (OPLS-DA), were carried out. The mass spectrometry was performed with the help of Dr. Peter Benke, Research Fellow in our laboratory.

In order to understand the metabolic functional potential and the changes in metabolic functions after rewetting, the metabolic features from oxic and

partial oxic zone were averaged within respective conditions over time-points. The features from C_1T_1, C_1T_2, and C_1T_3 were averaged, respectively from each zone (Fig. 5.2c). Similarly, C_2T_1, C_2T_2 and C_2T_3 were averaged at each zone. Quantitative data for the abundance of metabolites in field and rewetting conditions was included in the input file to be used in a web facility – Pathos (http://motif.gla.ac.uk/Pathos/) that that uses metabolic maps to display experimental changes in metabolites identified by mass spectrometry (Leader et al., 2011). A 10ppm was used as cut-off while input the data. Pathos employs Java servlets and is underpinned by a relational database populated from KEGG. The textual output lists the KEGG pathways on an XHTML page according to the number of metabolites or potential metabolites that they contain. The output result shows a colour-coded variation in the quantification of potential metabolite between after rewetting. Color coded change indication scale in this study was [increase GGGGGGG decrease]. The blue represents the metabolites that were up-regulated in rewetting conditions and the red shows the vice-versa.

5.3 Results

5.3.1 Subsidence rates and *insitu* environmental parameters

The average annual subsidence rate in oil palm plantations based on data monitored for 3.5 years was 4.4 cm/yr (Fig. 5.3). During low water table regimes (grey box in Fig. 5.3), peat oxidized at the subsidence rate of 5.7 cm/yr. During high water table regimes (green box in Fig. 5.3) subsidence rate is marginally lowered (5.4 cm/yr). However, subsidence did not stop in high water table times, mainly associated to high rainfall patterns. Based on this field data, it is clearly evident that even though subsidence rate is slightly lowered during high rainfall period, it does not stop during this rewetting. This data from field formed the basis to conduct this controlled microcosm study where the samples were monitored before and after rewetting. The in-situ environmental traits during the time of sampling are reported in Table 5.1, where it is indicated that water table was low during this season. This is owe to low rainfall in the precedent months (Section 5.2.1).

Table 5.1: *In-situ environmental traits during sampling time (February 2014)*
'±'denotes standard error

In-situ environmental parameters			
Water table depth		82±3 (cm) (n=4)	
Air temperature		32±0.8 (°C)(n=4)	
Air humidity		74±2.5 (%) (n=4)	
	Oxic zones	**Partial oxic zone**	**Anoxic zone**
Dissolved oxygen (pO_2 – mm Hg) (n= 16)	230±2.9	141±3.6	14±1.2

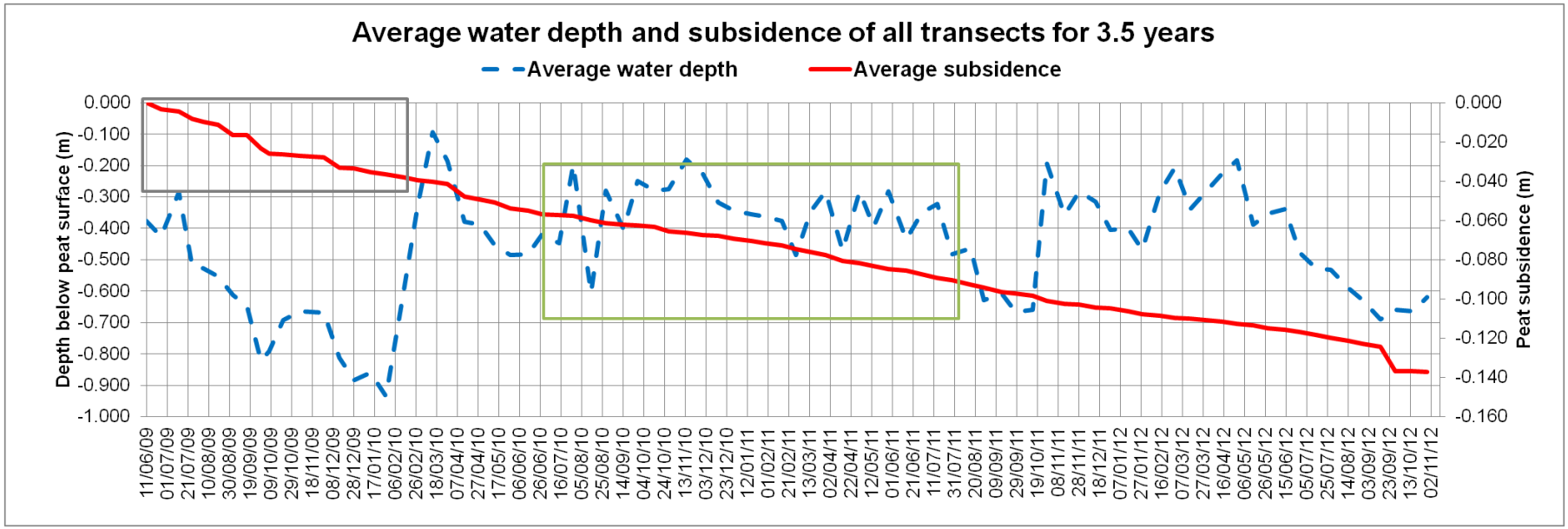


Fig. 5.3: *Subsidence measured in oil palm plantation sites (Site B – Fig. 3.1) between June 2009 and Nov 2012, averaged over 6 monitoring locations. The blue line reflected the changes in water table depth during this time period.*

5.3.2 Microbial physiological response

To estimate the microbial activity and their response after rewetting, we measured the amount of CO_2 (Fig. 5.4) and CH_4 (Fig. 5.5) emitted before and after rewetting. The autoclaved samples that served as 'controls' for this study had no measureable emissions of CO_2 (Fig. 5.4b) and CH_4 (Fig. 5.5b). The CO_2 emissions from all the three zones followed similar trend till 6 days (144 hrs) and after that the three zones showed continuous rise in the CO_2 emissions. CO_2 emissions were significantly higher than CH_4 emissions and CO_2 emissions from three zones studied followed an increasing order as Partial oxic<Oxic<Anoxic zones (Fig. 5.4a). Based on the r^2 coefficient, comparatively better fit was obtained post – wetting when compared to field conditions. The rates of emitted CO_2 (μLCO_2/day) during pre – and post – rewetting were 27 (r^2 = 0.985) and 33 (r^2 = 0.999) in the oxic zones, respectively. The rates were 20 (r^2 = 0.990) and 25 (r^2 = 0.999) in partial oxic zones during pre – and post – rewetting, respectively. This indicates that the rate at which CO_2 is emitted is increased upon rewetting. On the other hand, methane emissions were that were extremely low initially, further tapered down over time (Fig. 5.5a).

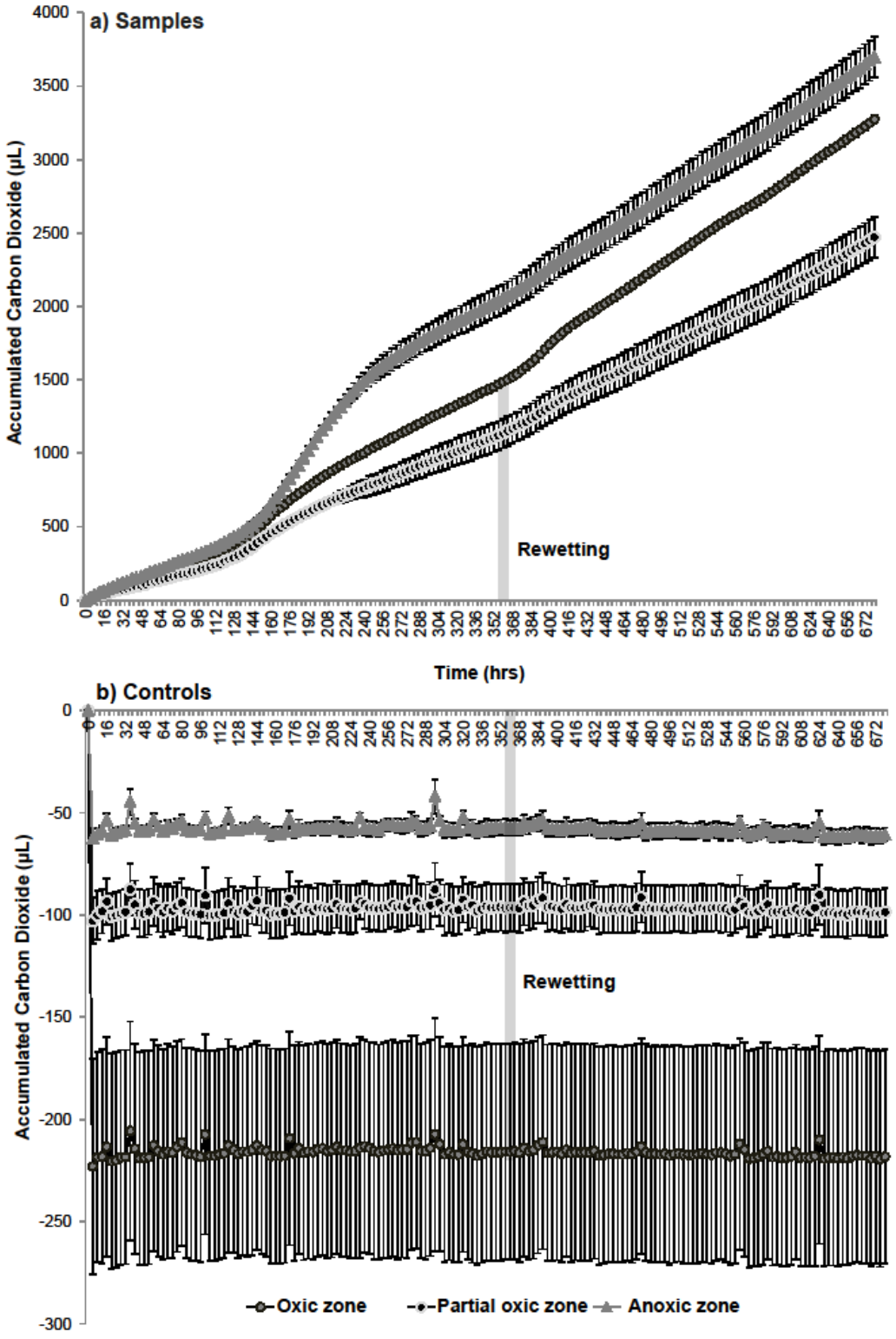


Fig. 5.4: *Net CO_2 emissions (expressed as µL), accumulated over the course of the experiment from (a) samples and (b) controls (n=6). The switch to rewetting in oxic and partial oxic zones was performed after 15days. The error bar denotes the standard error.*

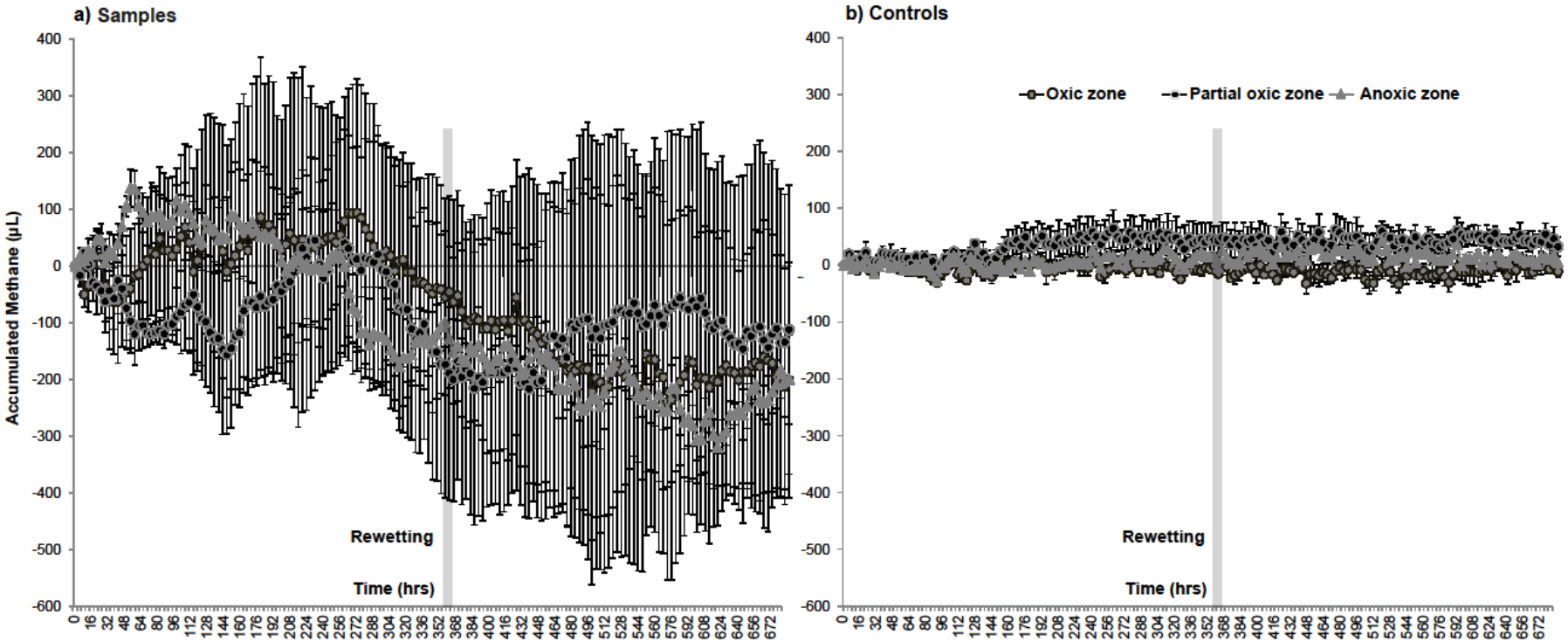


Fig. 5.5: *Net CH_4 emissions (expressed as µL), accumulated over the course of the experiment from (a) samples and (b) controls (n=6). The switch to rewetting was performed after 15days. The error bar denotes the standard error.*

5.3.3 Dynamics of physicochemical parameters

The physicochemical parameters, such as, nitrites and bromides were not detected in this study. The concentrations of nitrates were highest in the oxic zones, followed by partial oxic and anoxic zones, in that order (Fig. 5.6). The concentration of nitrates dropped upon rewetting and the dip was highest in the oxic zones. There was a drop in the concentrations of all cations (Fig. 5.6 – bottom panel) and anions (Fig. 5.6 – top panel) detected in the oxic and partial oxic zone, immediately after rewetting, except for potassium and chloride in oxic and partial oxic zone, respectively. The increase in concentration of potassium upon rewetting was high in the oxic zone when compared to partial oxic zones. Sulfates did not show a major difference in upon rewetting in the oxic and anoxic zones (Fig. 5.6 – top panel). The buildup in ammonia was marginal (Fig. 5.6 – bottom panel.

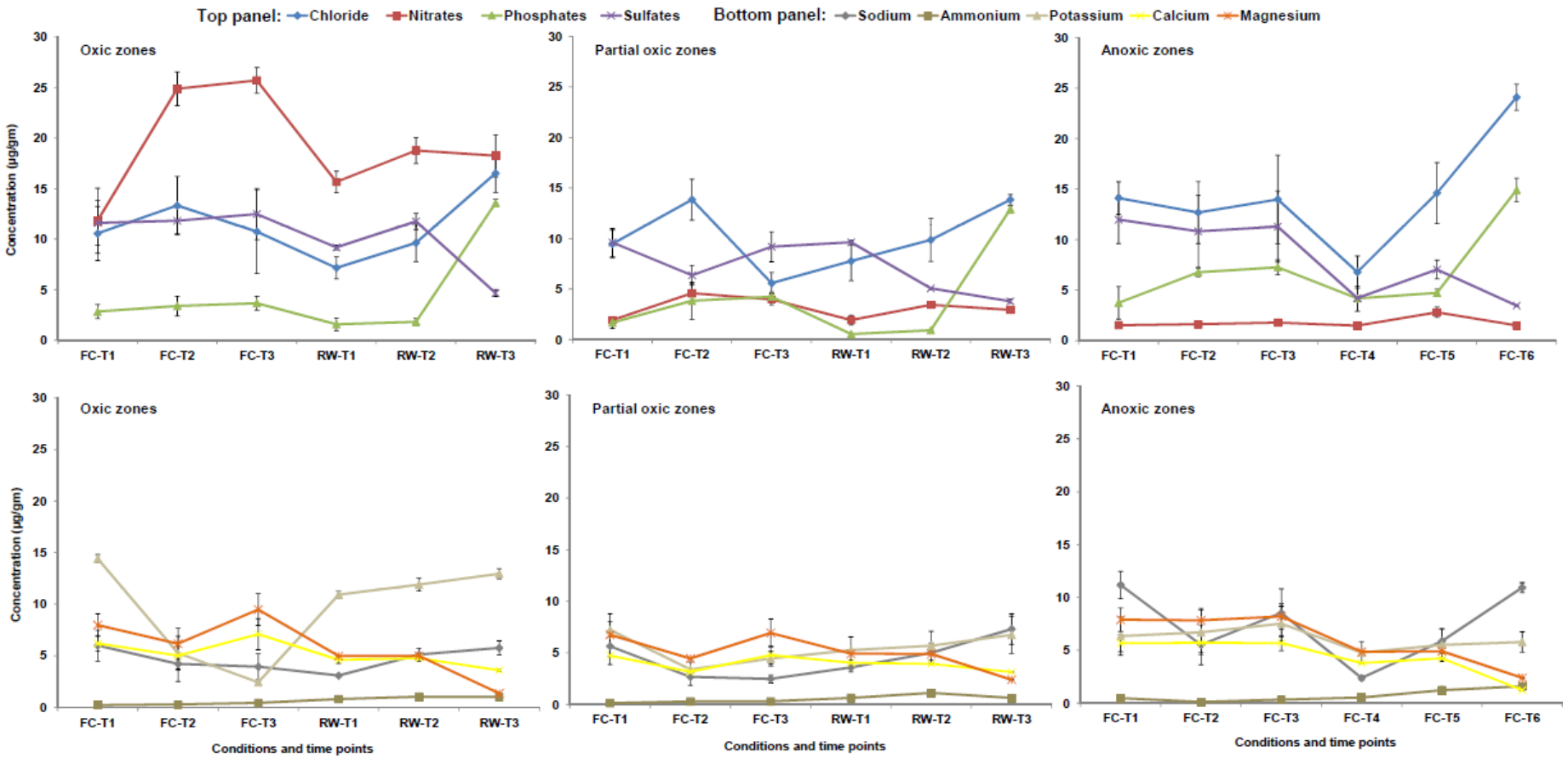


Fig 5.6: *Trends of physicochemical parameters in oil palm plantations over 30 days microcosm experiments. Each data points shows average of 6 data-points. Error bar shows the standard error. The top and bottom panel shows the fate of anions and cations, respectively, in the three zones studied in this study.* ***FC*** *denotes 'Field conditions';* ***RW:*** *'Rewetting';* ***T:*** *'time-points'. The error bar denotes the standard error.*

5.3.4 Metabolic functions of the microbial communities

Metabolic profiles of the entire data with blanks are show in Fig. 5.7a and it depicts that the blanks were highly distinguished from rest of the samples. The variation on x-and y-axis was 35% and 21%, respectively (Fig. 5.7a). OPLS–DA analysis showed that the metabolic profiles between field conditions and rewetting were slightly different at variation of 17% (Fig. 5.7b). There were a total of 111 (respectively in oxic as well as partial oxic zones – negative mode), 94 (in oxic zone – positive mode) and 86 (in partial oxic zone – positive mode) that had got annotated in the KEGG pathways. Out of these, selected pathways that had highest number of metabolites being shifted (up– or down–regulated) are shown in Table 5.2.The qualitative/quantitative changes during rewetting when compared to field conditions, shows that more than 3-fold of predicted metabolites (1321 of 6661 peaks – negative mode *vs* 473 of 2459 peaks – positive mode) was identified in negative mode when compared to positive (Table 5.2).

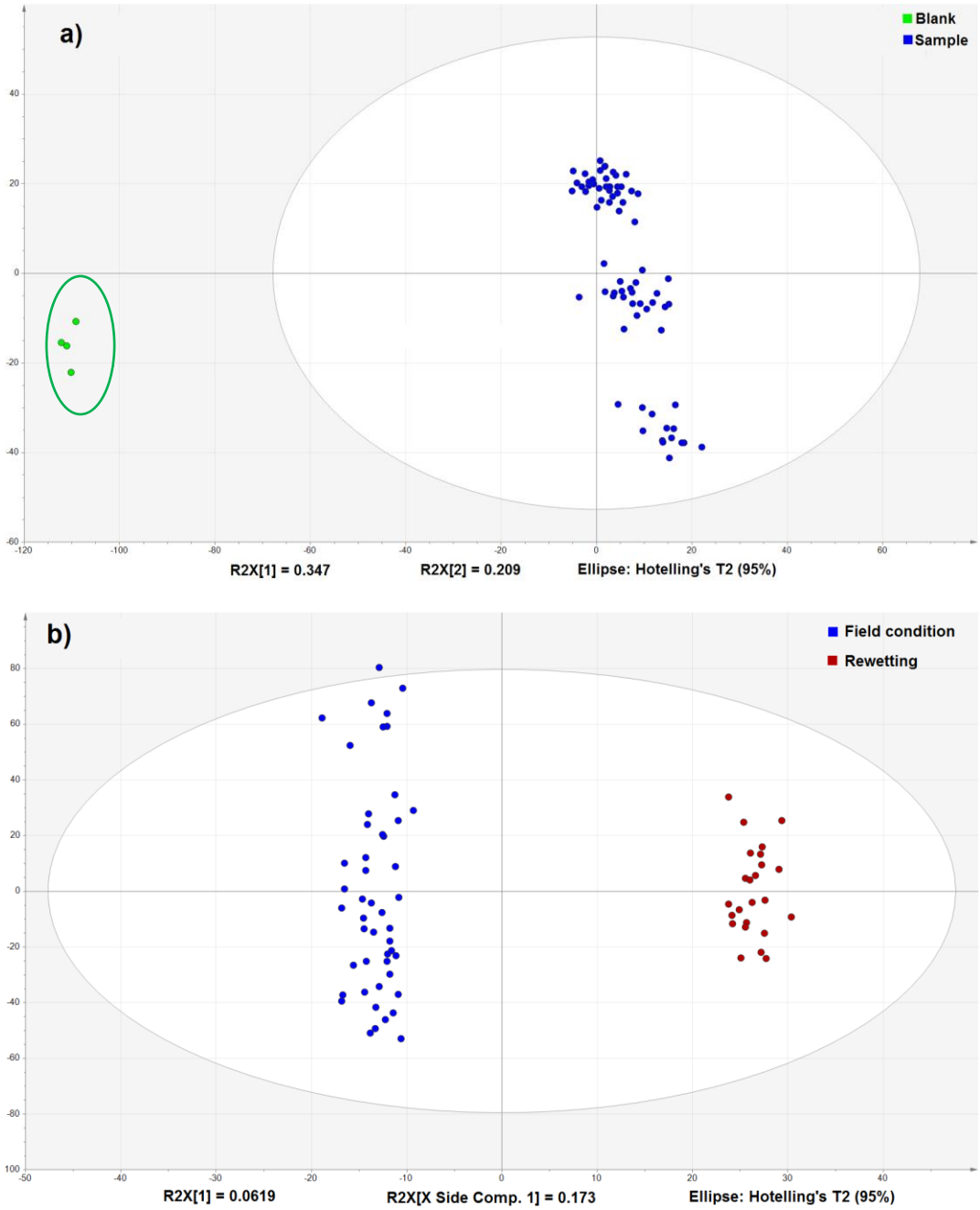


Fig. 5.7: a) *Principle Component Analysis (PCA) of the metabolic features for the entire dataset (blue) including blanks(green-towards left) – variation in X-and Y-axis are 34.7% and 20.9%, respectively;* ***b)*** *OPLS-DA analysis of the microbial metabolic features excluding blanks to estimate the variations between metabolic changes during field conditions (blue) and after rewetting (red) – Variation in X- and Y- axis are 17.3% and 6.2%, respectively*

Table 5.2: *Functional metabolic potential of microbial communities between field and rewetting conditions based on KEGG pathways using web facility - 'Pathos'. The second columns show the number of metabolites present in our data that were matched in respective pathways showing potential metabolites. The third and fourth column depicts the number of metabolites that got affected due to rewetting. The effect can either be up-regulated or down-regulated.*

Rewetting *vs* Field conditions (i.e.) (*Base condition:* Field conditions; *Experimental condition:* Rewetting)			
KEGG pathways	*Number of metabolites matched at 10 ppm cut-off*	*Number of metabolites changed/affected*	
Negative mode (1321 of 6661 peaks matched potential metabolites)		Oxic zone	Partial oxic zone
Arachidonic acid metabolism	69 out of 74	58	34
Tyrosine metabolism	36 out of 74	20	25
Phenylalanine metabolism	28 out of 64	15	20
Benzoate degradation	23 out of 66	11	12
Phenylpropanoid biosynthesis	20 out of 51	11	15
1,4-Dichlorobenzene degradation	31 out of 74	9	13
Methane metabolism	13 out of 58	5	5
Positive mode (473 of 2459 peaks matched potential metabolites)		Oxic zone	Partial oxic zone
Steroid hormone biosynthesis	27 out of 99	21	16
Indole alkaloid biosynthesis	13 out of 47	12	10
Brassinosteroid biosynthesis	21 out of 27	10	14
1,4-Dichlorobenzene degradation	19 out of 74	8	3
Galactose metabolism	15 out of 41	7	2
Fructose and mannose metabolism	15 out of 43	6	7
Carotenoid biosynthesis	24 out of 91	4	12

While running the sample in the negative mode, significant changes between the rewetting and field conditions in oxic and partial oxic zones were observed in amino acid (tyrosine), aromatic compounds (phenylalanine), xenobiotics (benzoate) and carbohydrate (methane) metabolism (Table 5.2; Fig. 5.8a, b and c). The changes metabolite concentrations in rewetting were either unaltered or down-regulated during rewetting in both oxic and partial oxic zone (Fig. 5.8a, b and c). Fatty acid (arachidonic acid) metabolism showed the highest number of metabolite changes during rewetting as well as the maximum difference of affected metabolites (24) between the oxic and partial oxic zones (58 and 34 metabolites – Table 5.2). The changes in rewetting over field conditions in the oxic and partial oxic zone aroused due to up-regulation and down-regulation of metabolites during rewetting in the oxic and partial oxic zone, respectively (Fig. 5.8d). As indicated, less number of metabolites got annotated (predictions) in the KEGG pathway for the positive mode (Table 5.2). The higher number of metabolites affected in partial oxic zone during rewetting were associated with up-regulation of those metabolites for carotenoid pathway, while running samples in positive mode (Fig. 5.8e)

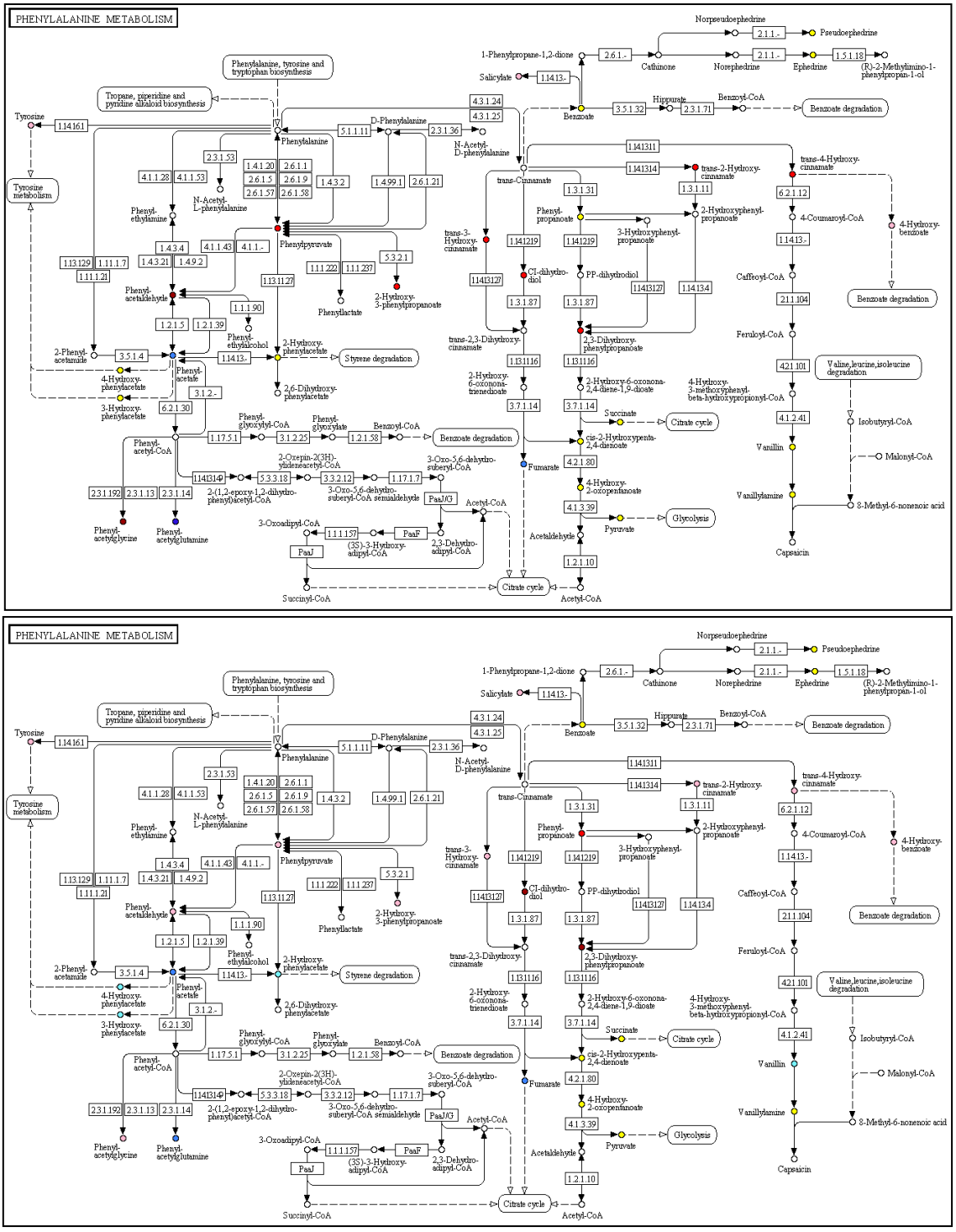


Fig. 5.8a: *Changes in metabolic functions in Phenylalanine metabolism pathway. The changes are based on rewetting over field conditions. The top and bottom chart denotes changes in oxic and partial oxic zones, respectively, after rewetting. Negative mode data presented in this Figure. Change indication scale — increase in quantification during rewetting to decrease (blue to maroon) is denoted by:* GGGGGGG

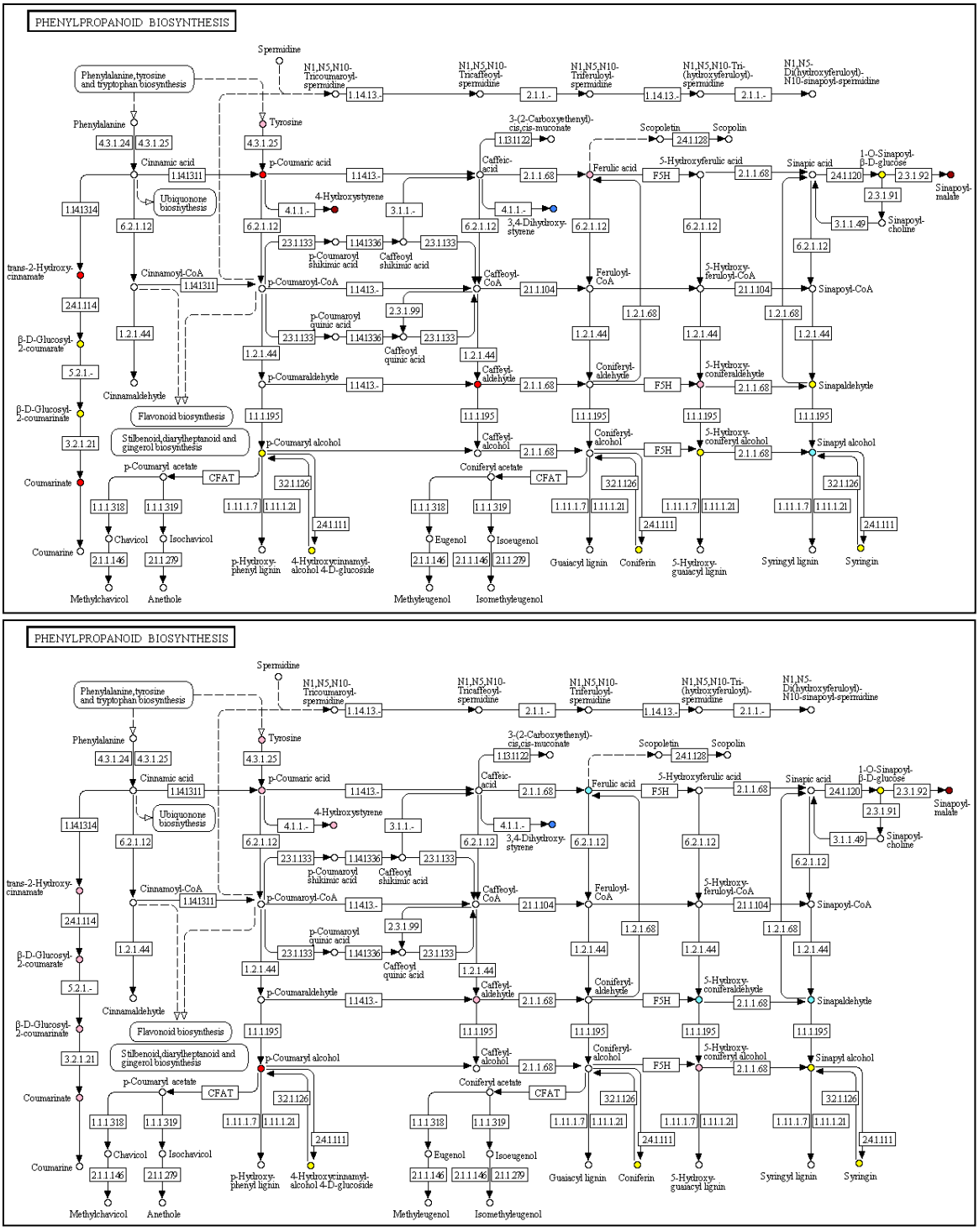


Fig. 5.8b: *Changes in metabolic functional potential in Phenylpropanoid biosynthesis pathway based on KEGG. The changes are based on rewetting over field conditions. The top and bottom chart denotes changes in oxic and partial oxic zones, respectively, after rewetting. Negative mode data presented in this Figure. Change indication scale — increase in quantification during rewetting to decrease (blue to maroon) is denoted by:* GGGGGGG

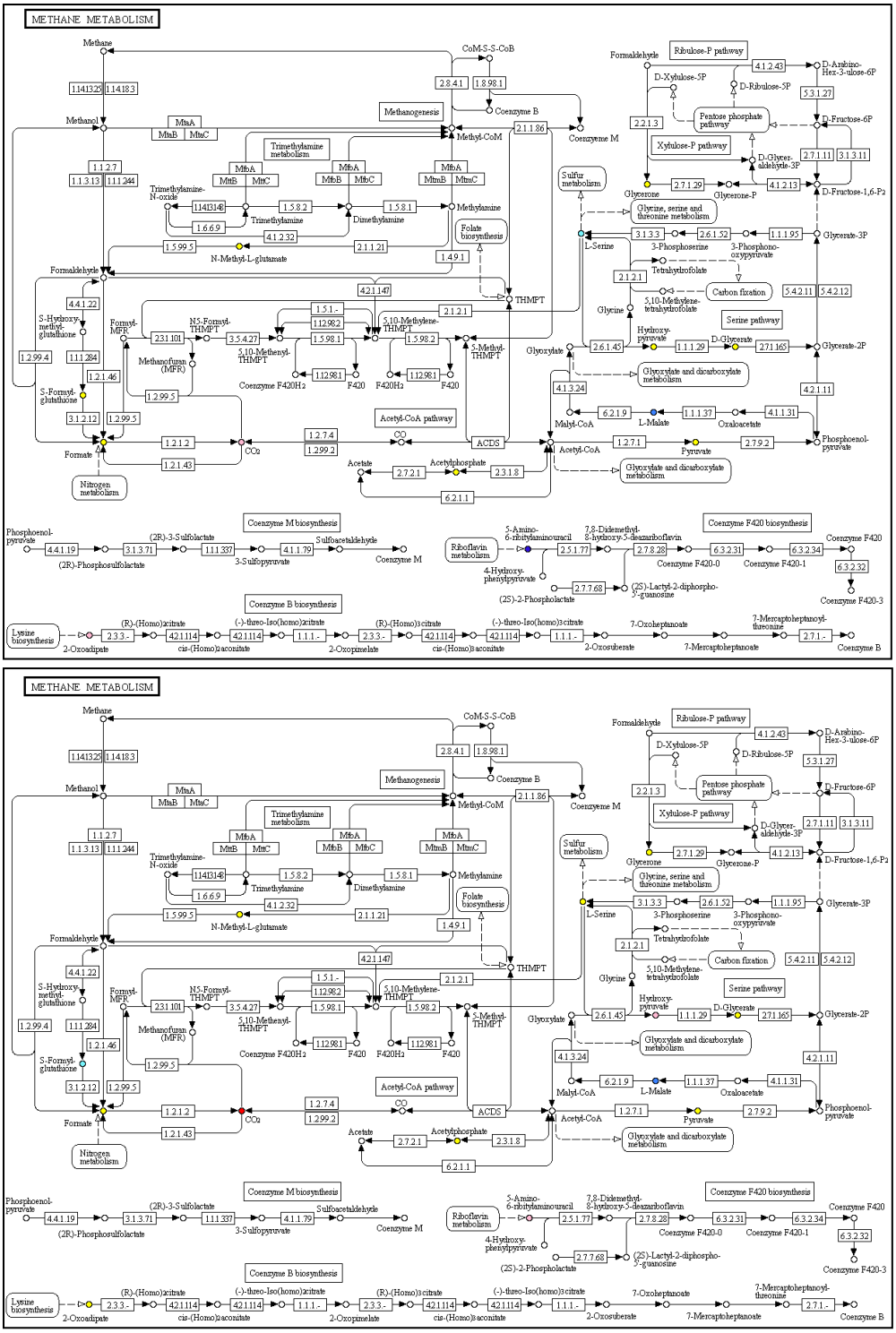


Fig. 5.8c: *Changes in metabolic functional potential in methane metabolism pathway based on KEGG. The changes are based on rewetting over field conditions. The top and bottom chart denotes changes in oxic and partial oxic zones, respectively, after rewetting. Negative mode data presented in this Figure. Change indication scale — increase in quantification during rewetting to decrease (blue to maroon) is denoted by:* GGGGGGG

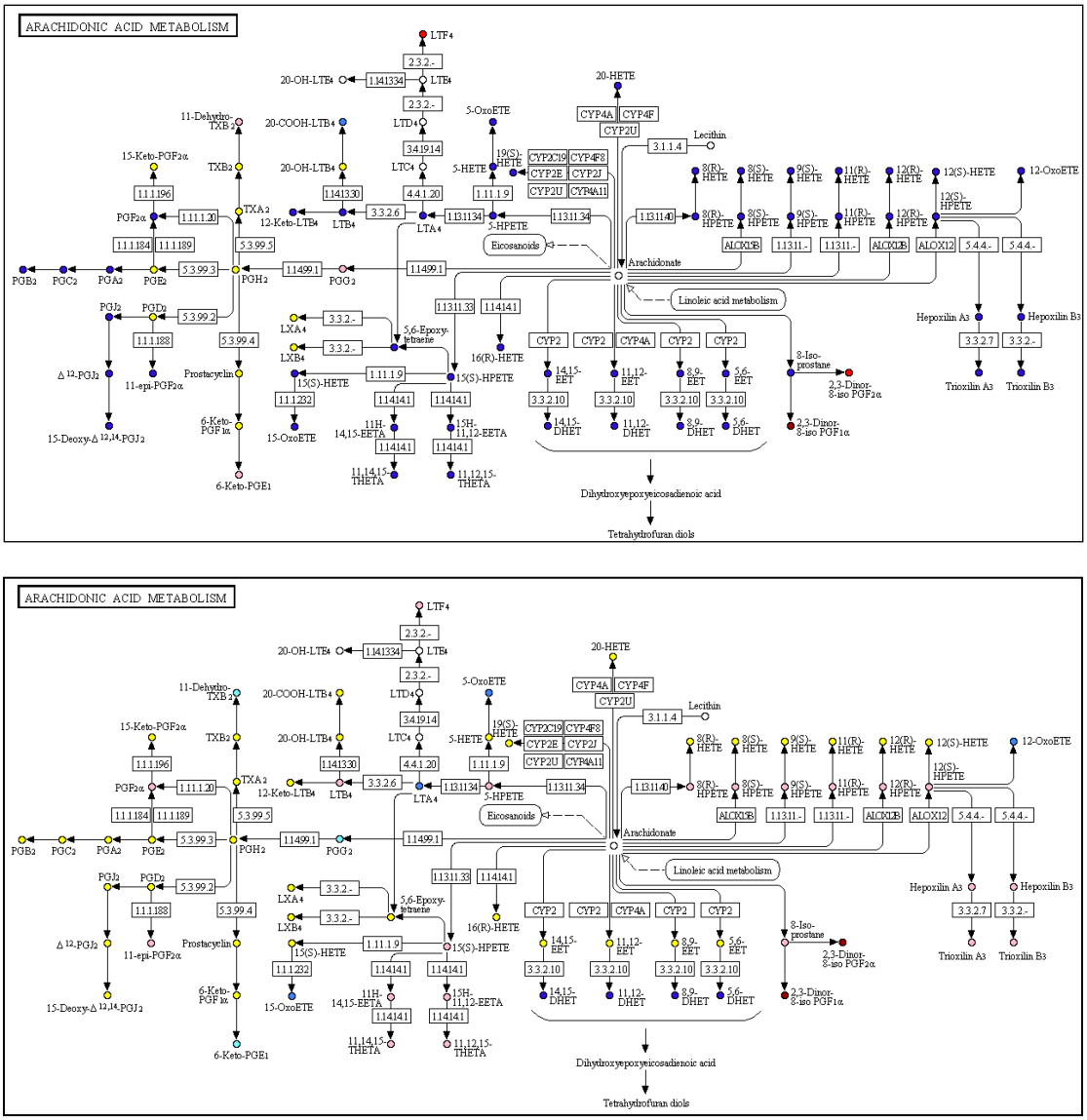


Fig. 5.8d: *Changes in metabolic functional potential in Arachidonic acid metabolism KEGG pathway. The changes are based on rewetting over field conditions. The top and bottom chart denotes changes in oxic and partial oxic zones, respectively, after rewetting. Negative mode data presented in this Figure. Change indication scale — increase in quantification during rewetting to decrease (blue to maroon) is denoted by:* GGGGGGG

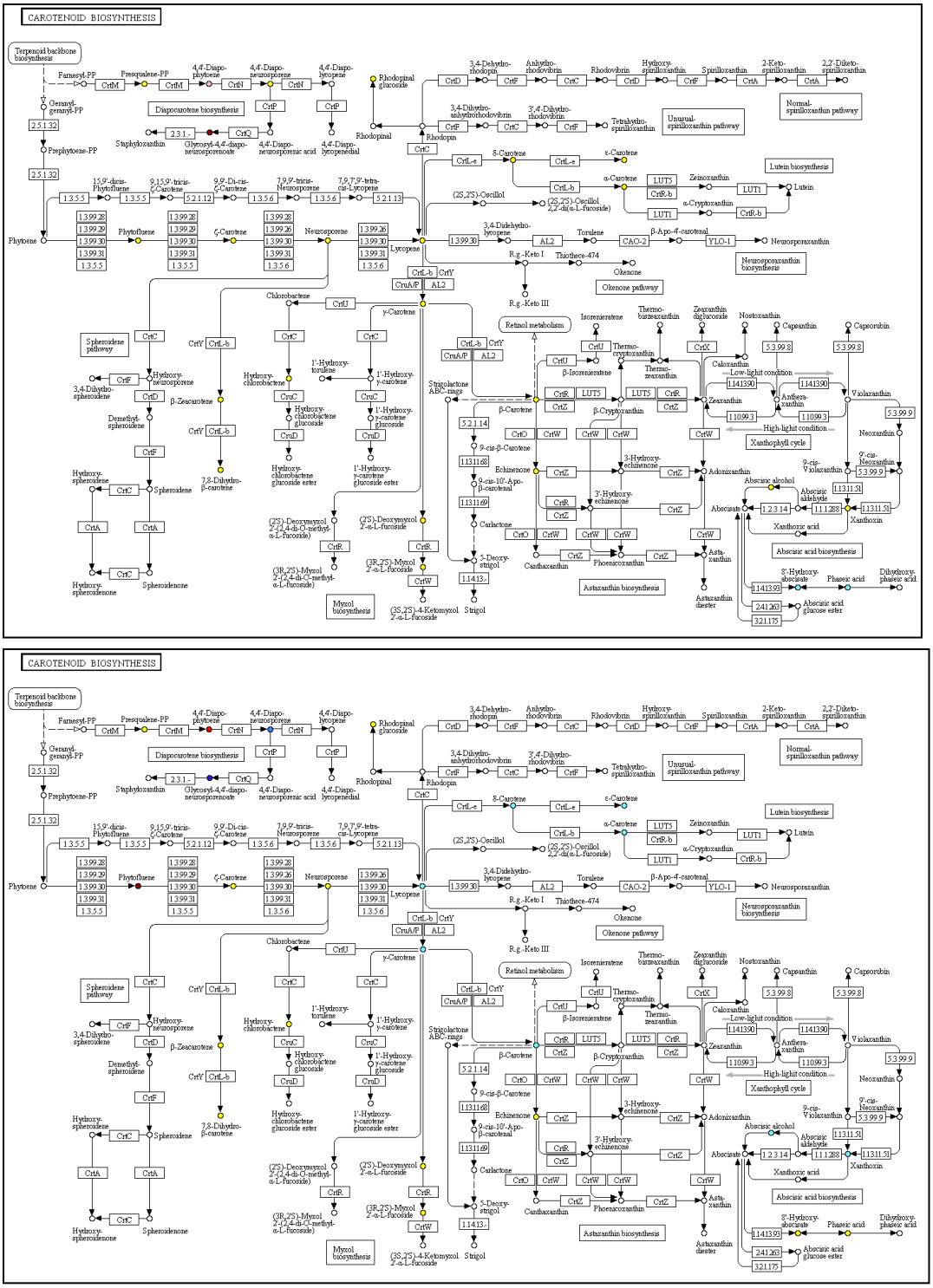


Fig. 5.8e: *Changes in metabolic functional potential in Carotenoid biosynthesis pathway based on KEGG. The changes are based on rewetting over field conditions. The top and bottom chart denotes changes in oxic and partial oxic zones, respectively, after rewetting. Positive mode data presented in this Figure. Change indication scale — increase in quantification during rewetting to decrease (blue to maroon) is denoted by:* GGGGGGG

5.3.4 Microbial community structure

The microbial community structure based on hierarchical clustering over group average using Bray-Curtis similarity indices showed that there was no change in microbial community structure pre- and post- rewetting (Fig. 5.9). The one-way ANOSIM global R statistics, equal to 0.33 (*p*-value: not significant) also validate the finding that no particular microbial assemblages showed any significant changes upon rewetting.

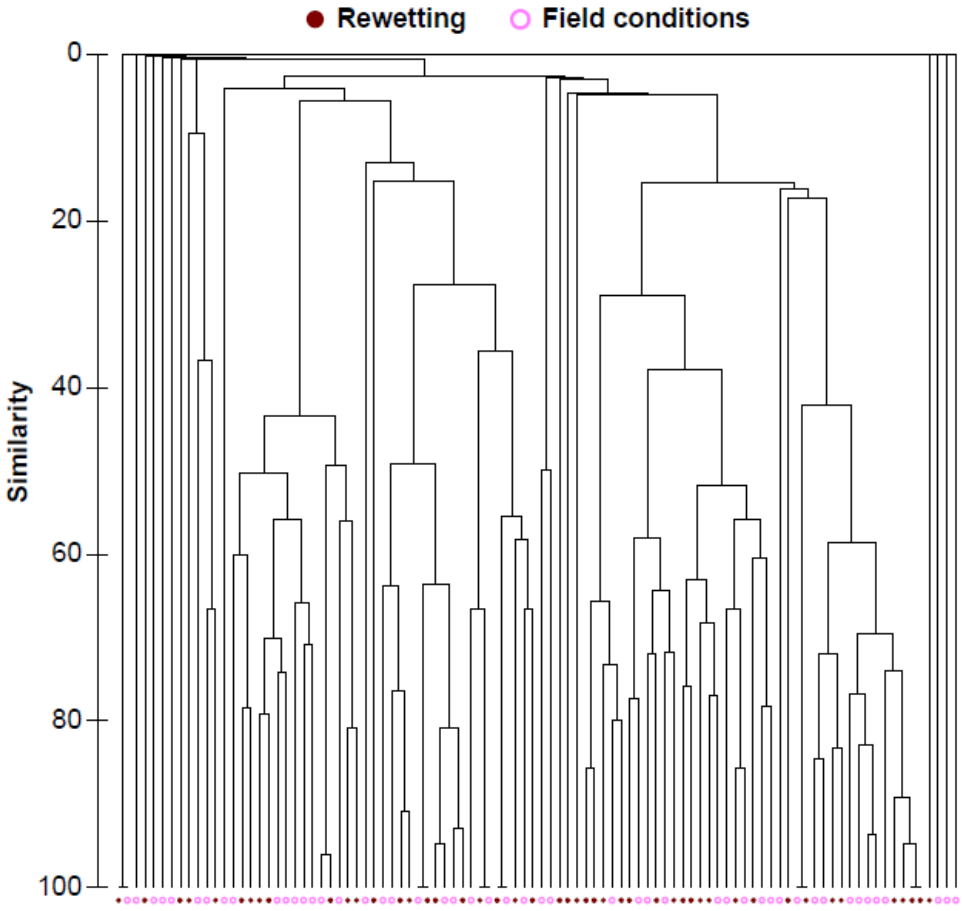


Fig. 5.9: *Hierarchical clustering of microbial communities during field conditions and rewetting. One- way ANOSIM value (R= 0.33)*

5.4 Discussions

This study was framed based on the observation that peat subsidence, a field – based estimation, which serves as a proxy of peat oxidation, did not stop even during high water table seasons (field data – Fig. 5.3). There have been multiple reports on the effects of hydrology on subsidence (Hooijer et. al., 2010 and 2012). In the oxic zones of our study sites (Mishra et al., 2014), it is shown that low water- table sites undergo more pronounced cycles of drying and wetting compared to high-water-table sites. Drying and wetting of peat leads to alternating aerobic and anaerobic physiological responses of the microbes. From sites in the Sumatran region, peat subsidence that is considered as proxy for carbon loss is indeed higher in low water sites (water table depth: -0.7 ± 0.2 m and subsidence rate: 5 ± 2 .2 cm y^{-1}), when compared to high water table sites (water table depth: -0.56 ± 0.06 m and subsidence rate: 3.9 ± 0.5 cm y^{-1}) (Couwenberg and Hooijer, 2013). Activation of different groups of enzymes in drying-wetting has been reported from mesocosms of peat in boreal region (Fenner and Freeman, 2011). Combined oxidation of recalcitrant and labile carbon, accompanied with out-gassing of carbon dioxide can lead to direct loss of peat. This extrapolation directly predicts that peat loss will be higher in low water sites, where these pronounced cycles of drying-wetting are prevalent, compared to high water table sites. Based on all these observations, we performed this controlled microcosm study to explore the influence of rewetting on microbial processes that are

responsible for high CO_2 emission rates leading to microbial-mediated peat oxidation (subsidence) in this region.

Microorganisms have a variety of evolutionary adaptations and physiological acclimation mechanisms that allow them to survive and remain active in the face of environmental stress. The stress in our study could be rewetting. Microbes acclimatize to immediate stress by altering their allocation of resources from growth to survival pathways. These microbes may use the material to support growth and survival (cryptic growth [Chapman and Gray 1986]), to enable attack on recalcitrant soil organic matter (priming [Fontaine et al. 2004; Battin et al., 2009]), or to fuel processes such as denitrification (Sharma et al. 2006) under stress conditions. During rewetting, microbes generally dispose osmolytes rapidly, either by respiring, polymerizing, or transporting them across the cell membrane (Wood et al. 2001). The consequence of disposing of osmolytes could lead to enhanced production of CO_2, DOC, and nutrients released on rewetting (Schimel et al., 2007), as found in this study.

The top 5 metabolic pathways that are affected by rewetting, as identified from microcosm study (Fig. 5.8), are also highly abundant in the field-based peat metagenome analysis (Table 4.2). The metabolic changes associated with aromatic, aminoacid, xenobiotics and carbohydrate metabolism after rewetting were down-regulated in the partial oxic zones. In the study about rewetting in organic soils, it is demonstrated that aromatic ring compound, amino-acids, glucose, and acetate metabolism

decreased after rewetting (Tate, 1979) with aromatic ring compound metabolism to be highly affected upon rewetting. The metabolism of such compounds has been reported to high in oil palm plantation sites based on metagenome data from the oxic zones of our study sites (Table 4.2, Chapter 4). Upon rewetting, those compounds, such as, arachidonic acid, linoleic acid and other lipid metabolism were found to be affected in the current microcosm study. The changes in metabolite concentration in phenylalanine metabolism upon rewetting are linked with adaption of tropical peat microbial communities to degrade recalcitrant lignocellulosic materials. Microbial community structure did not change significantly during rewetting, based on microbial profiling. So we can conclude that changes in metabolic functions that are distributed among diverse taxa are likely to govern the changes (non-reduced CO_2 emissions) upon rewetting, rather than specific microbial assemblages.

This and other study from tropical peatlands showed that out-gassing of CO_2 far exceeds the CH_4 emissions, in contrast to temperate peatlands. In our study, we demonstrated non-stop elevated CO_2 emissions, which did not stop upon hydrological restoration as well sites (Fig. 5.4). To add to this, the rates were also increased upon rewetting. On the other hand, negligible CH_4 emissions are demonstrated in our microcosm study sites (Fig. 5.5). There could be multiple reasons that could be attributed to this pattern. Some that will discussed in this Section are linked with (i) low

water table regimes in this region; (ii) pronounced cycles of drying-wetting interlinked with low water table; (iii) C and N mineralization.

Low water table depths

Lower water table positions resulted in increased CO_2, emissions, with a generally linear relationship between emission and water table. Higher amounts of CO_2 emissions is associated with low water table regimes as the gases stored in the pore-water in the peat profile can be released and emitted, presumably, through the increased diffusivity of these gases through the air-filled pore space created when the water table falls down (Moore and Knowles, 1989; Moore and Dalva, 1993; Jauhiainen et al., 2012c; 2014). Water table level drawdown during the dry season increases organic matter availability for aerobic (CO_2-releasing) decomposition in tropical peatlands (Jauhiainen et al., 2008). On the other hand, a pronounced decrease in CH_4 emission occurred from the peat columns with low water table positions (Jauhiainen et al., 2008; Jauhiainen et al., 2012c). Upward diffusing CH_4 can form an energy source to methanotrophic bacteria that are capable of oxidizing it to CO_2. The annual CH_4 flux estimates has been reported to be near-zero emission rates during dry periods, leading to low water table depths, in tropical peatlands of Malaysia (Melling et al., 2005) and Indonesia (Inubushi et al., 2003). Thus, these evidences demonstrate the low water table depths are linked with high CO_2 and less CH_4 emissions from tropical peatlands.

Pronounced cycles of drying-wetting

Drying-wetting mainly occur owing to low water table depths and high rainfall patterns, such as, in tropical peatlands. In our microcosm study which demonstrated that there was no affect on CO_2 emissions upon rewetting the oxic and partial oxic zones of peat, shows linkage of fluctuations of water table with peat oxidation. In Indonesia, land-use change from swamp and drained forest to cassava or coconut field, lowered groundwater levels and decreased CH_4 emission, while change to lowland paddy raised the groundwater level and increased CH_4 emission, depicting the C-dynamics during rewetting (Inubushi et al., 2005). Land-use change from wetland to upland crop lowered groundwater level and thus reduced CH_4 production and enhanced CH_4 oxidation (Inubushi et al., 2005). In another study from farmed organic soils in temperate region, it is shown that CO_2 emission rates increased up to 5-fold following wetting (Prieme and Christensen, 2001). In a field study from Kalimantan, it is reported that there was CO2 emission were not lowered pre- and post-hydrological restoration (Jauhiainen et al., 2008).

As seen in our study, there are other reports that have shown high variability of gas flux within treatments for CH4 emissions (Moore and Dalva, 1993). Methane consumption occurred in samples above or near to water table (Moore and Dalva, 1997). Methane flux remained at its experimentally induced low levels even after rewetting in a controlled perfusion system with temperate peat monoliths (Freeman et al., 1993). In

another study from farmed organic soils in temperate region, it is shown that methane emission rates were very low and there was no effect of wetting on any of the sites that were monitored in that study (Prieme and Christensen, 2001). Methane emission is largely dependent on the net emission between methane production and its consumption. It is largely studied as reported in a review (Segers, 1998) that these rates are weakly correlated with oxidation states (for example – oxic, partial oxic and anoxic zone in our study), temperature and other environmental traits. The large range in the methane: carbon dioxide production rates in the anaerobic zones indicate that a large part of the anaerobically mineralised carbon is used for reduction of electron acceptors, and, hence, is not available for methanogenesis (Segers, 1998).

C and N mineralization

Nitrates were one of the highest physicochemical parameter detected in this study. It is mainly due to fertilization of the oil palm plantation sites. However, the nitrate concentration lowers after rewetting. There are strong possibilities of high denitrification, reducing the nitrates upon rewetting. Also C and N mineralization may lead to pronounced CO_2 emissions that do not stop upon rewetting. Fertilization application has been reported before to increase average CO_2 fluxes from agriculture sites (Jauhiainen et al., 2014). Upon rewetting, denitrification measured in the re-wetted cores was much higher compared to field conditions in a wetland soil from a temperate region (Venterink et al., 2002). Drying–rewetting events can

induce significant changes in microbial C and N dynamics and these effects can last for more than a month after the last stress, as demonstrated in a controlled study of perennial oak (*Quercus agrifolia*) soil located in Santa Ynez, California, USA (Fierer and Schimel, 2002). It is also shown in many arid and semi-arid environments that rewetting of dry soils increase C- and N- mineralization that may dominate annual C and N production (Miller et al., 2005). Anaerobic nitrate consumption coupled with ammonium oxidation has been reported in studies on marine sediments (Thamdrup and Dalsgaard, 2002). Biodegradation of aromatic compounds has also been reported to be linked with nitrate reduction (Burland and Edwards 1999). We have found in our study pathways related to degradation of aromatic compounds and xenobiotics (Table 4.2, Chapter 4). The nutritional status (nitrates and ammonium) of the peat had a profound influence on the effect of low water-table N_2O emission (Aerts and Ludwig, 1997). Increased activity of autotrophic nitrifiers have also been reported during rewetting of perennial oak (*Quercus agrifolia*) and grassland (primarily *Bromus* sp.) soils located in Santa Ynez, California, USA (Fierer and Schimel, 2002). In another study, maximum N_2O emission rates occurred after rewetting of soil from temperate region (Ruser et al., 2006), suggesting that the rewetting that occurs frequently in tropical peatland sites will not only, enhance elevated CO_2 emission but may also contribute to high rates of other GHGs, such as nitrous oxide. This leads to importance of studying influence of agricultural practices,

particularly fertilization on bio-geochemical mechanisms of greenhouse gas emissions from tropical peatlands. Hence, we have proposed this study in the future direction for the continuation of this project (Chapter 6).

5.5 Conclusions

From this microcosm study, we conclude that elevated carbon dioxide emissions and negligible amount of methane emissions are attributed to low water table depths. At such low depth, there would be higher frequency of rewetting during rainfall events. Thus, sites with low water depths that undergo frequent rewetting are predicted to have continuous and elevated CO_2 emissions. Thus, it can be concluded from this study that once peatlands are degraded and drained, it is not possible to restore them by just elevating the water table. Peat oxidation do not stop as subsiding is continuous (field data), nor the CO_2 emissions were reduced upon rewetting (microcosm study data). Interestingly, the rates of CO_2 emissions are increased upon rewetting. Lastly, it can be concluded that metabolic functions, such as, metabolism of aromatic compounds, amino acids, xenobiotics and carbohydrate metabolism that are distributed among diverse taxa are likely to govern the changes (non-reduced CO_2 emissions) upon rewetting, rather than specific microbial assemblages.

Chapter 6: Overall conclusions and future perspectives

Major conclusions from this study suggest that once peatlands are degraded and drained, it is not possible to restore them by simple hydrological interventions, such as, elevating the water table height. Peat subsidence (oxidation) does not stop (field data), neither the CO_2 emissions are reduced upon rewetting (lab data). Our study suggests that better peatland management practices needs to be adopted, that can provide a basis to focus early efforts on hydrological interventions to reduce repeated drying-wetting and improving sustainability of oil palm plantations by multiple cropping practices.

From the field-based studies, it can be concluded that:

1. Rapid drying-wetting of peatlands is associated with high peat oxidation rates and that this process selectively enriches certain resilient microbial species/functions, which rapidly switch between aerobic and anaerobic conditions. Sites that are characterized as ‘low water table sites’ undergo more rapid fluctuations in water level than ‘high water table sites’ and thus need special attention.
2. A strong association of nitrates with microbial community structure was found in oil palm plantation sites. These inorganic fertilizers are routinely applied as part of plantation management practices,

indicating that land management practices increase the labiality of recalcitrant carbon and hence the rate of GHG emissions.

3. Mixed crop plantations, which have a more diverse plant cover than monoculture oil palm plantations, contain more DOC, have a high diverse metabolic profile, support a more diverse microbial community and importantly experience a lower rate of peat subsidence, a proxy for oxidation-led peat loss. Therefore, it provides a good basis to adopt microbial ecology principles to encourage mixed crop planting in the existing plantations, which can lead to sustainable use of these plantations.
4. Peat metagenome analysis revealed that one-carbon metabolism is positively correlated with subsidence rate and CO_2 emissions, indicating methane oxidation, as one of the possibility in the oxic zones of tropical peatlands. Also there is possibility of coupling of carbon oxidation with iron metabolism.
5. In degraded forest and oil palm plantations, *Rhizobiales* were abundant and were correlated with subsidence rates and ammonium concentrations, indicating linkages of these species with peat oxidation. Also *Actinobacteria* and *Firmicutes* were among the most abundant taxonomic groups, demonstrating their linkages in oxidation of tropical peatlands. The positive correlation with subsidence rates suggests, coupling of C- and N- cycling

leading to overall peat oxidation evident through high subsidence rates.

6. Phenylalanine metabolism leading to the biosysnthesis of aromatic compounds is positively correlated with CO_2 emissions. Flavone/ flavonol and phenylpropanoid biosynthesis that were abundant in degraded forest and oil palm plantations were positively correlated with ammonium but negatively associated with peat moisture content. These findings suggest that tropical peat microbes have adopted well to break-down such recalcitrant phenolics.

From the lab-based microcosm study, it can be concluded that:

7. The elevated carbon dioxide emissions and negligible amount of methane emissions are attributed to low water table depths. At such low depth, there would be higher frequency of rewetting during rainfall events. Thus, sites with low water depths that undergo frequent rewetting are predicted to have continuous and elevated CO_2 emissions.
8. It is also evident that methane consumption due to oxidation of CH_4 or incorporation into other carbon forms is high in degraded tropical peatlands, when compared to its production in all aerobic and anaerobic zones studied and this pattern does not change upon rewetting.
9. It also revealed that microbial metabolic functions, such as, metabolism of aromatic compounds, amino acids, xenobiotics and

carbohydrate metabolism that are distributed among diverse taxa are likely to govern the changes (non-reduced CO_2 emissions) upon rewetting, rather than specific microbial assemblages.

Future perspectives:

The current study established the fact that the CO_2 emissions are unaltered even upon rewetting and there were no significant methane emission from the peat systems. However, a substantial amount of carbon loss occurs as a result of leaching and subsequent out-gassing of CO_2 and CH_4 from fluvial systems. The labile dissolved organic carbon in peat that “primes” recalcitrant carbon for oxidation to CO_2 would increase in the fluvial peat systems. Hence, it would be interesting to elucidate the carbon and nitrogen dynamics in fluvial systems.

From the current study, we show strong associations of nitrates with microbial community structure in oil palm plantation sites. These inorganic fertilizers are routinely applied as part of plantation management practices. Hence, it would be important to understand the microbial metabolic basis behind GHG emissions, such as, N_2O in addition to CO_2 and CH_4 from peat systems, in response to agricultural practices, particularly nitrogen fertilizer inputs.

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