# SOIL MICROBIAL COMMUNITIES AND GHG EMISSIONS UNDER DIFFERENT LAND-USE TYPES IN MALAYSIAN PEATLANDS

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# Abstract

Tropical peatlands are complex and globally important ecosystems which are increasingly threatened by anthropogenic disturbances such as logging and expansion of oil palm plantations, particularly in South East Asia. Microbes in peatlands play an important role in governing overall functions e.g. carbon and nutrient cycling, yet their characteristics in these habitats are poorly understood. This research aimed to elucidate the response of tropical peatlands to disturbance by quantifying the peat properties, soil microbial community structure, nutrient content and greenhouse gas (GHG) emissions, and the functional interactions between these components under different land uses. Specifically, differences between primary peat swamp forest, secondary peat swamp forest, different land-use types and cropping systems associated with oil palm plantations in Peninsular Malaysia were investigated. Sampling was conducted between November 2014 - October 2018, covering both wet and dry seasons. Bacteria at all depths overwhelmingly dominated all land-use types but microbial phenotypic community structure responded to land-use, depth and changes in season. $CO_2$ emissions were lowest in the primary forest and highest in the secondary forest. $CO_2$ emissions in agricultural plantations were lower than the secondary forest, due to very low autotrophic contribution to net emissions in those habitats. Second-generation cropping systems exhibited distinct seasonal variations which were more pronounced in 2$^{nd}$ generation oil palm mono-cropping, where the $CO_2$ emission was reduced to half in the dry season. $CH_4$ emissions exhibited an opposite trend to that of $CO_2$, with highest $CH_4$ emissions in the primary forest and oxidation in the secondary forest, while the agricultural systems emitted low levels of $CH_4$. However, $CH_4$ emissions were very low in

comparison to $CO_2$ emissions. Both $CO_2$ and $CH_4$ emissions were significantly impacted by changes in moisture level, with 2nd generation oil palm exhibiting moisture limitation for $CO_2$ emissions, unlike all the other peat land-use studied. Temperature and pH also significantly influenced microbial community structure and GHG emissions. Gram-positive and Gram-negative relative abundance were positively correlated with $CO_2$ and $CH_4$ emissions, respectively. Almost all the nutrients were substantially lower in the secondary forest to that of the primary forest. Cu and Mo concentration were negatively correlated with $CO_2$ and $CH_4$, respectively. Past drainage in secondary forest reduced nutrient contents, including some with antimicrobial properties, and favoured Gram-positive bacteria over Gram-negative bacteria. In agricultural plantations the autotrophic contribution of the vegetation to $CO_2$ emissions was significant only for mature oil palm and yam plants, implying that most of the $CO_2$ emissions from other cropping systems is heterotrophic respiration, resulting in C loss. This is further supported by the 2nd generation mono-cropping having higher bulk density and the lowest organic content, while the 2nd generation intercropping maintained most of the organic content with relatively less change to other peat properties. Overall, the results demonstrate that peat properties, microbial communities and GHG emissions are affected by the land-use changes associated with oil-palm plantations. However, the impact was highest at prolonged oil palm mono-cropping, while the practice of intercropping ameliorated the damage caused by such plantations.

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# Table of contents

**Acknowledgements** ..... **iv**

**Table of contents** ..... **v**

**1. Introduction** ..... **1**

1.1 Land-use change and environmental impacts ..... 4

1.1.1 Oil palm plantations ..... 4

1.1.2 Logged forests ..... 11

1.1.3 Drained and Burnt Peatlands ..... 12

1.1.4 Other agricultural plantations ..... 14

1.2 Soil microbial communities and environmental controls ..... 15

1.2.1 Microbes in peatlands ..... 16

1.2.2 Microbes in disturbed peatlands ..... 19

1.3 Phospholipid Fatty Acid (PLFA) analysis ..... 22

1.4 Aims and objectives ..... 23

**2. Microbial community structure across the peat profile at different stages of conversion from forest to oil palm plantation** ..... **25**

2.1 Introduction ..... 25

2.2 Material and methods ..... 30

2.2.1 Study sites ..... 30

2.2.2 PLFA extraction ..... 32

2.2.3 Gas chromatography and peak identification ..... 32

2.2.4 Statistical analysis ..... 33

2.3 Results ..... 34

2.3.1 Effects of land use types ..... 34

2.3.2 Variations within land-use types along the depth ..... 35

2.3.3 Other ecological indicators ..... 43

2.4 Discussion ..... 45

2.4.1 Microbes at different land-use types ..... 45

2.4.2 Consistent and coherent change with depth irrespective of the land-use ..... 46

2.4.3 Bacterial dominance in community structure ..... 49

2.4.4 Higher nutritional stress under forest? ..... 51

2.5 Conclusion ..... 52

**3. Soil microbial community structure and GHG emissions under forest and different oil palm cropping systems....54**
3.1 Introduction....54
3.2 Material and Methods....58
3.2.1 Study sites....58
3.2.2 Sampling Strategy....62
3.2.3 Soil analysis....63
3.2.4 Phospholipid fatty acid analysis....64
3.2.5 Greenhouse gas measurement....66
3.2.6 Statistical analyses....66
3.3 Results....68
3.3.1 Peat properties....68
3.3.2 C and N content....71
3.3.3 Peat microbial communities....72
3.3.4 Microbial communities and environmental controls....77
3.3.5 Greenhouse gas emissions....81
3.3.6 GHG emissions and environmental controls....84
3.4 Discussion....87
3.4.1 Peat characteristics at different cropping systems....87
3.4.2 Microbial community structure at different land use types and environmental controls....89
3.4.3 Impacts of land-use on GHG emissions....92
3.4.4 Relationship between greenhouse gas emissions and microbial community structure....95
3.5 Conclusion....96
**4. GHG emissions, nutrient content and microbial community structure from a last-remaining pristine tropical peat swamp forest in Peninsular Malaysia....97**
4.1 Introduction....97
4.2 Materials and Methods....100
4.2.1 Study sites....100
4.2.2 Sampling strategy....103
4.2.3 Greenhouse gas measurement....103
4.2.4 Soil properties....104
4.2.5 Phospholipid fatty acid analysis....105
4.2.6 Statistical analysis....107

4.3 Results ..... 108
4.3.1 Greenhouse gas emissions ..... 108
4.3.2 Peat properties ..... 110
4.3.3 Nutrient content ..... 111
4.3.4 GHG emissions and environmental controls ..... 113
4.3.5 Peat microbial communities ..... 114
4.3.6 Microbial communities and GHG emissions ..... 119
4.4 Discussion ..... 120
4.4.1 Lower total GHG emissions in primary forest ..... 120
4.4.2 Higher nutrient content in primary forest and their impact on GHG emissions ..... 122
4.4.3 Tropical peatland microbial community structures and their relationship with GHG emissions ..... 124
4.5 Conclusion ..... 127
**5. Effect of proximity to plants on peat characteristics and GHG emissions under different cropping systems in tropical peatlands ..... 128**
5.1 Introduction ..... 128
5.2 Materials and Methods ..... 131
5.2.1 Study sites ..... 131
5.2.2 Sampling strategy ..... 133
5.2.3 Greenhouse gas measurements ..... 134
5.2.4 Soil properties ..... 135
5.2.5 Statistical analyses ..... 135
5.3 Results ..... 136
5.3.1 $CO_2$ emissions ..... 136
5.3.2 $CH_4$ emissions ..... 137
5.3.3 Peat characteristics ..... 138
5.4 Discussion ..... 140
5.4.1 Effect of plant proximity on $CO_2$ emissions ..... 140
5.4.2 Effect of plant proximity on $CH_4$ emissions ..... 141
5.5 Conclusion ..... 143
**6. General discussion and conclusions ..... 144**
6.1 Soil microbial communities under different land-use types and their responses to environmental change ..... 144
6.2 $CO_2$ emissions at different land-use types and environmental controls 146
6.3 $CH_4$ emissions at different land-use types and environmental controls 148

6.4 Soil microbial communities and GHG emissions.....149
6.5 Conclusions .....150
6.6 Implications for future work.....151
**Appendix 1.....154**
**Appendix 2.....156**
**References.....157**

# Chapter 1

## 1. Introduction

Peatlands are complex and globally important ecosystems for their distinctive biodiversity and high carbon storage, and because of their unique acidic, nutrient-poor and waterlogged conditions (Miettinen *et al.*, 2012). Even though peatlands are known for high $CH_4$ emissions, they store huge amounts of carbon and act as a net C sink (Schrier-Uijl *et al.*, 2013, Sjögersten *et al.*, 2014, Turetsky *et al.*, 2015). Sequestration of carbon in peatlands is mainly because of high primary production and low decomposition activity by microbes, due to anoxic, waterlogged conditions (Andersen *et al.*, 2013). Microbes in peatlands play an important role not only in the formation of peat itself, but also directly influence ecosystem carbon turnover and nutrient mineralization for high primary production, effecting overall ecosystem function.

Peatlands cover about 3% of total land area of the earth, distributed all around the world (Hooijer *et al.*, 2006, Strack, 2008). Though most of the peatland surface are in global north, there is a considerable area of tropical peatlands present in South America, Southern Africa and South East Asia, covering 0.25% of global land surface and accounting 3% of global soil carbon (Hergoualc'h and Verchot, 2011, 2014, Strack, 2008). About 56% of these carbon-rich tropical peatlands are present in South East Asia (SEA) with highest cover in Indonesia and Malaysia (Hergoualc'h and Verchot, 2011, 2014, Lo and Parish, 2013). Most of the peatland forests in SEA are in low-lying coastal plains (Lo and Parish, 2013, Rashid *et al.*, 2013). The high degradation of forests with lowland mineral soils has forced some of the endangered species like orang-utans and tigers to find refuge in peatland forests (Miettinen *et al.*, 2012). Other than serving as a home and refuge for unique biodiversity and ecosystem

services, the SEA peatlands store about 69 Gt of carbon (Miettinen and Liew, 2010, Page *et al.*, 2011b), and absorb at least 2.6 tonnes of $CO_2$ per hectare per year (Norwana *et al.*, 2011). The C has been gradually amassed in these peatlands since the last ice age for last 10,000 years, when the peatlands have played a major role in maintaining the C balance of the earth (Lo and Parish, 2013). This carbon accumulation is a result of delicate balance among different environmental and biological conditions, including hydrology, ecology, topography and landscape morphology (Miettinen *et al.*, 2012, Page *et al.*, 1999). This balance is perturbed by anthropogenic disturbances that are turning tropical peatlands from carbon sinks to carbon sources (Moore *et al.*, 2013). Degradation of peatlands would not only emit large amount of $CO_2$, but also still emit large amount of $CH_4$ through drainage ditches (Schrier-Uijl *et al.*, 2011). Conversion to oil palm agriculture and use of fertilizers were also known to increase $N_2O$ emissions (Mohd Kusin *et al.*, 2015). Thus, the degradation of peatlands would potentially accelerate climate change, via emissions of three potent greenhouse gases.

The SEA forests have been rapidly deforested in recent years, through agricultural expansion fuelled by increasing global population resulting in increasing need for food supply and renewable energy sources (Danielsen *et al.*, 2009, Indrarto *et al.*, 2012). The deforestation map by Hansen *et al.* (2013) shows SEA has very high deforestation rate in the 21$^{st}$ century, with Malaysia having the highest deforestation rate of all the countries in the world (Hansen et al., 2013). The peatlands are no exception to the deforestation in SEA (Hooijer *et al.*, 2006, Miettinen *et al.*, 2012). It is estimated that 25% of all forest degradation in SEA occurs in peatlands (Lo and Parish, 2013). Mapping of peatlands in SEA by high resolution LANDSAT satellite images, comparing peatland cover in 1990 and 2008, shows that nearly half of the peatland

area in 1990, were converted or degraded to other land cover by 2008 (Hooijer *et al.*, 2006, Miettinen and Liew, 2010). A report by Miettinen *et al.* (2012) shows that only 34% of the former peat swamp forest in western part of SEA remains intact, while the remaining were either converted to agricultural plantations or heavily degraded. The deforestation rate of peatland forests are found to be 4% per annum, which is higher than deforestation rate of all other forest types (Lo and Parish, 2013).

The major drivers for deforestation in peatlands are expansion of industrial plantations, timber extraction and agriculture (Lo and Parish, 2013). The expansion of oil palm plantations is the biggest cause of deforestation in SEA (Indrarto *et al*., 2012). There were a very little disturbance and degradation of peatlands in SEA, before the start of industrial plantation in the last few decades (Miettinen *et al*., 2012). However, after the 1980s the clearance of peatlands in the region was vigorous, due to the shortage of mineral soil for the expansion of oil palm plantations, fuelled by increasing global need for food and energy supply with increasing population (Danielsen *et al.*, 2009, Miettinen *et al.*, 2012). The forested lands were often cleared using fire to establish oil palm plantations, which results in high $CO_2$ emissions (Page and Hooijer, 2016). These human disturbances are turning SEA peatlands from carbon sinks to globally significant carbon sources (Moore *et al.*, 2013).

South East Asia is home to numerous endemic species, which were adversely affected by the installation of oil palm plantations and oil palm expansion remains as the biggest threat to remaining biodiversity in the region (Dhandapani, 2015, Fitzherbert *et al.*, 2008). All the past research have shown that the conversion of forest landscapes to oil palm plantations have resulted in drastic reduction of species richness of vertebrates and invertebrates (Danielsen *et al.*, 2009, Dhandapani, 2015, Fitzherbert

*et al.*, 2008, Savilaakso *et al.*, 2014). However there is minimal research carried out on microbial species richness and community structure on different tropical landscapes (Kerfahi *et al.*, 2014, Lee-Cruz *et al.*, 2013, Schimel and Schaeffer, 2012, Smith *et al.*, 2014, Tripathi *et al.*, 2012), despite the importance of the ecosystem services provided by them. Peatlands have been considered to have very high microbial population (Andersen *et al*., 2013), which are most likely to get affected by environmental change that influence and get influenced by climate change (Davidson and Janssens, 2006, Freeman *et al.*, 2004, Mastepanov *et al.*, 2008). The microbial influence on carbon cycling in the tropical forests is particularly important for climate change (Batjes and Sombroek, 1997, Smith *et al.*, 2014), as tropical forest soils are active throughout the year (Guo and Gifford, 2002).

## 1.1 Land-use change and environmental impacts

The major land-uses in SEA peatlands are intact peat forests, logged forest, burnt forest, drained forest, mixed croplands and shrublands, rice fields, oil palm plantations, acacia and sago palm plantations (Hergoualc'h and Verchot, 2011, 2014, Hooijer *et al.*, 2006, Miettinen and Liew, 2010, Silvius and Diemont, 2007). All these land-use changes, especially oil palm plantations, are potential threats to the remaining peatlands in SEA

### 1.1.1 Oil palm plantations

Oil palm is considered as one of the fastest expanding equatorial crops (Koh and Wilcove, 2008). The expansion of oil palm plantations is the biggest threat to any natural forest habitats in SEA, including peatlands (Hooijer *et al.*, 2006, Koh and Wilcove, 2008, Miettinen *et al.*, 2012). Palm oil is the leading source of vegetable oil and fat in the world (Turner *et al.*, 2008). Malaysia and Indonesia are the leading

producers of palm oil and also the home for numerous endemic species, leading to the association of high production of palm oil to a greater number of endangered species (Turner *et al.*, 2008). Some 8.4% of oil palm plantations in Malaysia are in peatlands (Rashid *et al.*, 2013). As the land become scarce for oil palm plantations, such cropping is expected to increase in peatlands (Miettinen *et al*., 2012). Hoojer *et al*. (2006) observed that one quarter of oil palm concessions in Indonesia are in peatlands and a major portion of planned oil palm expansions in Indonesia are located in Peatlands (Sheil *et al.*, 2009). The peatlands are usually scarcely populated, making it easy to claim and gain ownership for the oil palm industries, without any social conflict (Casson *et al.*, 2007).

Oil palm can affect forest by direct expansion into forested or degraded peatlands, or as a combined economic enterprise of paper and oil palm industry to cut costs of oil palm establishment (Miettinen *et al.*, 2012). They also indirectly affect peat forests by construction of roadways to the oil palm plantations and industries or by moving other crops to peatlands (Hirano *et al.*, 2007, Miettinen *et al.*, 2012). There was about 3 Million ha of industrial oil palm plantation in peatlands in SEA by 2010 (Miettinen *et al*., 2012), which is expected to increase to 4 Million ha by 2020 (Hooijer *et al.*, 2006).

Forested land should be completely cleared of vegetation to establish oil palm plantation (Bruhl *et al.*, 2003, Luskin and Potts, 2011). The clearing and draining of peatlands leave dead wood and debris that are easily susceptible to fire (Bruhl and Eltz, 2010). The forest fires in SEA are often associated with land clearing for oil palm plantations (Cao, 2000, Chazdon, 1998, Dennis *et al.*, 2005). It was claimed that there were more than 60,000 peatland fires in Indonesia between 1997 and 2007, causing harmful haze (Tan *et al.*, 2009). So, the oil palm plantations are directly responsible to two other land use changes i.e., burnt and drained peatlands. The ground is terraced

after clearing the forest, in preparation for establishing the oil palm plantations (Bruhl and Eltz, 2010; Luskin and Potts, 2011), causing further damage to the topsoil. The soil layer can become extremely hot during the process of establishing the plantations (Bruhl and Eltz, 2010). Oil palm plantations are usually well maintained, with regular harvesting, pruning, manuring and pest management, causing significant changes to the environment and microclimate (Chung *et al.*, 2000, Luskin and Potts, 2011).

The observations from various research show that oil palm plantations have very high temperature, less humidity, acidic pH and very high temporal variations of environmental characteristics (Table 1.1). Another common observation is a high degree of disturbance involving human movement/interference and complete lack of organic humus and leaf litter layer in the soil, severely altering the soil properties (Table 1.1). Though some researchers observed high canopy cover in oil palm plantations, the canopy was homogenous and single layered, unlike multi-layered heterogeneous canopy in old growth forest, backing stable and moderate microclimate that provide abode for understorey biodiversity (Peh *et al.*, 2006). The microclimate of oil palm plantations also varied with the age of the plantations and between industrial and small holders' plantations. The young plantations (8 years) were found to have open canopy and twice the understorey vegetation as old (22 years) plantations with closed canopy (Luskin and Potts, 2011). The habitats were also found to go through different microclimate changes, throughout the lifecycle of the oil palm plantations (Luskin and Potts, 2011).

**Table.1.1:** Summary of observations made by different researchers in oil palm plantations after forest conversion.

| **Observed environmental conditions at oil palm plantation in comparison with Forest conditions** | **Study** |
|---|---|
| High variation in microclimate.<br>Low floristic diversity and density, with number of plants and trees less than half to the number of plants and trees in forest sites, other saplings were almost completely absent in oil palm plantations.<br>Canopy cover was a bit lesser than forest.<br>Very low amount of leaf litter.<br>Bulk density and pH were similar to the forest sites, with higher variance within the habitat for oil palm plantations. | Chung *et al.* (2000) |
| Open habitat with very less ground cover and high visibility. | Rajaratnam *et al.* (2007) |
| Canopy cover was 67% while the canopy cover of forest was 90%.<br>Temperature was 30.9$^{o}$ C while the temperature in forest was 26.6$^{o}$ C.<br>Humidity was 72.1% while the humidity in forest was 84.9%.<br>Average dry weight of leaf litter was 310g while in forest it was 438g. | Turner *et al.* (2008) |
| Lack of humus and leaf letter layers, severely altering soil properties.<br>Less shade leading to hot and dry microclimate. | Bruhl and Eltz (2010) |
| Mean air temperature was 1.7$^{o}$ C higher, while the soil temperature was 1.9$^{o}$ C higher.<br>Greater variation of temperature within the plantations. | Lucey and Hill (2012) |

| | |
|---|---|
| Increased soil temperature. | Edwards *et al.* (2014) |
| Less complex vegetation with low ground cover and high visibility (>100 m).<br>The ground vegetation was either trimmed or applied with herbicide or grazed.<br>The plantations of different age had different microclimatic conditions. | Azhar *et al.* (2011) |
| High temperature flux between day and night.<br>Increased evaporation and lower humidity.<br>Pesticide use and periodical clearing. | Gillespie *et al.* (2012) |
| High temperature.<br>Reduced woody debris (leaf litter, fell log).<br>Less canopy cover.<br>Less dense and homogenised vegetation structure. | Faruk *et al.* (2013) |
| Hot and dry microclimate.<br>Simplification of canopy structure.<br>Reduced litter complexity. | Fayle *et al.* (2010) |
| Canopy cover, canopy height and leaf litter depth were significantly lower in oil palm than both logged and unlogged forests. | Cusack (2011) |
| Ground layer vegetation was absent.<br>Small holder plantations had different environmental conditions than industrial plantations.<br>Microclimate varied with different age of the plantations. | Azhar *et al.* (2013) |

| | |
|---|---|
| The plantations lacked habitat heterogeneity. | Fukuda *et al.* (2009) |
| Closed canopy, supporting very less understorey vegetation.<br>Simplification of vertical vegetation structure. | Peh *et al.* (2006) |
| Oil palm had the highest canopy cover, least complexity of vegetation among acacia, oil palm and selectively logged native forest habitats.<br>Oil palm plantation had simple botanical structure. | Sheldon *et al.* (2010) |
| High water surface temperature and high soil temperature. | Brant (2011) |
| Homogenous vegetation. | Singh *et al.* (2014) |
| Oil palm plantations are substantially hotter (+2.84$^0$C) and drier (+0.80 hPa vapour pressure deficit).<br>Young plantations have open canopy with high solar radiation and high wind exposure.<br>Old plantations have closed canopy with much lesser understorey vegetation.<br>There was high temporal variations in microclimatic conditions.<br>Plantation conditions were not distinguishable from forest during night time. | Luskin and Potts (2011) |
| Unfavourable change in soil properties leading to marked deficiency in most nutrients | Yeboua and Ballo, 2000 (From Bruhl and Eltz, 2010) |

A review by Hergoualc'h and Verchot (2014) found that oil palm plantations emitted 29.9 +/- 10.6 mg $CO_2$ $ha^{-1}$ $y^{-1}$, while the carbon loss associated with conversion of virgin peat swamp forest to oil palm plantations was found to be 188 +/- 29.8 mg $ha^{-1}$ $y^{-1}$ (Hergoualc'h and Verchot, 2011) . However, there are several studies using closed chamber methods that which show that the total soil $CO_2$ emissions from oil palm plantations were lower than total soil $CO_2$ emissions from forest (Couwenberg *et al.*, 2010). However, the proportion of heterotrophic contribution to $CO_2$ emissions were highest at plantations (Comeau *et al.*, 2016, Dariah *et al.*, 2014, Matysek *et al.*, 2017), while heterotrophic contribution in forest soils were substantially lower in comparison (Hergoualc'h *et al.*, 2017, Murdiyarso *et al.*, 2017). Thus, most of the $CO_2$ emissions in forest were autotrophic root respiration from immediate carbon cycling, while the $CO_2$ emissions in oil palm plantations were associated with loss of carbon that was stored over a long time. $CO_2$ emissions from oil palm plantations and the proportional contribution of heterotrophic respiration were also known to respond to the age and lifecycle of the plantations, with older plantations having higher $CO_2$ emissions, with larger more developed root systems contributing to greater proportion of autotrophic respiration to total $CO_2$ emissions (Hergoualc'h *et al.*, 2017, Melling *et al.*, 2005b). As the peatlands are generally drained for the establishment of oil palm plantations, the anoxic condition needed for $CH_4$ emissions are affected (Aben *et al.*, 2017, Couwenberg *et al.*, 2010). Thus, oil palm plantations in SEA tend to have lower $CH_4$ emissions than the already low $CH_4$ emissions observed in SEA peat swamp forests (Couwenberg *et al.*, 2010), and are low in comparison to carbon loss through $CO_2$ emissions in oil palm plantations (Page *et al.*, 2011a).

### 1.1.2 Logged forests

Logging and agricultural expansion are the two major drivers of tropical forest degradation (Edwards *et al.*, 2013). More than 20% of tropical forests were already selectively logged and even more are to be logged in the coming years (Bicknell *et al.*, 2015). As of 2011, 403 million hectares of tropical forests were designated for logging (Blaser *et al.*, 2011). Logging is often the pathway for other degrading land uses like oil palm expansion in SEA, where logged forests are readily converted to oil palm plantations (Koh and Wilcove, 2008, Woodcock *et al.*, 2011). The construction of roads for the transportation of logged timber, gives access to the remote forests for other land uses and have huge indirect effect on forest degradation (Forman and Alexander, 1998, Perz *et al.*, 2008). Governments granting permission for industrial timber plantations also poses a major threat to natural ecosystem. In Indonesia alone, there was 1.4 million ha of government permitted timber plantations in 1995, which increased to 1.8 million ha in 2000 and 2.3 million ha in 2003, with plans to reach 10.5 million ha by 2030 (Indrarto *et al.*, 2012). In addition to this, illegal logging also remains as a major threat to SEA rainforests (Indrarto *et al.*, 2012).

The peatlands in SEA are an important source of high quality timber (Posa *et al.*, 2011). The extent of logging in peatlands is so high, that pristine peatlands in Peninsular Malaysia and some other parts of SEA, are almost extinct, and often secondary logged forests are used as a standard to study peat swamp forests (Melling *et al.*, 2005b, Tonks *et al.*, 2017). The long term effects of logging on tropical peat swamp forests are poorly understood. However, timber extractions in SEA peatlands are often associated with peatland drainage, which hugely affects peatland functions (Lo and Parish, 2013). Even without drainage, logging alters forest species composition and impacts regeneration of primary forest tree species, similar to the

effects of logging observed in other forest types (Posa *et al.*, 2011). Logging reduces the complexity of the canopy cover, leaving some areas of logged forest with open canopy (Floren and Linsenmair, 2005). The reduction in complexity of canopy also have impacts on leaf litter, which was reduced in the logged forest, however it was higher than oil palm plantations (Chung *et al.*, 2000). This reduction in leaf litter after logging, did not recover even after 25 years (Bruhl and Eltz, 2010). Additionally, logging also resulted in lower quantities of dead wood, resulting in reduced number of ant and termite nests, which could impact other soil properties (Styring and bin Hussin, 2004).

Carbon loss from logging in virgin peat swamp forest was estimated to be 116.9 +/- 39.8 mg $ha^{-1}$ $y^{-1}$ (Hergoualc'h and Verchot, 2011). The impact of logging on $CO_2$ emissions in peat swamp forests is poorly understood. However, logging associated drainage of peatlands expose surface peat to aerobic decomposition, presumably resulting in higher $CO_2$ emissions. In parallel, $CH_4$ emissions were generally lower in disturbed peat forests than in pristine peat swamp forests, due to the decrease in ground water levels (Hergoualc'h and Verchot, 2011).

### 1.1.3 Drained and Burnt Peatlands

The drained and burnt peatlands in SEA are either the pathway to or a by-product of oil palm expansion and logging (Bruhl and Eltz, 2009). By 2010, 46% of the peatlands in Malaysia, Borneo and Sumatra were either drained or used as a cropland (Hergoualc'h and Verchot, 2014). The drainage of peatlands reduces the water table level, consequently exposing the organic peat to aerobic decomposition. This reverses the role of peatlands as a carbon sink to carbon source (Page and Hooijer, 2016). It is estimated that $CO_2$ emissions due to drainage of peatlands in SEA alone equals to 1.3% -3.1% of global $CO_2$ emissions from the combustion of fossil fuels (Rehman *et*

*al.*, 2014). Though $CH_4$ emissions from drained peat surface would be reduced, the drainage ditches have a potential for high $CH_4$ emissions and their impact on carbon loss is not well known (Page *et al.*, 2011a).

The drainage and drying of peat also makes it highly susceptible to fire, leading to another land use - burnt peatland, resulting in even higher $CO_2$ emissions with burning of carbon rich tropical peat swamp forests (Posa *et al.*, 2011). The dried peat in itself is highly flammable, thus fire in peatlands can sustain both above and below ground through smouldering (Turetsky *et al.*, 2015, Uda *et al.*, 2017). The fire belowground in peatlands could affect the underground seed banks thereby inhibiting forest regeneration, resulting in conversion of peatlands to open floodplains (Uda *et al.*, 2017). Peat fires are responsible for harmful haze in SEA, and the haze can indirectly influence carbon balance by affecting photosynthetic rates of above ground vegetation and suppressing plant $CO_2$ uptake (Davies and Unam, 1999). The $CO_2$ emissions from peat fire in Indonesia in 1997-98, amounting to 0.81-2.57 Gt of carbon, is the largest annual increase in atmospheric $CO_2$ concentrations recorded since the beginning of recording in 1957 (Page *et al.*, 2002). The average annual $CO_2$ emissions from fire in peatlands is estimated to be in the range of 500 - 1400 Mt (Hooijer *et al.*, 2006, van der Werf *et al.*, 2008). Hergoualc'h and Verchot (2011) estimated the total carbon loss from the conversion of virgin peat swamp forest to burnt forest by stock difference method and found it to be 151.6 +/- 36 Mg $ha^{-1}$ $y^{-1}$. However, GHG emissions from peat surface after fire would be dependent on the land use after the event of fire. The loss of above ground vegetation and shade provided by them may increase heterotrophic $CO_2$ emissions by 33-66%, depending on the degree of change in surface temperature and nutrient availability (Jauhiainen *et al.*, 2014). $CH_4$ emissions in burned peatlands were found to be higher than natural peat forests in SEA (Hatano *et*

*al.*, 2016). The long term effects of burning in peatlands on $CH_4$ emissions are less clear and do not appear to be well documented.

### 1.1.4 Other agricultural plantations

SEA was once known for high production of cocoa, rubber and coconuts (Basiron, 2007, Dhandapani, 2015). However, since the rise of oil palm industry, the plantations of these crops were replaced by industrial oil palm plantations (Basiron, 2007, Dhandapani, 2015). Due to the expansion of oil palm in all cultivable lands, small-scale agriculture has been gradually pushed into peatlands. The small-scale cultivation of rice, pineapple, yam, cassava etc. by independent farmers were reported in the SEA peatland, sometimes some of these crops are intercropped with oil palm by small holding farmers (Azhar *et al.*, 2011) . The environmental effects of these small scale cultivations and different cropping systems on peatlands are largely unknown. However, Hergoualc'h and Verchot (2014) estimated that mixed croplands and rice fields emitted 41.0 +/- 6.7 and 25.6+/- 11.5 Mg $Co_2$ $ha^{-1}$ $y^{-1}$ respectively. The $C_2$ loss from the conversion of peat swamp forest to mixed croplands and rice fields are estimated to be 204.1 +/- 28.6 and 214.9 +/- 28.4 Mg $ha^{-1}$ respectively (Hergoualc'h and Verchot, 2011). $CH_4$ emissions were generally higher in these croplands than in the forested peatlands, depending on the water table level (Hergoualc'h and Verchot, 2014). Notably, paddy fields in SEA were known to have substantially higher $CH_4$ emissions than any other land-use types in SEA peatlands (Couwenberg *et al.*, 2010). Acacia is another major industrial plantations found in peatlands (Hergoualc'h and Verchot, 2014). Chung *et al.* (2000) observed that acacia plantations had same amount of leaf litter as logged forests, which is slightly lesser than primary forest. Acacia plantations also had greater ground cover and higher leaf litter than oil palm plantations, while the canopy cover was lower (Chung *et al.*, 2000). Hergoualc'h and

Verchot (2011) estimated the carbon loss from the conversion of virgin peat swamp forest to Acacia plantations to be 191.7 +/- 28.5 Mg C $ha^{-1}$, while the emission from Acacia plantations is estimated to be 71.8 +/- 12.7 Mg $CO_2$ $ha^{-1}$ $y^{-1}$ (Hergoualc'h and Verchot, 2014).

## 1.2 Soil microbial communities and environmental controls

The response to environmental changes is much faster in microbes than any other flora and fauna in a habitat, owing to higher numbers and shorter life spans of the microbes (Andersen *et al*., 2013). The soil microbiota plays a key role in regulating nutrient availability, thereby modulating ecosystem processes and the successional progression of flora and fauna in a habitat (Andersen *et al*., 2013). The soil biota is in turn regulated by the physical and chemical properties of soil, which depends on various soil-forming and environmental factors (Bardgett, 2005). It is widely accepted that microbial activity increases with increasing temperature up to 30-35$^{o}$ C (Bardgett, 2005). Rainfall patterns directly influence soil microbial community structure with changes in moisture conditions (Bossio and Scow, 1998, Zhao *et al.*, 2016). Temperature and precipitation also indirectly influence soil microbial communities and soil formation through changes in soil organic matter composition and dynamics (Chen *et al.*, 2016, Deng *et al.*, 2017, Han *et al.*, 2017).

The composition of organic matter is a crucial factor, by which above ground diversity influences the below ground diversity (Hooper *et al.*, 2000). The soil biota depends on soil organic matter for their nutrients and C supply. It also affects soil structural stability, particularly protecting against erosion (Fullen and Catt, 2004). It also helps to retain water in soil. Soil water in itself plays different roles, forming the soil solution along with nutrients, serving the above-ground plant communities with water and

nutrients (Fullen and Catt, 2004). Water also serves as the medium for microbes to loco-mote around soil structures (Bardgett, 2005). Many soil fauna are sensitive to desiccation and some of them become inactive, if there is a lack of water (Yan *et al.*, 2015). Another important factor affecting the soil biota is soil pH (Rousk *et al.*, 2009). Most of the world soils, especially tropical soils, are acidic in nature (Slessarev *et al.*, 2016). Soil pH impacts nutrient availability especially P and thus directly influences the soil biota and above ground productivity (Bardgett, 2005). Acidity in soil can be caused by numerous factors like the mineral content of the parent material, climate, use of inorganic fertilizers, microbial oxidation of ammonium ions to nitrate, atmospheric pollution, formation of carbonic acid by the dissolution of $CO_2$ in soil water and decomposition of phenolic and carboxyl groups in soil organic matter (Bardgett, 2005, USDA, 2015). Phosphorus availability is low in acidic soil, affecting the P uptake by plants and their productivity (Antoniadis *et al.*, 2015). The microbes are found to grow between pH 4 and 9 (Bardgett, 2005). Very few microbes are found to inhabit and be active out of that pH range (Bardgett, 2005). pH was also found to affect other macroscopic soil biota, with neutral or near neutral pH being the optimal condition for high biodiversity in soil (Edwards and Bohlen, 1996). Thus, soil microbial communities heavily depend on environmental factors such as microclimatic conditions, the physical and chemical properties of the soil.

### 1.2.1 Microbes in peatlands

High carbon storage by photosynthetic accumulation and anaerobic conditions leading to low decomposition in peatlands, were known to support unique groups of microorganisms (Andersen *et al.*, 2013, Yule, 2010). The microbial communities in natural peatlands are found to be vertically stratified, because of variation in availability of oxygen and the quality of carbon along the peat depth (Dedysh *et al.*,

2006, Morales *et al.*, 2006, Sundh *et al.*, 1997, Williams and Crawford, 1983). However the vertical stratification of microbes depends on the stage of peat formation and the quality of carbon input (Andersen *et al.*, 2013). A study by Jackson *et al.* (2008) on bacterial communities in Malaysian peatlands show that the bacterial communities are vertically stratified, similar to the observations in northern peatlands. The microbes were concentrated in the upper layer with leaf litter than in deep older peats (Jackson *et al.*, 2008). Land-use change and other disturbances would further influence the stratification with more energy constraints that are influenced by changes in oxygen availability and carbon substrate quality (Andersen *et al.*, 2013).

In many terrestrial ecosystems, as carbon quality changes at the different stages of decomposition, different microbial communities with varied enzymatic capabilities found to dominate the different stages, the process commonly known as temporal niche partitioning (Andersen *et al.*, 2013, Lumley *et al.*, 2001). However, temporal niche partitioning is not observed in northern peatlands, where species of extensive enzymatic capabilities are found to share the spaces (Artz *et al.*, 2007, Thormann, 2006). The top layer of peat is observed to support aerobic decomposition, where fungal saprophytes are thought to initially dominate the decomposition process (Kuehn *et al.*, 2000, Newell *et al.*, 1995). There are other studies suggesting that bacterial communities dominate the top layers rather than fungal communities (Gilbert *et al.*, 1998, Winsborough and Basiliko, 2010). However, all of this research were in northern peatlands and there appears to be no published research into microbial community structure as a whole, integrating both fungal and bacterial communities in tropical peatlands. Therefore, it is unknown whether the soil microbial communities in tropical peatlands are dominated by fungi or bacteria. The lack of data and understanding of the mechanisms of microbes and their response to disturbances in

peatlands in general (Artz *et al.*, 2007, Elliott *et al.*, 2015), makes it difficult to clearly speculate the structure and functions of tropical peatland microbial communities in different land-use types.

The bacterial communities vary in size and structure with peat depth (Xiang *et al.*, 2014). Morales *et al.* (2006) found more bacterial cells with increasing depth up to 1 metre than in the surface, but the bacterial diversity was lesser at 1 metre depth. The lower bacterial diversity at deeper layers may be due to lack of competition from aerobic bacteria in anoxic deeper peat (Andersen *et al.*, 2013). However, the bacterial diversity appears to be highest at the intermediate depths and both diversity and density decreased with depth from there (Andersen *et al.*, 2006, Dedysh *et al.*, 2006, Jaatinen *et al.*, 2007, Kotsyurbenko *et al.*, 2004, Sundh *et al.*, 1997). Actinobacteria followed a similar trend of decrease after intermediate depth (Golovchenko *et al.*, 2007). The highest diversity in intermediate depths can be explained by the meeting and co-existence of oxic and anoxic layers in intermediate depths, where there is simultaneous presence of CH4 and oxygen, harboring methanotrophic bacterial communities (Andersen *et al.*, 2013, Clymo and Bryant, 2008). The deeper anaerobic peat layers contain a functionally important group of microbes called methanogens (Offre *et al*., 2013). Methanogens, a specialized group of archaea, produce $CH_4$ by the process called methanogenesis (Andersen *et al.*, 2013), which plays a major role in global geochemical cycles (Offre *et al.*, 2013). Archaea other than methanogens are also found in deep peats, but their metabolic pathways and functional importance are largely unknown (Andersen *et al.*, 2013, Jackson *et al.*, 2009).

The spore numbers of fungal communities are found to decrease with depth (Golovchenko *et al*., 2002), while the richness remained the same (Preston *et al.*, 2012). The reduction in spore numbers might be due to the energy constraints and the

quality of C with increasing depth. However the density of yeast remained the same with different depth of peats (Golovchenko *et al.*, 2002). Some studies observed that the litter quality is the main control for fungal communities in peatlands (Thormann *et al.*, 2004, Trinder *et al.*, 2008, Trinder *et al.*, 2009), while mycorrhizal fungal communities are linked to the above ground vegetation (Andersen *et al*., 2013). A study by Kachalkin *et al*. (2008) showed species specific interaction between plants and yeast. Although, the role of yeast in peatlands function is unclear, as they are unable to degrade complex organic matters (Andersen *et al*., 2013). Archaeal communities in peatlands were also found to show substrate and plant specificity (Cadillo-Quiroz *et al.*, 2009, Lin *et al.*, 2012, Yavitt *et al.*, 2012), though most of the archaeal communities found in peatlands were unknown and uncultivated (Cadillo-Quiroz *et al*., 2008; Yavitt *et al*., 2012). To our knowledge, there is no previous research on seasonal variations of microbial communities in tropical peatlands. However, Golovchenko *et al.* (2005) found multi-fold increases in bacterial biomass in northern peatlands during summer. The climatic conditions are dramatically different in the tropics in comparison to northern hemisphere, as tropical seasonal change are characterized by changes in the pattern of rainfall, unlike northern peatlands, where temperature change plays a major role in seasonal variations.

### 1.2.2 Microbes in disturbed peatlands

Research detailing the effects of disturbance on microbial communities in peatlands is scarce. Fire is one of the major disturbances in peatlands, both tropical and northern peatlands (Hergoualc'h and Verchot, 2014, Turetsky *et al.*, 2002). Curlevski *et al.* (2011) found that there was no effect on fungal communities in peatlands, 5 years after fire, while some studies suggest that bacteria to fungi ratio increases after fire, as bacteria are more resistant to heat (Chen *et al.*, 2008, Hart *et al.*, 2005). While there

are few studies on the effect of peatland disturbance such as fire on microbes in northern peatlands (Curlevski *et al.*, 2011, Treseder *et al.*, 2004), the effect of different disturbances on microbes in disturbed tropical peatlands are yet to be investigated.

Another important disturbance is the changes in water table depth resulting from drainage of peatlands, draught, changes in rainfall, timber extraction or other land conversions. The microbial reaction to changes in hydrology depends on the intensity of change, changes in other environmental factors and the type of peatlands (Andersen *et al.*, 2013, Kim *et al.*, 2008). Persistent hydrological changes can affect above ground biodiversity, which in turn could influence microbial communities belowground (Yrjala *et al.*, 2011). The anaerobic bacterial communities, methanogens and methanotrophs are most likely to be affected by changes in hydrology, as they require anaerobic niche, which depends on water table. So, the lowering of water table could increase aerobic communities, while pushing the anaerobic communities deeper down the surface (Francez *et al.*, 2000). However these communities are found to be very tolerant to stress, and had been found active through relocating to smaller microhabitat refuges (Kotiaho *et al.*, 2010, Valentine, 2007), suggesting that they can tolerate and redevelop through periodical variation of water table. However, long term change by land conversion would be detrimental to these communities (Andersen *et al.*, 2013). Jaatinen *et al.* (2008) observed that prolonged decrease in water table depth in the top layers would be beneficial to fungal and Gram-negative bacterial communities, while being detrimental to Gram-positive bacterial communities in northern boreal peatlands.

The conversion of peatlands to other agricultural landscapes would have considerable effect on nutrient deposition in peat by anthropogenic activities (Agamuthu and Broughton, 1985, Comte *et al.*, 2013), which in turn would affect microbial

communities in peatlands (Mello *et al.*, 2016). The understanding of the long term impact of nutrient deposition or fertilization on tropical peatland microbial communities is limited, since there were only very few studies on relation between nutrient deposition and microbial communities, and none on tropical peat microbial communities. The changes in nutrient deposition by anthropogenic activities can indirectly affect microbial communities in peat by changing the above ground vegetation (Andersen *et al.,* 2012). A study by Liu *et al.* (2012) on old growth forests in tropical mineral soil found that the total microbial biomass increased with the addition of phosphorus, though it cannot be assumed to have the same effect on tropical peatlands, as peatlands are physically and chemically distinct. Observations in northern peatlands show that increased N deposition, decreased community diversity and reduced mycorrhizal colonization of mycorrhizal fungi, while increasing the general fungal biomass and altering the fungal community structure (Basiliko *et al.*, 2006, Yesmin *et al.*, 1996). Bragazza *et al.* (2012) found that bacterial biomass increase with increased nutrient deposition, which was also in agreement with Basiliko *et al.* (2006). The latter also found that the $CO_2$ emission increased with nutrient input.

Most of the studies on microbes in peatlands and their response to disturbance, were focused on northern temperate and boreal peatlands. While there are a few studies on microbes and land-use change in tropics (Kerfahi *et al.*, 2014, Krashevska *et al.*, 2015, Wood *et al.*, 2017), they are all focussed on mineral soil. It cannot be assumed that tropical peatlands would follow similar cause-and-effect patterns to those associated with the mineral soil environments or even northern peatlands, as the tropical peatlands are dramatically different from the northern peatlands, and are chemically and physically distinct from mineral soil (Yule, 2010). Hence the microbial

communities in tropical peatlands and their response to disturbance remain largely unknown, despite their functional importance in tropical peatlands.

## 1.3 Phospholipid Fatty Acid (PLFA) analysis

All living cells contain lipids in their cell membranes, which rapidly degrades after cell death (Bloem et al, 2008; Gattinger et al., 2003; Zelles and Bai, 1993; Kaur et al., 2005). Lipids are highly variable in structure. The wide spectrum of this structural and chemical heterogeneity in each lipid molecules determines the various functions of each molecules (Dowhan et al., 2016). The major function of lipids is to form a bilayer permeability barrier of cells and organelles (Dowhan et al., 2016). Phospholipids constitute 75% of the membrane lipids in a cell membrane (Dowhan et al., 2016). The PLFAs respond to both intracellular and extracellular stress (Kaur et al., 2005), asserting the importance of PLFA analysis in monitoring soil microbes and their response to changes. The Phospholipid bilayer of the cell wall contains hydrophilic and hydrophobic region (Singer and Nicholson, 1972). The variations in the hydrophobic region results in increased diversity of the membrane (Dowhan et al., 2016; Kaur et al., 2005). The individual species of phospholipids ranges from hundreds to few thousands depending on the complexity of the organisms (Dowhan et al., 2016). PLFAs are classified as ester linked PLFAs and non- ester linked fatty acids making up 60-90% and 10-40% of total phospholipids respectively (Kaur et al., 2005). Only ester linked phospholipids, which make the most of the biomass but not the number of phospholipids, are analysed in this procedure (Bloem et al., 2008). A normal soil profile obtained following PLFA analysis contains about 20 to 40 PLFAs (Bloem et al., 2008).

Analysis of ester linked Phospholipid fatty acid is an established and well acknowledged method for phenotypic characterization of microbial communities (Broem et al., 2008; ISO, 2014; Frostegard, 2011; Kaur et al., 2005). In the past, the soil physico – chemical characteristics were used to study the effect of disturbances and changes in land use. Considering the microbial communities respond faster to stress (Kaur et al., 2005), there is an increasing need for the effective monitoring of microbial communities in evaluating the ecosystems. Even though PLFA analysis, unlike advanced molecular methods, do not give information on specific species compositing, it is an effective, rapid and sensitive tool for monitoring the microbial communities' response to the environmental changes (Kaur et al., 2005; Frostegard et al., 2011; Bloem et al., 2008). The Genetic diversity of soil microbes are so high, that it is hard to detect fine scale impacts of ecosystem disturbance from the total diversity obtained using DNA based techniques (Kaur et al., 2005). The phenotypic diversity provided by the PLFA analysis is a better tool for monitoring the ecosystem and the impact of disturbances on soil microbes (Zelles, 1999; Kaur et al., 2005). However a major limitation for using PLFA analysis in this study is that PLFA analysis does not detect methanogenic archaea, functionally important group in peatlands. Methanogenic archaea contains ether linked fatty acids in their cell wall (Gattinger *et al.*, 2003). Phospholipid fatty acid analysis extracts only ester linked fatty acids, thus not detecting archaeal communities responsible for methane production.

## 1.4 Aims and objectives

Oil palm expansion and related land-use change is a cause for degradation of pristine peatlands in SEA region. While there are few studies on the impact of these land-use changes on $CO_2$ emissions and C loss, microbial communities underlying the process are previously unexplored, despite their importance for peatland development and

functions. This study aims to elucidate the response of tropical peatlands to disturbance by exploring peat properties, peat microbial communities, nutrient content, GHG emissions and the functional interactions between these components. The objectives were:

i) To explore the variation of tropical peat microbial communities associated with different land-uses corresponding to different stages of oil palm expansion and their variations with depth within those land-use types. (Chapter 2, *Microbial community structure across the peat profile at different stages of conversion from forest to oil palm plantation*)

ii) To assess the impacts of different oil palm cropping systems on peat properties, microbial community structure and GHG emissions across wet and dry seasons. (Chapter 3, *Soil microbial community structure and GHG emissions under forest and different oil palm cropping systems*)

iii) To evaluate the difference between primary and secondary peat swamp forest in microbial community structure and GHG emissions. (Chapter 4, *GHG emissions, nutrient content and microbial community structure from a last-remaining pristine tropical peat swamp forest in Peninsular Malaysia*)

iv) To understand the environmental controls on peat microbial community structure and GHG emissions and evince a relationship between microbial community structure and GHG emissions, (Chapters 3 and 4)

v) To examine the effect of proximity to a plant, on peat properties and GHG emissions in different oil palm cropping systems. (Chapter 5, *Effect of proximity to plants on peat characteristics and GHG emissions under different cropping systems in tropical peatlands*)

# Chapter 2

## 2. Microbial community structure across the peat profile at different stages of conversion from forest to oil palm plantation

### 2.1 Introduction

Tropical peatlands are complex and globally important ecosystems supporting distinctive biodiversity and high carbon (C) storage capacity, related to their unique acidic, nutrient-poor and waterlogged environmental conditions (Miettinen *et al.*, 2012, Parish *et al.*, 2008). The formation of these C-rich peatlands is a result of a delicate balance between different environmental and biological conditions, including hydrology, ecology, topography and landscape morphology (Miettinen *et al.*, 2012, Page *et al.*, 1999). The microbes in peatlands play an important role not only in the formation of peat itself, but also directly influence ecosystem C turnover and nutrient mineralization required to support high primary production, affecting overall ecosystem function and sustainability (Andersen *et al.*, 2013). Different anthropogenic disturbances impact different environmental and biological factors, affecting the overall balance of peatlands, and can turn the peatlands from C sinks to C sources (Lo and Parish, 2013).

Agricultural expansion, particularly plantation-scale growth of oil palm, is the biggest cause of deforestation and land-use change in South East Asia (Dhandapani, 2015, Hooijer *et al.*, 2006, Indrarto *et al.*, 2012, Koh and Wilcove, 2008, Miettinen *et al.*,

2012) and the peatlands are no exception to this. About 8.4% of oil palm plantations in Malaysia are in peatlands (Rashid *et al.*, 2013) and as the land becomes scarce for oil palm plantations, this proportion is expected to increase (Miettinen *et al.*, 2012). A major portion of planned oil palm expansions in Indonesia are located in peatlands (Hooijer *et al.*, 2006, Sheil *et al.*, 2009). There was about 3 M ha of industrial oil palm plantation in peatlands in South East Asia by 2010 (Miettinen *et al.*, 2012), which is expected to increase to 4 M ha by 2020 (Hooijer *et al.*, 2006). During the conversion, the forest land is usually completely cleared of vegetation to establish the plantation (Bruhl and Eltz, 2010, Luskin and Potts, 2011). Such clearing and draining of peatlands leaves dead wood and debris that are prone to fire (Bruhl and Eltz, 2010, Sumarga *et al.*, 2016), and forest fires in South East Asia are often associated with land clearing for oil palm plantations (Cao, 2000, Chazdon, 1998, Dennis *et al.*, 2005). Consequently, oil palm plantations are direct drivers for change in ecosystem function through drainage and burning of peatlands.

Oil palm plantations are generally maintained with regular harvesting, pruning, fertilization and pest management, causing significant changes to the environment and microclimate (Azhar *et al.*, 2011, Chung *et al.*, 2000, Luskin and Potts, 2011). The observations from various studies show that oil palm plantation have relatively high soil and air temperature (Lucey and Hill, 2012, Turner and Foster, 2009), lower humidity (Gillespie *et al.*, 2012, Turner *et al.*, 2008), low pH similar to tropical forest (Chung *et al.*, 2000) and very high temporal variation of such environmental characteristics (Gillespie *et al.*, 2012, Lucey and Hill, 2012). Though most of mature oil palm plantations have a closed canopy (Peh *et al.*, 2006), such canopies are typically homogenous and single layered. This is unlike the multi-layered heterogeneous canopy structure characteristic of old growth forests, which underpin a

stable and moderate microclimate that provides a more appropriate habitat to provision understorey biodiversity. Oil palm plantations of different ages were observed to have different microclimatic conditions and they tend to vary with the lifecycle stages of the palm (Azhar *et al.*, 2011, Faruk *et al.*, 2013, Luskin and Potts, 2011). Another common feature is the high degree of soil disturbance involving human movement and interference and a complete lack of leaf-litter or humus layer in oil palm plantations (Cusack, 2011, Peh *et al.*, 2006).

Drained and burnt peatlands in South East Asia are either a pathway or by-product of oil palm expansion and logging (Bruhl and Eltz, 2009; Wetlands International, 2010). By 2010, 46% of the peatlands in Malaysia, Borneo and Sumatra were either drained or used as a cropland (Hergoualc'h and Verchot, 2014). The drainage of peatlands reduces the water table level, consequently exposing the organic peat to microbial activity and aerobic decomposition. The lowering of water table could directly impact soil temperature, aeration, pH, and salinity (Coyne, 1999, Turetsky *et al.*, 2015), therefore affecting the soil microbial communities.

The lowering of water table in peatlands also make them susceptible to fire, as the dry peat itself is highly flammable (Posa *et al.*, 2011). Unlike contexts involving mineral soils, peat fires can burn both above and below ground, profoundly affecting the biota and seed bank underground and damaging the vegetation structure (Posa *et al.*, 2011, Turetsky *et al.*, 2015, Uda *et al.*, 2017). Fire in tropical peatlands can have a severe impact on moisture content, nutrient availability quality of organic matter and above-ground biodiversity, by turning peat forest into an open floodplain (Chokkalingam *et al.*, 2005, Turetsky *et al.*, 2015). Impacts belowground are less clear and do not appear to be documented in tropical peatlands. All of these are expected to have severe effects on soil microbial communities.

Responses to environmental change is typically more rapid for soil microbes than other flora and fauna because of their short generation times and higher population number per area or volume of a surface (Andersen *et al.*, 2013). It is widely demonstrated that soil microbes respond to environmental changes induced by anthropogenic land use changes (Bossio *et al.*, 2005, Hartman *et al.*, 2008, Kerfahi *et al.*, 2014, Krashevska *et al.*, 2015, Wood *et al.*, 2017). A general inference from the observed pattern of the microbial responses is that soil chemical properties, especially pH, plays a key role in governing bacterial community structure (Hartman *et al.*, 2008, Wood *et al.*, 2017) while fungal community changes are more dependent on soil moisture level, C quality and composition (Asemaninejad *et al.*, 2017, Thormann *et al.*, 2004, Trinder *et al.*, 2008, Trinder *et al.*, 2009). Bacteria were found to be more tolerant to fire in peatlands, with the ratio of bacteria to fungi increasing after an event of fire (Chen *et al.*, 2008, Hart *et al.*, 2005).

Microbes in northern peatlands were found to vary with depth and be vertically stratified because of energy constraints, which become further influenced by disturbances (Andersen *et al.*, 2013, Dedysh *et al.*, 2006, Morales *et al.*, 2006, Sundh *et al.*, 1997). Some studies on northern peatlands found fungal communities to be dominant on surface peat layers (Kuehn *et al.*, 2000, Newell *et al.*, 1995) while other studies found bacterial communities to be dominant (Gilbert *et al.*, 1998, Winsborough and Basiliko, 2010). However for tropical forest, such studies are sparse. Hadi *et al.* (2001) measured greater bacterial than fungal biomass in both forest and paddy fields in Indonesian peatlands. In a rare study into microbial communities in Malaysian tropical peatlands, Jackson *et al.* (2009) found Acidobacteria to be the most dominant species in the bacterial community up to 50 cm depth, but did not study any eukaryotic soil communities. Similar trends of Acidobacteria dominance was also observed in

forest, oil palm plantations and other disturbed peatlands in Indonesia (Mishra *et al.*, 2014). Bacterial communities in Central American peatlands also had high proportion of Acidobacteria, but Proteobacteria were slightly higher in proportion than Acidobacteria (Troxler *et al.*, 2012). Other than these four mentioned studies, all the rest of the very few studies on microbes in tropical peatland focused on specific microbial functions such as activity and decomposition (Valenzuela *et al.*, 2017, Yule and Gomez, 2009), or on specific microbial groups (Arai *et al.*, 2014a, Hashidoko *et al.*, 2008, Rahman *et al.*, 2010, Voglmayr and Yule, 2006). The lack of data and research on poorly understood peat microbial communities and considerable differences in dominant communities, variations with depth etc., within the limited studies, make it difficult to infer the structure and functions of tropical peatland microbial communities and their response to disturbances in relation to land use change.

Most of the studies on microbes in peatlands and their response to disturbance, were also focused on northern temperate and boreal peatlands. The tropical peatlands are dramatically different from the northern temperate and boreal peatlands (Yule, 2010). While there are a few studies on microbes and land-use change in tropics (Kerfahi *et al.*, 2014, Krashevska *et al.*, 2015, Wood *et al.*, 2017), they are all focussed on mineral soil. It cannot be assumed that tropical peatlands would follow similar cause-and-effect patterns to those associated with the mineral soil environments, as tropical peatlands are physically and chemically distinct. Hence the microbial communities in tropical peatlands and their response to disturbance remain largely unknown. The changes in soil microbial communities after disturbance can have potential impact on their ability to utilize different organic matter for decomposition and mineralization (Kerdraon *et al.*, 2017). The changes in proportions or dominance of different

functional microbial groups such as methanotrophs and methanogens, can significantly alter the composition of greenhouse gas emissions. These changes in carbon utilization and pathways in a carbon rich tropical peatlands could have severe impact on climate change. So, it is absolutely essential to understand the peatland microbial communities and their response to disturbances.

The objectives of this research were to investigate and explore the variation of tropical peat microbial communities associated with (i) different land-uses corresponding to different stages of oil palm expansion (ii) different depth within those land use types. We hypothesised that microbial community structures are affected and altered by the disturbances involved with different stages of conversion from forest to oil palm plantation, considering the dramatic changes in hydrology, microclimate and above ground biodiversity associated with the forest conversion. Similarly, microbial communities were hypothesised to change with depth due to the difference in carbon quality between surface layers and deeper layers along with changes in hydrology and oxygen availability with depth.

## 2.2 Material and methods

### 2.2.1 Study sites

Study sites were selected in North Selangor peat swamp forest (NSPSF) in Peninsular Malaysia (3º34’32N 101º15’44E) to represent land-use types involved in different stages of peatland conversion to oil palm namely: (i) Historically drained secondary peat forest , hereafter denoted as ‘forest’; (ii) Recently drained peatland forest, denoted ‘drained’; (iii) Burnt peatland, denoted ‘burnt’; (iv) Recently cleared peatland with young oil palm plantation, denoted ‘young oil palm’; (v) Mature oil palm plantation, denoted ‘mature oil palm’.

The North Selangor peatlands are about 10 km from the west coast of Malaysia (Yule and Gomez 2009). The forest and drained sites had similar above ground vegetation comprising *Macaranga pruinosa* (Miq.) Müll.Arg, *Campnosperma coriaceum* (Jack) Hallier f., *Blumeodendron tokbrai* (Blume) Kurz, *Shorea platycarpa* F.Heim, *Parartocarpus venenosus* Becc., *Ixora grandiflora* Ker Gawl, *Pternandra galeata* Ridl., *Stenoclaena palustris* (Burm. f.) Bedd., *Asplenium longissimum* Baker, *Nephrolepsis biserrata* (Sw.) Schott, *Crytostachys sp., Cyperus rotundus* L., and *Pandanus atrocarpus* Griff. (Yule and Gomez 2009). The major difference between the two forested land use types being the deep drainage in drained land use type with 2 to 3 m wide ditches dug at every few hundred metres (Tonks *et al*., 2017). The burnt sites have been subject to regular burning, with the most recent being 1.5-2 years before sampling, resulting in open scrubland, dominated by ferns, some grasses and bare soil and no forested areas. The young and mature oil palm sites have undergone both drainage and deforestation with oil palm plants 9 months, and 10-15 years old respectively.

Sampling was carried out during the wet season from November – December 2014, with five independent replicate sites for each land use type: precise locations are listed in Appendix 1. Forest replicate sites were spatially distributed over the whole NSPSF. Mature oil palm sites were spatially distributed on the southern edge of NSPSF. Drained forest and young oil palm were constrained to two areas on the north eastern edge of NSPSF. Burnt sites were constrained to one area on the southern edge of NSPSF. A total of 25 soil cores of up to 2 metres were collected from 5 different land-use types using a Russian peat corer. The peat depth varied between sites and the core depth covered 2 metres for all sites except 3 of the mature oil palm sites (*viz*. 100 cm, 50 cm and 150 cm for Sites 3 , 4 and 5 respectively) and 2 forest sites (150 cm each

for Sites 2 and 3). The peat cores were then separated into 20 cm segments and freeze-dried. During the time of sampling, forest sites had water level close to the surface, drained at 10-42 cm below surface, young oil palm at 25-60 cm below surface and mature oil palm at surface to 47 cm below surface (Tonks *et al*., 2017).

### 2.2.2 PLFA extraction

Microbial community phenotypic structure was determined by phospholipid fatty acid (PLFA) analysis. PLFAs were extracted from replicate 1 g freeze-dried tropical peat samples using a modification of the method described by (Frostegard *et al.* 1991). The lipids from peat were extracted using Bligh & Dyer extraction (Bligh and Dyer 1959). The extracted lipids were then separated into neutral lipids, glycol lipids and polar lipids (containing phospholipids) fractions using Megabond Elut® silica gel column. The extracted polar lipids were then methylated by mild alkaline methanolysis and converted into fatty acid methyl esters, which were then run on gas chromatography.

### 2.2.3 Gas chromatography and peak identification

The dried fatty acid methyl esters were suspended in 200 µL of hexane, ready for GC injection. One µL of each sample was injected into the GC in split-less mode. The column used in the GC for phospholipid analysis was 'ZB-FFAP' column, supplied by Phenomenex®. The column was 30 m length x 0.25 mm inner diameter x 0.25 µm film thickness. The carrier gas was helium with the constant pressure of 18 psi. The initial oven temperature in GC was 120°C; this was maintained for 1 min and then programmed to 250°C at the rate of 5°C $min^{-1}$. The constant temperature of 250$^{o}$C was maintained throughout the run. The results were displayed as a chromatogram of retention times of the compounds and the mass spectroscopy provides the ion profile of each compounds.

The fatty acids *i15:0, a15:0, i16:0, i17:0, a17:0* were considered as Gram-positive biomarkers (Wilkinson *et al.* 2002). The fatty acids *10me16:0* and *10me18:0* were described as the biomarkers for actinomycetes (Wilkinson *et al.* 2002; Moore-Kucera and Dick 2008), a group that belongs to Gram-positive bacteria. The relative abundance of Gram-negative bacteria were calculated using *16:1n9, 16:1n7, cyc17:0, 18:1n7* and *cyc19:0* as biomarkers (Wilkinson *et al.* 2002; Kaiser *et al.* 2010). *18:2n6* and *18:1n9* were used as fungal biomarkers (Vestal and White 1989; Wilkinson *et al.* 2002; Kaiser *et al.* 2010). *14:0, 16:0, 18:0, a17:1* and *20:0* were non-specific fatty acids (Wilkinson *et al.* 2002). The fatty acids with similar mass spectrum *18:1n9* and *18:1n7* were differentiated with the help of neutral lipid fatty acid analysis, by the findings that fungal biomarker *18:1n9* should have much higher NLFA/PLFA ratio that the Gram-negative biomarker *18:1n7* (Baath 2003). The ratio of Cyclopropane fatty acids (*cyc17:0& cyc19:0*) to their monoenoic precursors (*16:1n7 & 18:1n7*) and the ratio of total saturated fatty acids (*14:0, 16:0, 18:0, 20:0*) to mono-unsaturated fatty acids (*16:1n9, 16:1n7, a17:1n, 18:1n9, 18:1n7*) were used indicators of stress and other ecological conditions (Bossio and Scow 1998).

### 2.2.4 Statistical analysis

Principal component analysis (PCA) was performed on PLFA data using Mol% normalised spectra. Resultant PCs were analysed by one way analysis of variance (ANOVA). Relative abundance of individual microbial groups, and ratios between groups, were calculated and were subjected to statistical analysis using restricted maximum likelihood (REML) models, to identify the interactions of individual microbial groups with land use type, depth and combination of land use type and depth. Similar REML were also performed for PCs. REML was carried out using

'land use type' and 'depth' as fixed model, while 'core number' was used as random model. The statistical analyses were carried out using Genstat® 17th edition.

## 2.3 Results

In PCA of the PLFA profiles, PC1-4 collectively accounted for 57.1% of the variation, with PC2-4 each accounting for similar proportions of variation between 13-10% (Fig. 2.1). There were no significant interactions between site and depth for any of PC1-4 (Table 2.1) and hence main effects of treatments are considered directly.

### 2.3.1 Effects of land use types

Across all depths, there was highly significant discrimination between land-use types across all of PC1-4 (Table 2.1; Fig. 2.1a-b). The primary nature of the discriminations were that PC1 separated burnt from the other land-use types, and PC2 further separated forest from these. Young and mature oil palm were strongly separated by PC3, with the latter being distinct from all other types. Burnt treatments were intermediate to the others with respect to PC3. PC4 then separated oil palm from all others, which otherwise were clustered with respect to this component. The loadings for individual PLFAs associated with each of the PCs were generally dispersed, with no particular dominance of any single PLFA, and *20:0, 18:2n6* and *16:0* having very low loading scores (Fig. 2.1c-d). Notable exceptions were that 16:0 was particularly associated with the separation of forest from all other land-use types with respect to PC1 and 2 (Fig. 2.1a, c). Likewise, *14:0, 10me18, 17:1n* and *i17:0* were strongly associated with the distinctness of both oil palm land-use types with respect to PC4 (Fig. 2.1b, d).

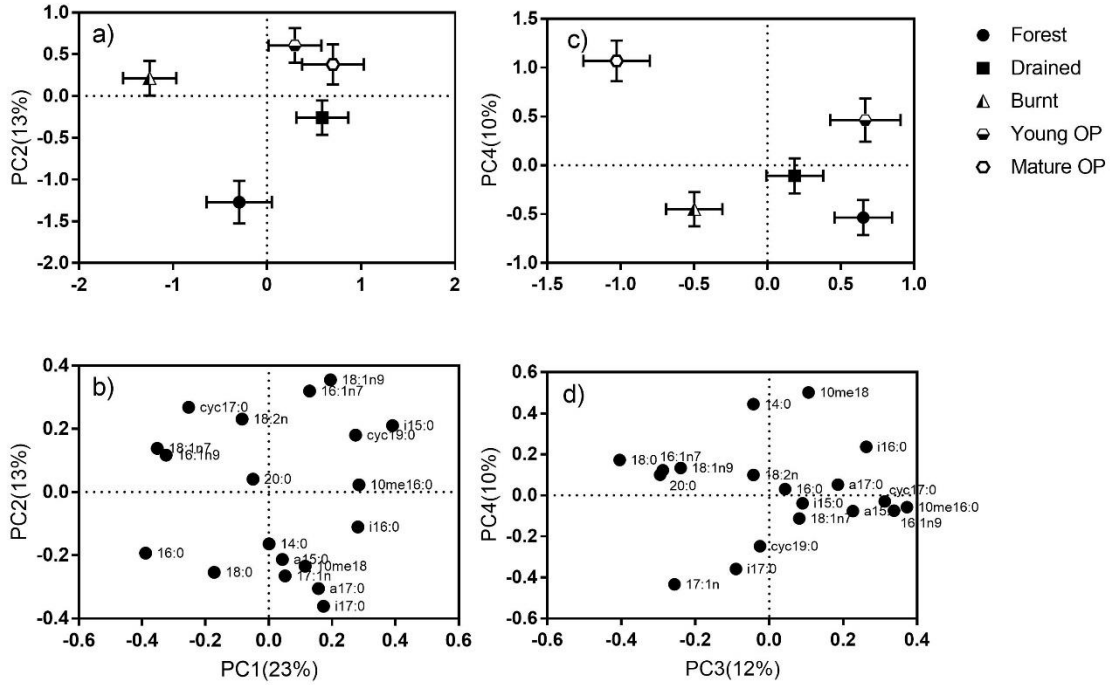



**Figure 2.1:** Effect of land-use type upon phenotypic structure of soil microbial communities determined by PLFA analysis, as shown by principal component (PC) analysis. (a, b) ordination of PC1 and 2 and associated loadings for individual PLFAs; (c, d) ordination of PC3 and PC4 and associated loadings, point labels denote PLFA type. For (a, c) points denote means (n varies, see text), whiskers denote standard errors.

### 2.3.2 Variations within land-use types along the depth

Across all treatments, PC1 and 3 (collectively accounting for 35% of the variation; Fig. 2.2), varied significantly with respect to depth (Table 2.1). Both PCs showed a consistent and coherent relationship to depth, resulting in a distinct trajectory in the biplot (Fig. 2.2a). There was a distinct shift in this trajectory at around 1 m depth, below which PC2 showed little change (Fig. 2.2a). The loadings for individual PLFAs associated with each of the PCs were generally dispersed, with no particular

dominance of any single PLFA and 18:2n and 14:0 having very low loading scores (Fig. 2.2b).

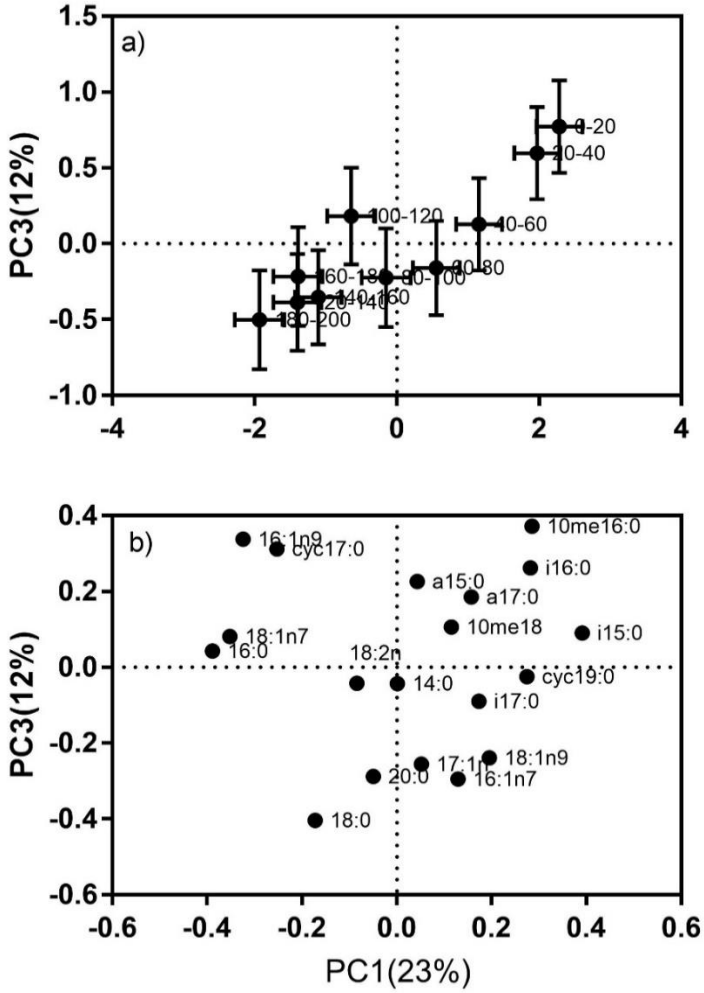



**Figure 2.2:** Relationship between soil depth and phenotypic structure of soil microbial communities determined by PLFA analysis, as shown by principal component (PC) analysis. (a) ordination PC1 and PC3; points denote means across all land-use types (n varies, see text), whiskers denote standard errors. Point labels denote depth of sample in cm from surface; (b) associated loadings for individual PLFAs, point labels denote PLFA type.

**Table 2.1:** Linear mixed model (REML) for aggregated PLFA data with respect to microbial groups, showing statistical significance of the effects of land-use type, depth and the interactions between land-use type and depth.

| REML | PC1 | | | PC2 | | | PC3 | | | PC4 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Df | F ratio | P | Df | F ratio | P | Df | F ratio | p | df | F ratio | p |
| Land-use type | 4, 165 | 14.64 | <0.001 | 4, 165 | 8.59 | <0.001 | 4, 165 | 12.57 | <0.001 | 4, 165 | 12.29 | <0.001 |
| Depth | 9, 165 | 23.62 | <0.001 | 9, 165 | 0.69 | 0.721 | 9, 165 | 2.87 | 0.004 | 9, 165 | 0.65 | 0.752 |
| Land-use type*Depth | 36, 165 | 0.77 | 0.817 | 36, 165 | 0.48 | 0.994 | 36, 165 | 0.92 | 0.608 | 36, 165 | 1.42 | 0.072 |

**Relative abundance and microbial community structure**

Only the proportion of Gram-negative bacteria, and fungi varied significantly between land-use type, with a significant interaction between type and depth for fungi (Table 2.2). The difference between the land uses for fungal relative abundance was mainly driven by forest, as here the fungal communities reduced with depth, while for all the other land-use types, the proportion of fungal markers was maintained across all depths (Fig. 2.3). Removing the forest land-use type from the model, fungal relative abundance did not significantly vary between other land-use types ($F_{(3,15.8)} = 1.31$, $p = 0.306$) or depths ($F_{(9,128.1)} = 1.32$, $p = 0.234$). Gram-negative bacteria was higher in the burnt than the rest of the land use types effecting the statistically significant difference in Gram-negative relative abundance between the land-use types. Removing the burnt site, the Gram-negative relative abundance did not significantly vary between sites ($F_{(3,15.5)}= 1.66$, $p=0.217$).

All the microbial groups showed significant changes with depth (Table 2.2). All of the land-use types were dominated by bacteria at all depths. The Gram-positive bacteria (including actinomycetes) was the dominant group at the surface peat for all land-use types except for the burnt peatland, where Gram-negative bacteria was the most dominant group at the surface by a large margin (Fig. 2.3). The proportion of Gram-positive bacteria decreased and that of non-specific fatty acids increased with depth across all land uses. This decrease in proportion of Gram-positive biomarkers made the Gram-negative bacterial group the dominant one with increasing depth, starting at 40-60 cm for forest and 20-40cm for the others (Fig. 2.3). The non-specific fatty acids increased with depth at all land-use types. The relative abundance of fungal markers

was less than 10% and remained at a similar lower level across all depths, irrespective of the land-use types. The proportions of actinomycetes were also less than 10% across all land-use types, and followed a similar trend of decrease with depth as other Gram-positive bacteria.

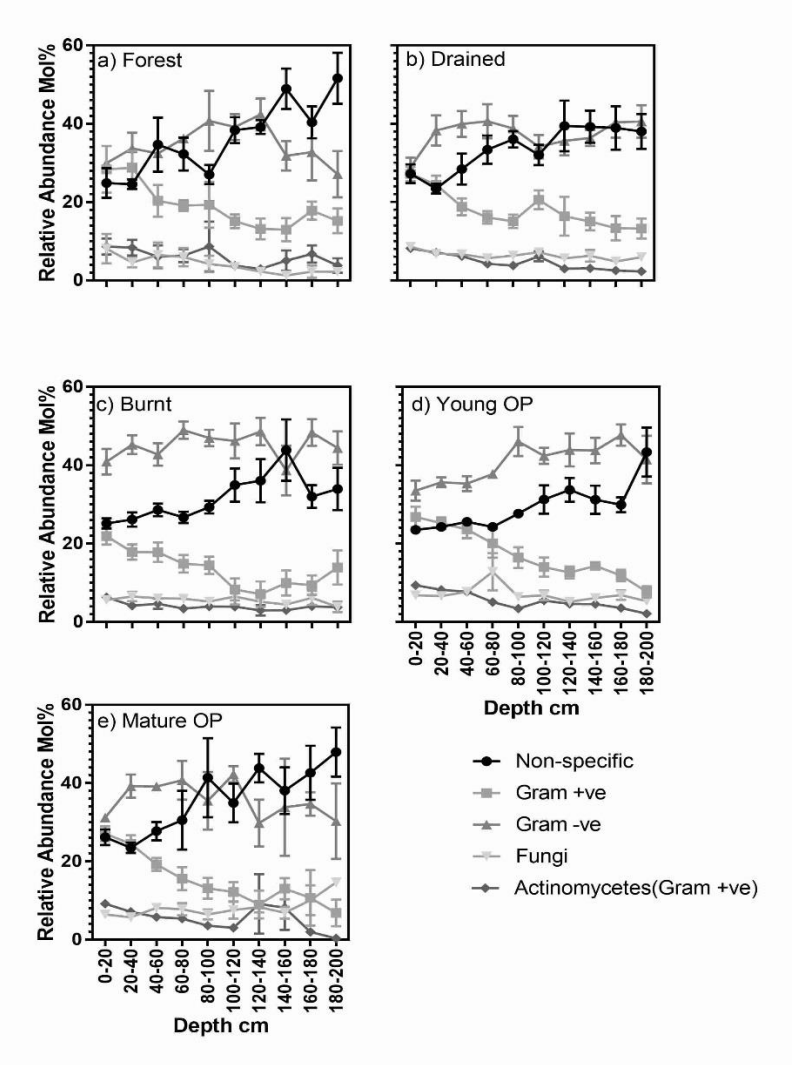



**Figure 2.3:** Relative abundance of different microbial groups as determined by PLFA analysis in relation to soil depth ranges under (a) forested peatland; (b) drained peatland; (c) burnt peatland; (d) young oil palm (OP) plantation; (e) mature oil palm (OP) plantation. Points denote means (n varies, see text), whiskers denote standard errors.

**Table 2.2**: Linear mixed model (REML) for prinicipal component scores, showing statistical significance of the effects of land-use type, depth and the interaction between land-use type and depth.

| | Gram-positive | | | Actinomycetes | | | Gram-negative | | | Fungi | | | Non-specific | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| REML | Df | F ratio | P | Df | F ratio | P | Df | F ratio | P | Df | F ratio | p | df | F ratio | p |
| Land-use type | 4, 19.6 | 1.61 | 0.211 | 4, 18 | 1.43 | 0.265 | 4, 19.6 | 4.11 | 0.014 | 4, 19.8 | 3.87 | 0.018 | 4, 17.4 | 2.65 | 0.069 |
| Depth | 9, 146.6 | 19 | <0.001 | 9, 146.2 | 7.47 | <0.001 | 9, 146.8 | 3.31 | 0.001 | 9, 147.5 | 2.29 | 0.02 | 9, 145.2 | 13.26 | <0.001 |
| Land-use type*Depth | 36, 147 | 1.23 | 0.198 | 36, 146.6 | 1.23 | 0.194 | 36, 147.1 | 0.168 | 0.168 | 36, 147.9 | 1.65 | 0.021 | 36, 145.7 | 0.97 | 0.531 |

In general, the ratio between fungal and bacterial relative abundance (F:B) did not vary significantly with respect to depth in drained, burnt or cleared sites, notwithstanding there was a distinct peak in the ratio at 60-80 cm for cleared sites (Fig. 2.4a). There was a trend for a decline in the ratio across all depths in forest soils (Fig. 2.4), driven by a general reduction in fungal relative abundance with depth (Fig. 2.3). There was a marked increase in fungal to bacterial ratio in oil palm soils beyond 140 cm depth (Fig. 2.4a).

The ratio of Gram-negative to Gram-positive bacteria (G-:G+) generally increased with depth for all land-use types, but with different patterns of behaviour according to land-use type. Burnt sites involved a rapid increase in ratio up to 140 cm and decline thereafter, with a similar trend for forest although more attenuated (Fig. 2.4b). Cleared and oil palm sites showed a distinct increase with depth commencing at 160 cm (Fig. 2.4b).

PCA using relative abundance of microbial groups strongly reinstated the variations between sites observed via such analysis of individual fatty acids (Appendix 2). Here, PC1 and PC2 together accounted for 73% variations. PC1 separated out burnt from the other of the land-use types, while PC2 separated forest from the other land use types. Burnt peatland was separated farthest away from forest by both the PCs, the changes with depth and a shift in pattern of change after a metre depth was also apparent in microbial group level PCs (Appendix 2).

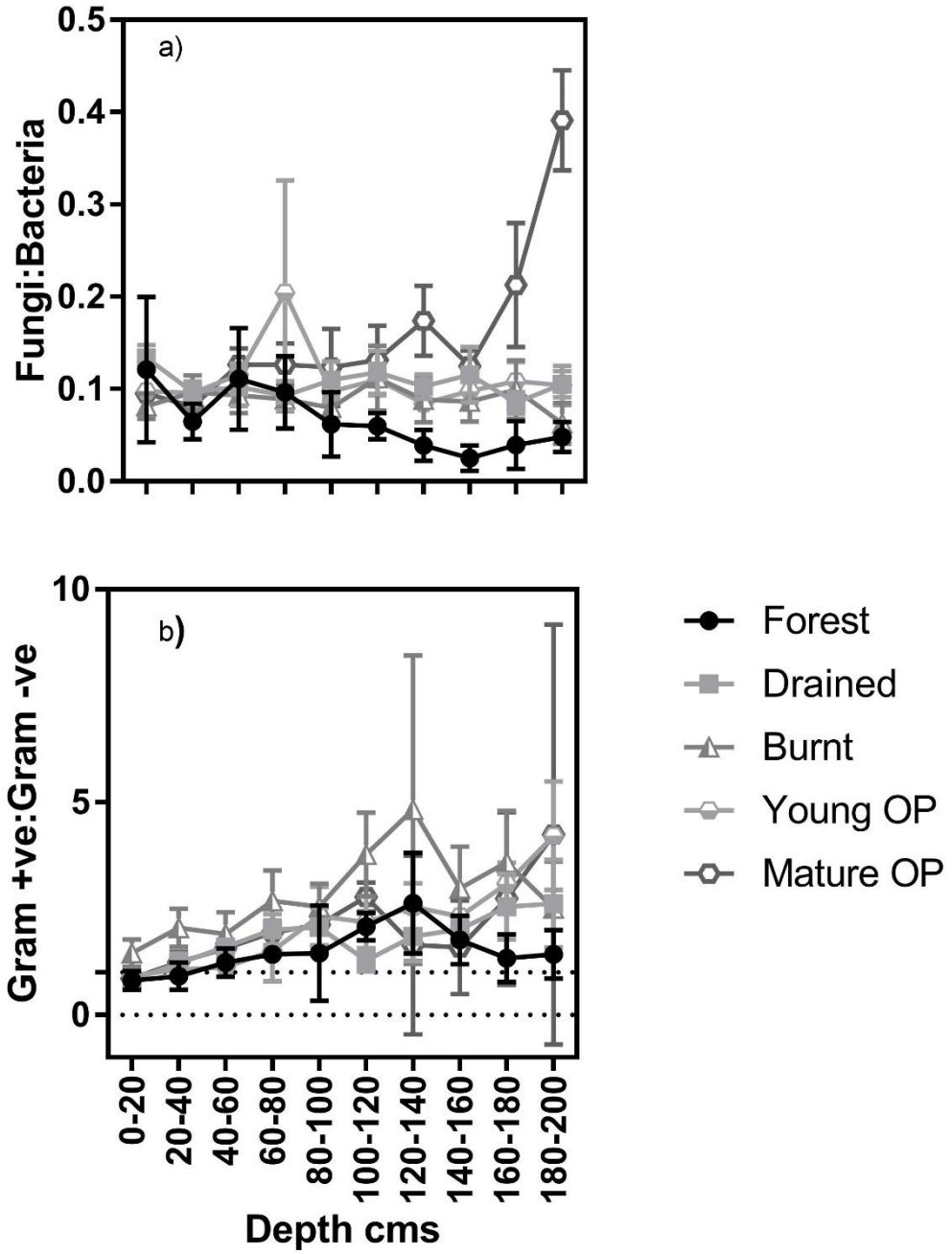



**Figure 2.4:** Ratios of relative abundance of microbial groups across soil depth range under different land-use types (OP = oil palm). (a) ratio between the relative abundance of fungi and bacteria; (b) ratio between the relative abundance of Gram-negative bacteria and Gram-positive bacteria. Points denote means (n varies, see text), whiskers denote standard errors.

### 2.3.3 Other ecological indicators

The ratio between cyclopropane fatty acids and their precursors (cyc:pre) decreased with depth irrespective of the land use type ($F_{9,144}$ =15.75, $p<0.001$), while the difference among land-use types and interactions between land-use type and depth were not significant (Fig. 2.5). Burnt land-use type had the lowest ratio in the surface and also least changes with depth. Overall, the ratio decreased with depth for all land-use types.

The ratio of total saturated fatty acids to mono-unsaturated fatty acids (sat:mono) varied with depth ($F_{9,140}$=1.98, $p<0.05$) and land-use types ($F_{4, 15}$= 3.76, $p<0.005$), but the interactions between land-use type and depth was insignificant. Forest, drained and mature oil palm had identical ratio at the surface, while burnt and young oil palm had identical, lower ratio at the surface layer. The ratio increased for forest and it either decreased or remained the same with depth for other land-use types. The oil palm site had similar higher ratios at and after 1 m depth. Burnt land-use type had the lowest ratios across all depth ranges except for 140-160 cm.

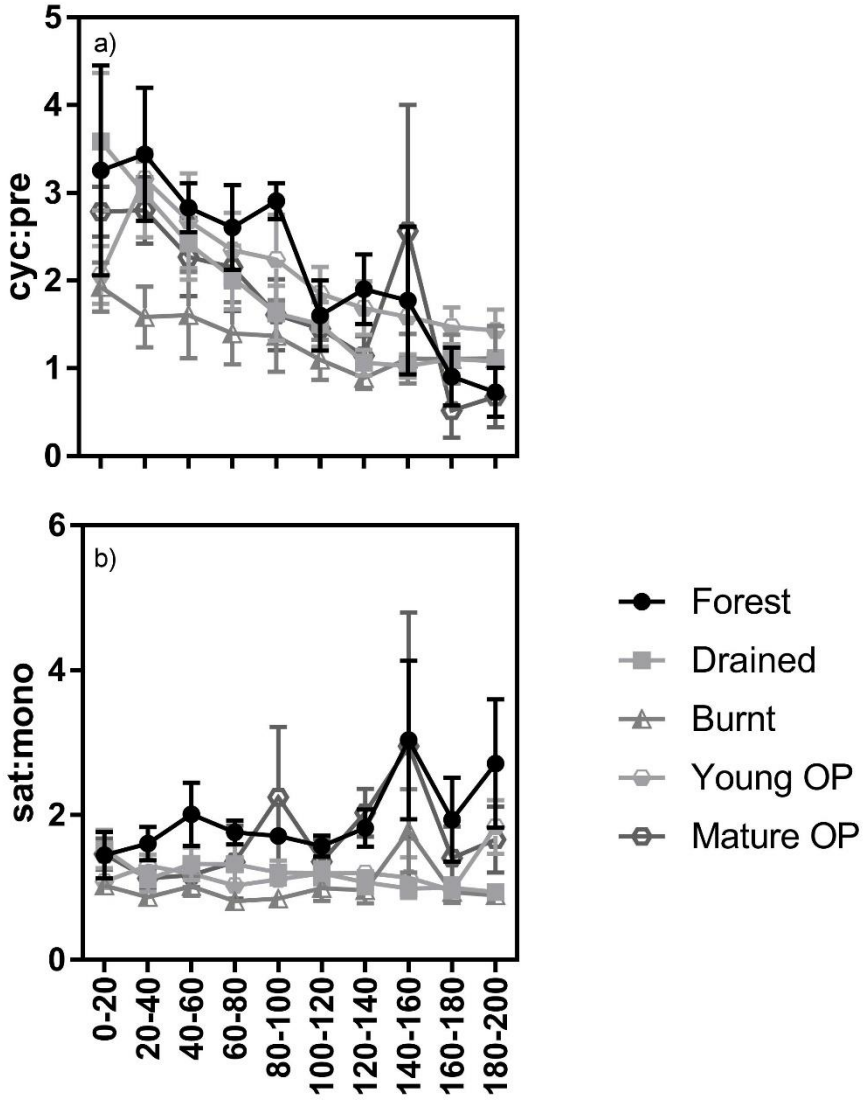



**Figure 2.5:** PLFA ratios used as stress indicators as determined by PLFA analysis in relation to soil depth ranges under different land-use types (OP = oil palm). (a) ratio between cyclopropance fatty acids and their precursors; (b) ratio between saturated fatty acids and mono-unsaturated fatty acids. See text for further details regarding derivation and interpretation. Points denote means (n varies, see text), whiskers denote standard errors.

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## 2.4 Discussion

### 2.4.1 Microbes at different land-use types

This research compared sites at different stages of oil palm conversion from forest, including draining of forest, followed by clearing, or burning in some cases, and establishing oil palm plantations. These five types of land uses along with other managed production forest, cover most of South East Asian Peatlands (Miettinen *et al.*, 2012, Miettinen *et al.*, 2016). Given the environmental changes that accompany these land uses and changes in physico-chemical properties at different stages of conversion (Tonks *et al.*, 2017), it is not surprising to see that they have different microbial communities from each other, confirming the first hypothesis. As expected, forest had different microbial communities, while the drained peatlands had microbial communities that was similar to those in oil palm plantations. The young oil palm plantation site in this study is a site that is recently cleared of forest without the use of fire. It is not uncommon to use fire to clear sites for oil palm plantation or use/claim fire affected site for plantations, even though legislation in both Indonesia and Malaysia prohibits it (Varkkey, 2013, 2016). It is highly likely that oil palm plantations established on fire affected site would have completely different microbial community structure to that of oil palm plantation in this study, as our results have shown that oil palm plantations and burning changes microbial communities in different ways.

The burnt site was distinct from other sites because of high relative abundance of primarily Gram-negative biomarkers namely *18:1n7*, *16:1n9* and *cyc17:0*. The mono unsaturated fatty acids such as *18:1n7* and *16:1n9* were found to decrease with flooding and increase with increased C availability (Bossio and Scow, 1998). This explains the dominance of Gram-negative bacteria in burnt peatland, as they have increased C availability and experience less flooding (Turetsky *et al.*, 2015). The forest

site was separated out by both the principal components, showing increased abundance of most of the non-specific and Gram-positive biomarkers. This is also explained by Bossio and Scow (1998), showing higher abundance of branched fatty acids, which are generally Gram-positive biomarkers, with increased flooding. The forest sites had relatively higher water table and were more prone to flooding (Tonks *et al.*, 2017). A study by Blaško *et al.* (2015) showed that newer ecosystems tend to favour Gram-negative bacteria over Gram-positive bacteria. This remains true with forest being the oldest ecosystem in this study which is separated out from other sites mostly by Gram-positive fatty acids. It is also in agreement with Jaatinen *et al.* (2008), who found that prolonged decrease in water table depth in the top layers would be beneficial to fungal and Gram-negative bacterial communities while being detrimental to Gram-positive communities in temperate peatlands.

These results differ from the findings of Krashevska *et al.* (2015) that show increased abundance of *i17:0* and decrease in Gram-negative bacteria with rainforest conversion in mineral soil. Previous studies in mineral soil surface also reported that converted systems had higher proportion of Gram-negative bacteria, than the forest (Krashevska *et al.*, 2015, Lee-Cruz *et al.*, 2013), but our study in peatlands show that the ratio between Gram-negative and Gram-positive bacteria was highest for the burnt peatlands, while all the other managed peatlands and forest had identical ratios, on the surface. Thereby leading to an inference that microbial communities in tropical peat swamp forests and their response to disturbances are different from that of tropical lowland rainforests in mineral soil.

### 2.4.2 Consistent and coherent change with depth irrespective of the land-use

The clear grouping and separation of fatty acids that belong to a particular microbial group in loadings and clear changes in related principal component scores with depth,

show that there is change in microbial communities as a whole, rather than changes in few random fatty acids with depth. The results clearly confirm the second hypothesis. The observed change in community structure with depth, may be influenced by several factors such as water table level, availability of oxygen and other nutrients, quality of C, and other energy constraints, along the peat depth. This change with depth in forest site was anticipated, as the surface microbial communities in North Selangor peat forest were dependent on C leached from leaf litter (Yule, 2010), and the microbial communities were expected to change with changes in C source and quality with depth (Artz *et al.*, 2007). However, similar change with depth were also observed in other sites, including plantation and burnt sites which lack leaf litter layer in the surface, indicating that there are more factors than altered litter input driving this change in community structure.

Here we characterised microbial community phenotypes, which essentially integrate the genotypic components of the community with their environmental context. The generally observed pattern in genetic structure of vertical stratification of microbes in northern peatlands, is for greatest diversity and abundance at intermediate depths, where co-existence of oxic and anoxic layers support wide range of microbial communities, and reduction in diversity thereafter with greater depth (Andersen *et al.*, 2013). It is not known whether tropical peatlands follow similar pattern and was beyond the scope of this study. However, 16S rRNA sequencing by Jackson *et al.* (2009) found that the microbial diversity decreased with depth in North Selangor peatlands, suggesting vertical stratification at least up to 50 cm depth in tropical peatlands. The decrease in this diversity may be due to loss of some Gram-positive bacteria with depth, observed in this study.

Increase in non-specific fatty acids, with decrease in Gram-positive bacteria influenced the change with depth below the surface, which also imply the possibility of larger proportion of unknown, novel microbes with increasing depth. The fungal relative abundances were low throughout the depth, contrary to the finding in northern hemisphere where fungi dominate the surface aerobic layers in natural forest ecosystems (Bardgett, 2005, Winsborough and Basiliko, 2010). The Gram-negative bacteria either remained unchanged or increased slightly with depth, this is likely be due to the increase in C:N ratio with depth, which favours Gram-negative bacteria (Bossio and Scow, 1998, Liu *et al.*, 2015, Tonks *et al.*, 2017). A sudden spike in F:B and G-:G+ ratios at the deepest layers of mature oil palm plantations may be due to the reaching of mineral layer at the highest depths, as the depth of the peat cores in mature oil palm were very shallow compared to the other land use types (Tonks *et al.*, 2017).

A distinct shift in community structure after a metre depth in all land use types, may be associated with water table depths at the studied sites. However, water table levels varied between different land use types during the time of sampling and they were not at a metre depth at any of the studied sites (Tonks *et al.*, 2017), suggesting the shift in the community structure after a metre depth was not directly related to the water table. Reasons for the shift in microbial communities after about a metre depth are therefore unclear. However, it is possible that the old peat deposits below a metre depth at the catotelm (Morris *et al*., 2011) are dense and recalcitrant, causing changes in microbial communities through changes in C quality and blocking water movement. The other plausible explanation for this shift is changes in microbial community structure due to the occurrence of methanogenic communities at 1 metre depth. Jackson *et al.* (2009) did not find methanogens in North Selangor peat swamp forest, but their research did

not cover the layers below 50 cm. However, methanogens are found to be abundant at 30-40 cm below the water table (Galand *et al.,* 2002, Lin *et al.,* 2014), which is at about 1 metre after the long term water table draw down levels in most of our studied sites (Tonks *et al.,* 2017). Though PLFA analyses does not detect methanogenic archaeal communities, due to their ether linked phospholipid cell membranes (Gattinger *et al.,* 2003), rather than ester linked fatty acids found in other bacteria and fungi, it is possible that occurrence of methanogenic archaea at about a metre depth have altered the rest of the microbial community structure. The water table level at all the studied land-use types in North Selangor were known to fluctuate to 60 cm below surface (Tonks *et al.,* 2017), which supports this thesis.

### 2.4.3 Bacterial dominance in community structure

The generally observed pattern in previous studies at different non-peat ecosystems are for bacterial dominance in heavily disturbed or intensive agricultural sites, while the sites with lesser disturbance and higher input of complex organic matters in natural habitats tend to favour fungal dominance (Bardgett, 2005). This did not hold true for the Malaysian tropical peatlands, as all of the studied land-uses were clearly dominated by bacterial groups across all depths.

All the surface layers, except for the burnt land-use type were dominated by Gram-positive bacteria, but soon were superseded by Gram-negative bacteria, with increasing depth. Jackson et al. (2009) also found similar results with Acidobacteria, a phylum belonging to Gram-negative bacterial group, being the single biggest group from surface to 50 cm, with considerable increase in their proportion at 50cms, the deepest layer in their study. Tonks et al. (2017) found that all the studied peat sites had higher nitrogen content in the surface, which might favour Gram-positive bacteria. This hypothesis is in agreement with study by Liu *et al.* (2012), who found that Gram-

negative PLFA abundance decreased with the addition of nitrogen irrespective of the land use type in humid tropical forests in Southern China. Similar trend was also observed in Hawaiian forests (Balser, 2001).

The dominance of Gram-positive bacteria over Gram-negative bacteria on forest and plantation peat surface layers, is in contradiction with what was observed in Panamanian peatlands, where Acidobacteria and Proteobacteria, both Gram-negatives, together constituted 68-80% of bacterial communities on surface of peat forest (Troxler *et al.*, 2012). The extracellular enzyme activities were found to be highest at the peat surface and decrease with depth at the North Selangor peat swamp forest (Jackson *et al.*, 2009), which corresponds with Gram-positive group's relative abundance. This likely suggests that Gram-positive is the most dominant group involved in extracellular enzyme activity and C mineralization in this tropical peatland. Actinomycetes were found to be more active at higher pH (Charousová *et al.*, 2017). Higher pH is also known to support higher bacterial diversity in oil palm plantations on mineral soil (Tripathi *et al.*, 2012, Wood *et al.*, 2017). Even though the pH were higher in the plantations than at the forest, it was still under pH 4 (Tonks *et al.*, 2017) and did not affect the relative abundance of actinomycetes. This slight increase in pH with establishment of plantations in peatlands, may not be high enough to support increased bacterial diversity, as seen on plantations in mineral soil. Though this study does not indicate bacterial diversity, the ratio of fungi to bacteria were generally higher in mature oil palm plantations (Fig 5), showing that the slight increase in pH did not favour the bacterial community in peatlands. Actinomycetes, despite being Gram-positive bacteria, are more similar to fungi in structure and functions, while being much smaller in size to that of fungi (Barka *et al.*, 2016). It is interesting to see that they exhibit similar trends throughout the depth at different land uses. The

results are in agreement with Bossio and Scow's (1998) results and conclusion that fungi are not important in submerged systems.

### 2.4.4 Higher nutritional stress under forest?

*Cyc 17:0* and *cyc 19:0* are formed by trans-methylation of *16:1n7* and *18:1n7* (Kaur *et al.*, 2005). This trans-methylation was found to be a modification in cell membrane to minimize cellular degradation and membrane losses at the times of stressful conditions (Kaur *et al.*, 2005).The higher ratio between *cyc17:0+cyc19:0* and their precursors *16:1n7+18:1n7* is used as a stress indicator for starvation and nutritional stress, due to low C, high acidity and low oxygen (Bossio and Scow, 1998, Kieft *et al.*, 1994, Liu *et al.*, 2015, Wilkinson *et al.*, 2002). The ratio was found to decrease with high substrate availability (Bossio and Scow, 1998). In our study, this ratio was highest at the forest and lowest in burnt site on most depth ranges. The ratio also decreased with depth, implying decrease in stress level with depth. However, this might not be an indicator of stress in these tropical peat habitats but might just indicate increase in anaerobic bacteria, as anaerobes contain *cyc17:0* and *cyc19:0*, and aerobic bacteria contains *16:1n7* and *18:1n7* (Vestal and White, 1989). The increased ratio of anaerobes, in forest to the burnt site, and with depth at all sites, is more plausible explanation of the ratio rather than an indication of stress. However, tropical peatlands are naturally low oxygen, low pH, nutrient poor microbial habitats and those conditions may be stressful to microbial communities, explaining the higher ratio in the forest. Additionally, Aw *et al.* (2016) identified a novel anaerobic bacterial strain with potential antimicrobial property in NSPSF, adding to the stress in already nutrient poor environment. Conversely, burning in peatlands were found to increase C availability (Turetsky *et al.*, 2015), which results in increase of monounsaturated fatty

acids (Borga *et al.*, 1994) resulting in reduced cyclopropane/monounsaturated fatty acid ratios in burnt sites.

Several studies suggest that an increase in the ratio of saturated fatty acids to mono-unsaturated fatty acids is an indicator for nutritional stress (Bossio and Scow, 1998, Kieft *et al.*, 1994, Moore-Kucera and Dick, 2008, Zelles *et al.*, 1992). Similar to the ratio between cyclopropane and monounsaturated fatty acids, the ratio between saturated and mono-unsaturated fatty acids was highest at the forest and lowest at the burnt site at most depth ranges. The ratio increased with depth for forest and mature oil palm while rest of the land-use types maintained a similar ratio throughout the depth. Bossio and Scow (1998) found that this ratio increased with flooding. The higher ratio in forest and increase in ratio with depth for forest and mature oil palm sites may be due to flooding.

Both the ratios then suggest the forest sites to be nutritionally stressed. It is known that burning makes more nutrients available in peatlands (Turetsky *et al.*, 2015) and the natural tropical peatland habitats are naturally nutrient poor, explaining the results (Sjögersten *et al.*, 2011). The combination of all the results show that burning impacts the natural peatland microbial communities the most, among the studied anthropogenic disturbances.

## 2.5 Conclusion

This study has demonstrated that the phenotypic structure of microbial communities strongly respond to the anthropogenic disturbances and vary with peat depth irrespective of the land-use. The microbial communities were distinct at each land use type. Burnt peatland and oil palm plantations were found to differently affect the soil microbial communities. Though the fatty acid compositions varied the most from

forest in both mature oil palm plantations and burnt peatlands, changes in overall phenotypic structure of microbial communities were minimal in oil palm plantation, while the burnt peatlands had the most different microbial phenotypic structure to the forest. Fatty acid ratios used as ecological indicators also point towards burnt peatland to have the most different microbial communities to that of peat forest. In addition to higher emissions and loss of old C with burning (Turetsky *et al.*, 2015), these findings demonstrate that the burning of peatlands also have detrimental effect on natural peat microbial communities. Anthropogenic fire is considered a major threat to tropical peatlands and fire affected peatlands in South East Asia are increasing in alarming rate (Dohong *et al.*, 2017, Page and Hooijer, 2016), yet their effect on tropical peat microbial communities are virtually unknown. Future research should focus on understanding species level alterations in these microbial communities with land use change and fire, and environmental controls on these microbial communities. Understanding these peat microbial processes and incorporating them into wider peat management plan is critical for improving peat restoration efforts in South East Asia.

# Chapter 3

## 3. Soil microbial community structure and GHG emissions under forest and different oil palm cropping systems

### 3.1 Introduction

Tropical peatlands are globally important, yet are endangered ecosystems with high C storage capability and endemic biodiversity (Dohong *et al.*, 2017, Jackson *et al.*, 2009, Yule, 2010). Acidic, nutrient-poor and water-logged conditions in natural peatlands inhibit aerobic microbial decomposition, resulting in the accumulation of partially-decomposed plant materials arising from their inherently high primary production (Miettinen *et al.*, 2012, Parish *et al.*, 2008, Sjögersten *et al.*, 2011). Even though natural peatlands are known to have high methane emissions, higher C storage means that they act as important long-term C sinks (Page *et al.*, 2011b, Schrier-Uijl *et al.*, 2013). About 56% of all tropical peatlands are in South East Asia (SEA), with greatest cover in Indonesia and Malaysia, mostly on low-lying coastal plains (Hergoualc'h and Verchot, 2014, Lo and Parish, 2013, Rashid *et al.*, 2013, Xu *et al.*, 2018, Yule, 2010). SEA peatlands store about 69 Gt of C (Dohong *et al.*, 2017, Miettinen and Liew, 2010) and absorb at least 2.6 tonnes of $CO_2$ per hectare a year (Norwana *et al.*, 2011).

Anthropogenic disturbances in SEA peatlands in the last few decades increasingly affect the balance of the environmental, biological and climatic conditions that maintain peatlands, resulting in peatland degradation and C loss (Couwenberg *et al.*, 2010). The SEA region has experienced relatively high deforestation rates in the 21$^{st}$ century, with Malaysia having the greatest deforestation rate of all the countries in the world (Hansen *et al.*, 2013) and now undisturbed peat swamp forest are nearly extinct

in Peninsular Malaysia (Miettinen *et al.*, 2016). It is estimated that 25% of all forest degradation in SEA occurs in peatlands (Lo and Parish, 2013). Most of these anthropogenic disturbances were associated with agricultural expansion, especially oil palm plantations. Oil Palm plantations currently cover 7.6 million hectare and 4.6 million hectare in Indonesia and Malaysia, respectively (Dislich *et al.*, 2017) and are only expected to increase (Miettinen *et al.*, 2016). Oil palm expansion is a major threat to SEA peatlands, with most of the planned expansion is projected on peatlands (Dohong *et al.*, 2017, Miettinen *et al.*, 2012). Around 75% of all peat forest loss in Peninsular Malaysia, Sumatra and Borneo between 2007 and 2015 were due to oil palm expansion (Miettinen *et al.*, 2016). The establishment of oil palm in peatlands requires draining and clearing of vegetation, severely altering the peatlands' physical and environmental conditions (Luskin and Potts, 2011). Degradation of these peatlands would not only emit large amounts of C, but also maintain high methane emissions through drainage ditches (Schrier-Uijl *et al.*, 2011). Increased use of fertilizers in oil palm plantations were known to increase both $N_2O$ and $CO_2$ emissions (Comeau *et al.*, 2016, Mohd Kusin *et al.*, 2015). Thus, the expansion of oil palm plantations into peatlands would likely contribute to climate change, with increased emissions of three potent greenhouse gases.

Though industrial plantations are the leading land-use type for oil palm cultivation (Azhar *et al.*, 2011), about 3.5 million ha (22.4%) of peatlands in peninsular Malaysia, Sumatra and Borneo are small-holders plantations, and make up half of the managed peatlands in Peninsular Malaysia (Miettinen *et al.*, 2016). Unlike industrial plantations, smallholder farmers follow a diverse range of management practices and cropping systems such as intercropping, depending on personal convenience and their local needs. The smallholders' plantations are less productive and lack modern

infrastructure (Azhar *et al.*, 2011), but have greater landscape heterogeneity (Azhar *et al.*, 2015). However, small-scale multiple cropping systems are prone to more frequent human disturbances, with use of pesticides, fertilizers, and extra labour required for cultivation and management of different crops (Azhar *et al.*, 2014). The multiple cropping systems could influence the microbial communities in broadly two ways: (1) Above- and below-ground linkages through rhizosphere microbial communities, root exudates and through quality and quantity of vegetative C sources added to the soil; (2) changes in microhabitat and microclimatic conditions due to the heterogeneity of aboveground plant structure.

Microbial communities are an important biological factor for the formation and maintenance of peatlands and peat functions, by directly controlling C turnover and nutrient mineralization supporting high primary production (Andersen *et al.*, 2013). Most of the C utilised by bacterial groups in tropical peatlands are obtained via dissolved organic C leaching from the surface leaf-litter layers (Yule, 2010). Several studies have shown that oil palm plantations lack leaf litter and a humus layer (Bruhl and Eltz, 2010, Cusack, 2011, Faruk *et al.*, 2013, Fayle *et al.*, 2010), and lack the leaf-litter heterogeneity that forests provide through high plant biodiversity. This could have detrimental effects on peat microbial communities, impacting nutrient cycling and biodiversity dependent on the microbial C food chain.

The loss of organic matter from soils can change the path of global climate change, as soils contain three times more C than the atmosphere (Davidson *et al.*, 2000). The net annual C emission by natural microbial processes in soil is estimated to be more than ten times larger than the annual global emissions from fossil fuel burning and the cement industry (Oertel *et al.*, 2016). The microbial influence of C cycling in the tropical forests is particularly important (Smith et al, 2014; Batjes and Sombroek,

1997), as the tropical forests are biologically active throughout the year (Guo and Gifford, 2002). Therefore, even a slight increase in the microbial activities in C rich tropical peat may result in a rapid increase in the rate of $CO_2$ emission and C loss, if the lost C is not replaced by primary production.

Given the importance of microbial communities in tropical peatlands and the anthropogenic pressure they are exposed to, there is an increasing need to understand how land-use change in tropical peatlands affect these microbial communities and their consequent effects on biogeochemical cycles. Microbial communities has been found to vary among different stages of conversion of forest to oil palm monoculture and also vary with depth within the conversion stages (Chapter 2). However, the impact of different oil palm cropping systems in peatlands remain poorly understood.

Most of the published studies on GHG emissions have been limited to peat forest and industrial oil palm monoculture, ignoring the different kinds of management and cropping systems increasingly used by oil palm smallholders throughout SEA. Despite the importance of microbes in peatlands and their importance in GHG emissions, they are largely unknown in tropical peatlands, let alone their response to different oil palm cropping systems, seasonal variations and their relationship with GHG emissions. The aim of this study was therefore to determine the changes in peat properties and the responses of peat microbial community structure and functions, specifically GHG emissions and C storage in different oil palm cropping systems.

We hypothesised that peat properties are severely altered by conversion of forest to oil palm plantations, and predict that peat properties such as organic matter content, moisture content, bulk density, pH and temperature are progressively affected more from 1$^{st}$ to 2$^{nd}$ generation cropping systems, while the intercropping systems are

anticipated to ameliorate such damage to peat properties. We postulated the damage to peat properties in turn influences soil microbial communities and GHG emissions, which are additionally impacted by the difference in above ground vegetation in different cropping systems. We hypothesised seasonal changes in rainfall also impact soil microbial communities, subsequently effecting changes in GHG emissions, given the short response time of microbial communities to environmental changes.

## 3.2 Material and Methods

### 3.2.1 Study sites

The study sites are located in the state of Selangor in Peninsular Malaysia. The North Selangor peatlands are the largest peatlands in the state of Selangor with an area of 81,304 ha comprising 4 protected forest reserves namely Raja Musa forest reserve, Sungai Karang forest reserve, Sungai Dusun forest/wildlife reserve and a part of Bukit Belata forest reserve extensions (Selangor State Forestry Department, 2014). The protected forest reserves are surrounded by industrial oil palm plantations and villages dependent on smallholding agriculture, which have resulted in diverse land uses of peatlands in and around the reserves.

The forest site is located in the northern edge of Sungai Karang forest reserve. All the other study sites are small scale agricultural plantations in Kampung Raja Musa village, located at the southern edge of Raja Musa forest reserve. The sites are described below in order of age of conversion from forest, starting with secondary forest. The peat depth at the sites ranged from 1.3 to 6 m. The mean annual rainfall in NSPSF varies from 1359 mm to 2480 mm, peaking at October- November and driest at May- September (Global Environmental Centre, 2014). In the sampling periods on 2016-2017, it rained several times a week during wet season measurements

(November 2016 – January 2017), while it had rained only twice during the whole sampling period for the dry season (July 2017). All the agricultural sites in Kampung Raja Musa village was about 2 ha. The management practices were different with each individual smallholder and specific management practices in the different study sites were unkown. However, addition of chicken waste is a common practice followed as a fertilizer addition to the plantations.

#### *3.2.1.1 Forest site*

The forest site (3°42'03.5"N 101°10'58.9"E) is protected and managed by Kelang forestry office. The site is bordered with oil palm plantations. The forest received protection status from the state at 1990 (Tonks, 2017) and it was subjected to selective logging before that (Jackson *et al.*, 2009). The site has not been logged for at least last 40 years, but still contains old channels for timber extraction, many of which are blocked today. The forest vegetation is composed of *Macaranga pruinosa* (Miq.) Müll.Arg, *Campnosperma coriaceum* (Jack) Hallier f., *Blumeodendron tokbrai* (Blume) Kurz, *Shorea platycarpa* F.Heim, *Parartocarpus venenosus* Becc., *Ixora grandiflora* Ker Gawl, *Pternandra galeata* Ridl., *Stenoclaena palustris* (Burm. f.) Bedd., *Asplenium longissimum* Baker, *Nephrolepsis biserrata* (Sw.) Schott, *Crytostachys sp., Cyperus rotundus* L., and *Pandanus atrocarpus* Griff. (Yule and Gomez, 2009). The forest surface was covered with leaf litter.

There was no observable change in the physical environment above ground such as vegetation, water level and leaf litter, at this site between wet season and dry season sampling periods.

#### *3.2.1.2 First generation oil palm mono-cropping site*

The oil palm site (3°25'25.8"N 101°20'12.9"E) was located on a border right next to Raja Musa natural reserve on the southern tip of the North Selangor peatlands. The oil palm plantations were about 15 years old. The surface was uneven flat with numerous peat heaps and hollows. The site borders the oil palm plantations which were left for forest regeneration inside Raja Musa protected forest reserve. A drainage ditch, running in the middle, divides the site into two halves. The site had a sizeable cover of understorey vegetation, predominantly ferns. There were abundant decaying dead wood of non-oil palm plants on the site. There were some dead cut stems of other plants and trees of previous land use, visibly protruding on the surface.

There was no observable change in the physical environment such as vegetation, surface flooding etc., at this site between wet season and dry season sampling periods. This site was denoted as '1$^{st}$ generation oil palm'.

#### *3.2.1.3 Cleared site*

The cleared site (3°25'23.9"N 101°20'09.0"E) was located in Kampung Raja Musa village, adjacent to the oil palm plantations study site. The site was recently cleared of 1$^{st}$ generation oil palm plantations. Chemical fertilisers along with chicken wastes were used on the previous oil palm plantations. The dead oil palm wood and branches were left in rows along the site. Saplings of lemon were planted throughout the site after the first week of sampling. No chemical fertilisers were applied to the lemon plantations during the sampling period. There were a few other saplings such as guava, pineapple etc, growing at the site, which were not intentionally planted. The site was surrounded by 1$^{st}$ generation oil palm plantations on all sides. The surface is relatively flat, with four ditches draining water from the site.

In between the wet and dry season measurements, most of the lemon plants had died off and oil palm saplings were planted about a month before the dry season measurements. Some of the remaining lemon and other plants were not cleared from the site. Most of the surface area were covered by grass, unlike the previous wet season when the surface was completely clear. The site was still referred as ‘cleared’ for both wet and dry season results.

#### *3.2.1.4 Second generation oil palm and yam intercropping site*

The oil palm and yam intercropping site (3°25'22.7"N 101°18'46.7"E) was a site containing one year-old oil palms planted in rows with ample space in between, where four to six rows of yam were planted. There was a cleared open space between each paired yam and oil palm rows. The surface of the site was relatively dry and flat with numerous dead oil palm roots of the previous generation of plantation. The edge of the site were cropped with pineapple plants on the available spaces in between yam and oil palm rows.

During the dry season, yam crops were harvested and a new pineapple crop were planted in the open areas. There were smaller yam saplings in place of the older harvested yam. The site is denoted as ‘yam intercropping’ for both wet and dry season results.

#### *3.2.1.5 Second generation oil palm and pineapple intercropping site*

The oil palm and pineapple intercropping site (3°25'20.6"N 101°19'56.6"E) in Kampung Raja Musa consisted of *circa* one year-old oil palm plants in rows with pineapple planted densely between the oil palm rows. There were two drainage ditches in the border on the either side of the site, but none within the site. There were stagnant

water on most part of the site during the wet season measurements. Some open regions without any stagnant water were covered with grass.

During dry season measurements, the pineapple plants were fully grown and covered any remaining open spaces between pineapple rows and there was no stagnant water at the surface. The site is denoted as 'pineapple intercropping' in the results.

#### *3.2.1.6 Second generation oil palm mono-cropping site*

This young oil palm site (3°24'51.3"N 101°19'42.7"E) was located in Kampung Raja Musa village. The plantation itself is second generations with the age of 3-5 years old. The first generation oil palm trees were killed off chemically and were still standing on the site. Most of the site surface was covered with dead, dried and brown grass. During the wet season, there were green algal growths on the surface under the shades of young oil palm trees.

During the dry season measurements, most of the grass on surface were cleared and there were no algal growth on the surface. This site is denoted as '2$^{nd}$ generation oil palm'

### 3.2.2 Sampling Strategy

Sampling was carried out during both the wet and dry seasons. The wet season sampling was carried out during November 2016 to January 2017 and the dry season sampling was done during July 2017. Each site was visited three times during each season. At each time, samples were collected from 25 random points distributed over the site. At each sampling point, greenhouse gas measurements were taken and soil samples were collected for laboratory analyses. This resulted in 150 independent sampling points per site, with 75 samples from each season. Of these samples, 5 random samples were taken from each visit for phospholipid fatty acid (PLFA)

analysis and a different 10 random samples from each visit were used for nutrient analysis.

### 3.2.3 Soil analysis

#### *3.2.3.1 Temperature, volumetric moisture and bulk density*

Soil temperature and moisture were measured *in situ*, using a digital thermometer from Fischer Scientific (Loughborough, UK) and a theta probe® (Delta-T Devices, Cambridge, UK) digital volumetric moisture meter respectively. For some sampling without *in situ* volumetric moisture meter, additional soils were collected for measuring gravimetric moisture. Fresh soils were then transported to the laboratory, where gravimetric moisture was calculated. Approximately 10-15 g of fresh soil was taken in an aluminium tin and dried in an oven at 105$^{o}$C for 48 hours. The gravimetric moisture was calculated as follows: Gravimetric Moisture (GM) = (Weight of water in fresh soil/ Weight of dry soil) × 100. Bulk density samples were collected by inserting a tube of known volume (20 ml) into peat surface. The collected soil was then dried in an oven at 105$^{o}$C for 48 hours and the dry weight was recorded. The bulk density was then calculated using the following formula: Bulk density (g/cm$^3$) = Dry soil weight (g) / Soil volume (cm$^3$). The calculated gravimetric moisture was then converted to volumetric moisture using bulk density data.

#### *3.2.3.2 pH*

About 5 ml volume of peat sample was diluted in 10 ml deionised water in a centrifuge tube and shaken in a rotary shaker for 30 minutes. The pH of the supernatant was then measured using a pH meter supplied by Mettler Toledo (Leicester, UK).

#### *3.2.3.3 Organic matter content*

Oven-dried peat samples were placed in silica crucibles and then transferred to a muffle furnace and maintained at 550$^{o}$C for 4 hours. The loss on ignition was then calculated as follows: Loss on ignition (%) = (weight of oven dried soil – weight of ash) / weight of oven dried soil ×100.

#### *3.2.3.4 Total C and N*

All samples were oven-dried and finely ground using a ball mill. Approximately 10 mg of sample was weighed into a tin cap and the exact weight was recorded. The samples were then transferred to an auto sampler on Flash 2000 CHNS-O elemental analyser supplied by Thermo Scientific (Leicester, UK) to measure total C and N. The analyser was set at 55$^{o}$C oven temperature, with helium as the carrier gas at the flow rate of 140 ml/min. L-aspartic acid supplied by Sigma Aldrich (St Louis, USA) was used as quality control and peaty soil standard supplied by Elemental Microanalysis (Okehampton, USA) was used as a standard.

### 3.2.4 Phospholipid fatty acid analysis

#### *3.2.4.1 PLFA extraction*

Microbial community phenotypic structure was determined by phospholipid fatty acid (PLFA) analysis. PLFAs were extracted from replicate 1 g freeze-dried tropical peat samples using a modification of the method described by (Frostegard et al., 1991). The lipids from peat were extracted using Bligh & Dyer extraction (Bligh & Dyer, 1959). The extracted lipids were then separated into neutral lipids, glycol lipids and polar lipids (containing phospholipids) fractions using Megabond Elut® silica gel column. The extracted polar lipids were then methylated by mild alkaline methanolysis and

converted into fatty acid methyl esters, which were then analysed using gas chromatography.

### *3.2.4.2 Gas chromatography and peak identification*

The dried fatty acid methyl esters were suspended in 200 µl of hexane, ready for GC injection. One µl of each sample was injected into the GC in split-less mode. The column used in the GC for phospholipid analysis was 'ZB-FFAP' column, supplied by Phenomenex®. The column was 30 m length x 0.25 mm inner diameter x 0.25 µm film thickness. The carrier gas was helium with the constant pressure of 18psi. The initial oven temperature in GC was 120°C; this was maintained for 1 min and then programmed to 250°C at the rate of 5°C /min. The constant temperature of 250°C was maintained throughout the run. The results were displayed as a chromatogram of retention times of the compounds and the mass spectroscopy provides the ion profile of each compounds.

The fatty acids *i15:0, a15:0, i16:0, i17:0, a17:0* were considered as Gram-positive biomarkers (Wilkinson et al., 2002). The fatty acids *10me16:0* and *10me18:0* were described as the biomarkers for actinomycetes (Wilkinson et al., 2002, Moore-Kucera & Dick, 2008), a group that belongs to Gram-positive bacteria. The relative abundance of Gram-negative bacteria were calculated using *16:1n9, 16:1n7, cyc17:0, 18:1n7* and *cyc19:0* as biomarkers (Wilkinson et al., 2002, Kaiser et al., 2010). *18:2n6* and *18:1n9* were used as fungal biomarkers (Vestal & White, 1989, Wilkinson et al., 2002, Kaiser et al., 2010). *14:0, 16:0, 18:0, a17:1* and *20:0* were non-specific fatty acids (Wilkinson et al., 2002). The fatty acids with similar mass spectrum *18:1n9* and *18:1n7* were differentiated with the help of neutral lipid fatty acid analysis, by the findings that fungal biomarker *18:1n9* should have much higher NLFA/PLFA ratio that the Gram-negative biomarker *18:1n7* (Baath, 2003).

### 3.2.5 Greenhouse gas measurement

$CO_2$ and $CH_4$ emissions from soil surface were measured using a Los Gatos® (San Jose, USA) ultraportable greenhouse gas analyser. The gas analyser works on the principle of laser absorption spectroscopy. The instrument gives the readings of $CH_4$, $CO_2$ and moisture in ppm and gas temperature. The measurements were made using closed chamber method using a chamber with a height of 15 cm and the inner diameter of 13.5 cm. The chamber had an inlet and an outlet port that were connected to the gas analyser, using a quarter inch outer diameter polytetrafluoroethylene (PTFE) tube. During each measurement about 1 cm of the chamber was carefully inserted into the ground to avoid disturbance until it was sealed to the ground surface, and gas measurements were taken for 5 minutes. There was no surface vegetation in any of the measurement points. The gas analyser was set to record gas flux every 20 seconds, resulting in at least 12 recorded measurement points for each plot. The first minute of each measurement was ignored allowing the gas flux to settle down after initial disturbance of placing the chambers. The gas measurements in ppm were converted to mg $CO_2$ $m^{-2}hr^{-1}$ and µg $CH_4$ $m^{-2}hr^{-1}$ for $CO_2$ and $CH_4$ respectively, as described in (Samuel and Evers, 2016), using the ideal gas law. PV=nRT. Where: P = atmospheric pressure; V = volume of headspace; n = number of moles (mol); R = universal Gas Constant law (8.314J. $K^{-1}mol^{-1}$) and T = temperature in kelvin (K), with conversion factor, 1 mol of $CO_2$ = 44.01g and 1 mol $CH_4$ = 16.02g.

### 3.2.6 Statistical analyses

All the statistical analyses were carried out using Genstat® $17^{th}$ edition (VSN international, 2017). The significance of differences between sites for greenhouse gas emissions and other environmental parameters were evaluated using linear mixed models with restricted maximum likelihood (REML) incorporating seasons and sites

as fixed effects and individual visits as random effects. For the data sets that were not normally distributed, the data were log transformed. If the log transformed data were still not normally distributed, the data were power transformed, using Boxcox transformation.

Principal component analysis (PCA) was performed on PLFA data using Mol% normalised spectra and the correlation matrix. Resultant PCs were analysed by one way analysis of variance (ANOVA). Relative abundance of individual microbial groups, and ratios between groups, were calculated and were subjected to statistical analysis using restricted maximum likelihood (REML) models, to identify the interactions of individual microbial groups with site, season and combination of site and season. Similar REML were also performed for PCs. REML was carried out using 'site' and 'depth' were used as fixed model

Backward stepwise multiple regression was performed with relative abundance of each individual microbial groups and ratios as response variate and other environmental parameters as fitted terms. Similar backward stepwise multiple regression was also performed with $CO_2$ as response variates. To meet the normality assumptions means of each visit were used to find correlations between $CH_4$ and other environmental parameters. Linear regression was also performed to predict $CO_2$ emissions at each individual site from the measured volumetric moisture. Backward stepwise multiple regression was also carried out to determine the relationship for $CO_2$ and $CH_4$ emissions with relative abundance of different microbial groups.

## 3.3 Results

### 3.3.1 Peat properties

The organic matter content in first generation plantation was significantly reduced by ~10% from the forest (Table 3.1, Fig. 3.1a). In 2nd generation systems, the cleared and pineapple intercropping sites maintained similar level of organic matter content as forest, yam intercropping site had similar level of organic content as 1st generation mono-cropping, while organic matter content was almost halved to 50% in the 2nd generation oil palm mono-cropping. No seasonal changes in organic matter content was observed in any of the sites.

Volumetric moisture varied significantly between the sites (see Table 3.1, Fig. 3.1b). The first generation mono-cropping had the lowest moisture level of all sites at *ca.* 31%. All the 2nd generation systems maintained moisture levels that were significantly higher than 1st generation but lower than forest, except for pineapple intercropping site that had moisture level higher than the forest during wet season. Volumetric moisture significantly varied between seasons and these seasonal changes were observed only in the 2nd generation agricultural systems, which is more pronounced in 2nd generation oil palm mono-cropping. The changes in moisture content between seasons were higher with increasing age from conversion. For example, forest and 1st generation oil palm maintained their respective levels of moisture throughout the seasons but at the 2nd generation oil palm, the moisture level was almost reduced to half during the dry season.

The conversion of forest to plantations had increased pH at all sites (Table 3.1, Fig. 3.1c). The 1st generation oil palm had pH <3.5 closer to forest. For 2nd generation systems, cleared and pineapple intercropping sites had similar pH as 1st generation oil

palm. There was a significant site by season interaction with respect to pH, where there was an increase in pH between wet and dry seasons under forest, 1st generation oil palm and 2nd generation oil palm, a decrease under cleared and pineapple intercropping, and no change under yam intercropping (Fig. 3.1c).

Peat surface temperatures were significantly higher at all agricultural sites, with forest having the surface temperature at *ca.* 27° C and 1st generation oil palm having the highest temperature at *ca.* 29.5° C (Fig. 3.1d, Table 3.1). Among the second generation sites, the cleared and yam intercropping sites maintained similar temperature level as 1st generation oil palm, while the pineapple intercropping and 2nd generation oil palm had lower surface temperatures in comparison, yet significantly higher than forest. During the dry season, the temperature increased significantly at some agricultural sites such as 1st generation oil palm, yam intercropping and 2nd generation oil palm, while soil temperature decreased significantly for the forest and pineapple intercropping sites.

**Table 3.1:** Linear mixed model (REML) for environmental parameters with respect to microbial groups, showing statistical significance of the effects of site, season and the interactions between site and season.

| | **Site** | **Season** | **Site*season** |
|---|---|---|---|
| **Organic matter %** | $F_{(5,835.3)}$=485,***p<0.001*** | $F_{(1,4)}$=0.23,p=0.658 | $F_{(5,835.9)}$=2.19,p=0.053 |
| **pH** | $F_{(5,836.5)}$=35.4,***p<0.001*** | $F_{(1,4)}$=1.26,p=0.325 | $F_{(5,837)}$=15,***p<0.001*** |
| **Moisture** | $F_{(5,887.2)}$=124,***p<0.001*** | $F_{(1,4)}$=8.26,***P<0.05*** | $F_{(5,887.2)}$=15.2,***p<0.001*** |
| **Temperature** | $F_{(5,879.2)}$=180,***p<0.001*** | $F_{(1,4)}$=0.20,p=0.681 | $F_{(5,879.3)}$=9.2,***P<0.001*** |
| **$CO_2$** | $F_{(5,845.1)}$=37.8,***p<0.001*** | $F_{(1,3.9)}$=80.3,***p<0.001*** | $F_{(5,843.2)}$=12.5,***p<0.001*** |
| **$CH_4$** | $F_{(5,861.8)}$=27.5,***P<0.001*** | $F_{(1,3.8)}$=37.5,***p<0.005*** | $F_{(5,854.6)}$=6.7,***p<0.001*** |

Bulk density was significantly greater in all agricultural plantations than at forest ($F_{(5,118)}$=45.1, p<0.001; Fig. 1e). The 1st generation oil palm and all the 2nd generation sites except 2nd generation oil palm, had bulk densities around 0.3 g $cm^{-3}$, while the 2nd generation oil palm mono-cropping had the highest bulk density at >0.4 g $cm^{-3}$.

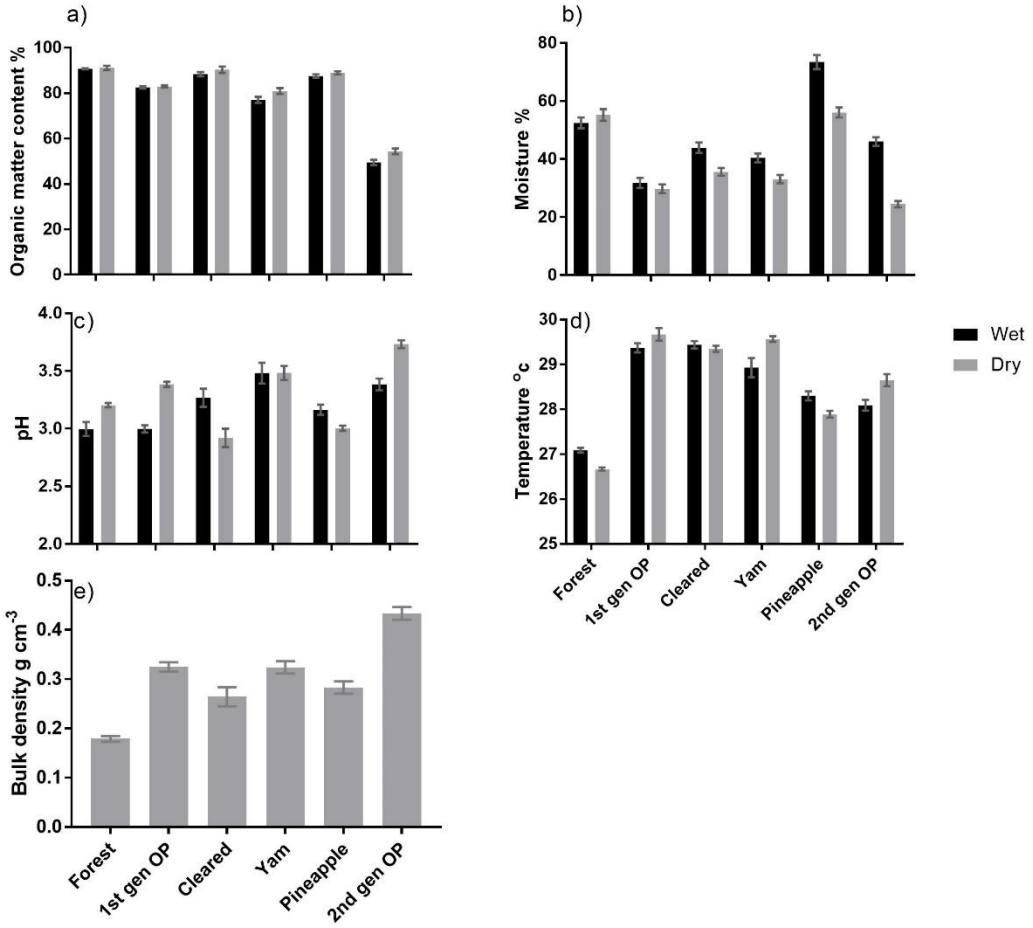



**Figure 3.1:** Effect of site and season upon (a) organic matter content, (b) moisture, (c) pH, (d) temperature and (e) bulk density between different study sites during wet (black) and dry (grey) season. Bars denote mean values (n varies, see text) and whiskers denote standard errors. Note 1st gen OP denotes 1st generation oil palm monocropping, yam denotes, 2nd generation oil palm and yam intercropping, pineapple denotes 2nd generation oil palm and pineapple intercropping, 2nd gen OP denotes 2nd generation oil palm mono-cropping.

### 3.3.2 C and N content

Total C varied significantly between the sites ($F_{(5,54)}$=39.0, p<0.001). The forest and first generation oil palm site had similar level of C content at *ca*. 50% (Table 3.2). The second generation oil palm showed substantial variations depending on whether it was a mixed cropping or mono-cropping system. The C content was highest at >60% in cleared and pineapple intercropping sites, while it was lowest at the 2nd generation oil palm at *ca.* 25%.

Total nitrogen content also varied significantly between the sites ($F_{(5,54)}$=25.47, p<0.001). The nitrogen content was highest in forest at 2.5%, which was reduced to Ca 2% at the first generation oil palm. In second generation agricultural systems, cleared and pineapple intercropping systems had higher nitrogen content than the 1st generation oil palm, but the 2nd generation oil palm had lowest nitrogen content at 0.8%.

The ratio between C and nitrogen (C:N) also varied significantly between the sites ($F_{(5,54)}$=8.41, p<0.001). C:N was lowest at the forest, which was increased in 1st generation oil palm plantations, and the ratio was further increased in all the 2nd generation cropping systems with the 2nd generation oil palm containing the highest C:N ratio at 32.

**Table 3.2:** Total C, N and C:N under different sites at surface layers. Note 1st gen OP denotes 1st generation oil palm mono-cropping, yam denotes 2nd generation oil palm and yam intercropping, pineapple denotes 2nd generation oil palm and pineapple intercropping, 2nd gen OP denotes 2nd generation oil palm mono-cropping.

| Site | C % | N % | C:N |
|---|---|---|---|
| **Forest** | 48.9 | 2.47 | 19.8 |
| **1st gen OP** | 51 | 1.97 | 26 |
| **Cleared** | 64.1 | 2.13 | 30 |
| **Yam** | 46.3 | 1.49 | 31 |
| **Pineapple** | 60.2 | 2.27 | 26.5 |
| **2nd gen OP** | 26.4 | 0.83 | 32 |

### 3.3.3 Peat microbial communities

#### *3.3.3.1 Variations between sites across the seasons*

PC1 and 2 collectively accounted for 39% of the variation, with PC1 accounting for about 5% more of the variations than PC2 (Fig. 3.2). There were no interactions between site and season for either PC1 and 2 (Table 3.3). Hence main effects of the treatments were considered directly.

**Table 3.3:** Linear mixed model (REML) for prinicipal component scores, showing statistical significance of the effects of site, season and the interaction between site and season.

| | PC1 | PC2 |
|---|---|---|
| **Site** | $F_{(5,48)}$= 7.53, p=0.138 | $F_{(5,48)}$= 3.81, ***p<0.005*** |
| **Season** | $F_{(1,48)}$= 65.64, ***P<0.001*** | $F_{(1,48)}$= 3.91, p=0.054 |
| **Site*Season** | $F_{(5,48)}$= 1.77, p=0.138 | $F_{(5,48)}$= 0.53, p=0.753 |

Both PC1 and 2 showed highly significant discrimination between sites, while only PC1 showed significant discrimination between seasons. PC1 separated the two seasons, and also separated the forest site from the agricultural sites within each season. PC2 separated yam intercropping site in both seasons, 2nd generation oil palm mono-cropping and pineapple intercropping in wet season from the rest of the sites. The loading for individual PLFAs associated with each of the PCs were generally dispersed, with no particular dominance of any single PLFA (Fig. 3.2b). A notable characteristic of the loadings bi-plot was the association of saturated fatty acids with discrimination of wet season sites from the dry season sites.

Overall, the microbial community structure was different in agricultural plantations from the forest. Two of the 2nd generation systems such as cleared and pineapple intercropping had similar microbial structure as the 1st generation oil palm, while the other two 2nd generation systems such as yam intercropping and 2nd generation oil palm had different microbial community structure between each other that were also different from the 1st generation oil palm

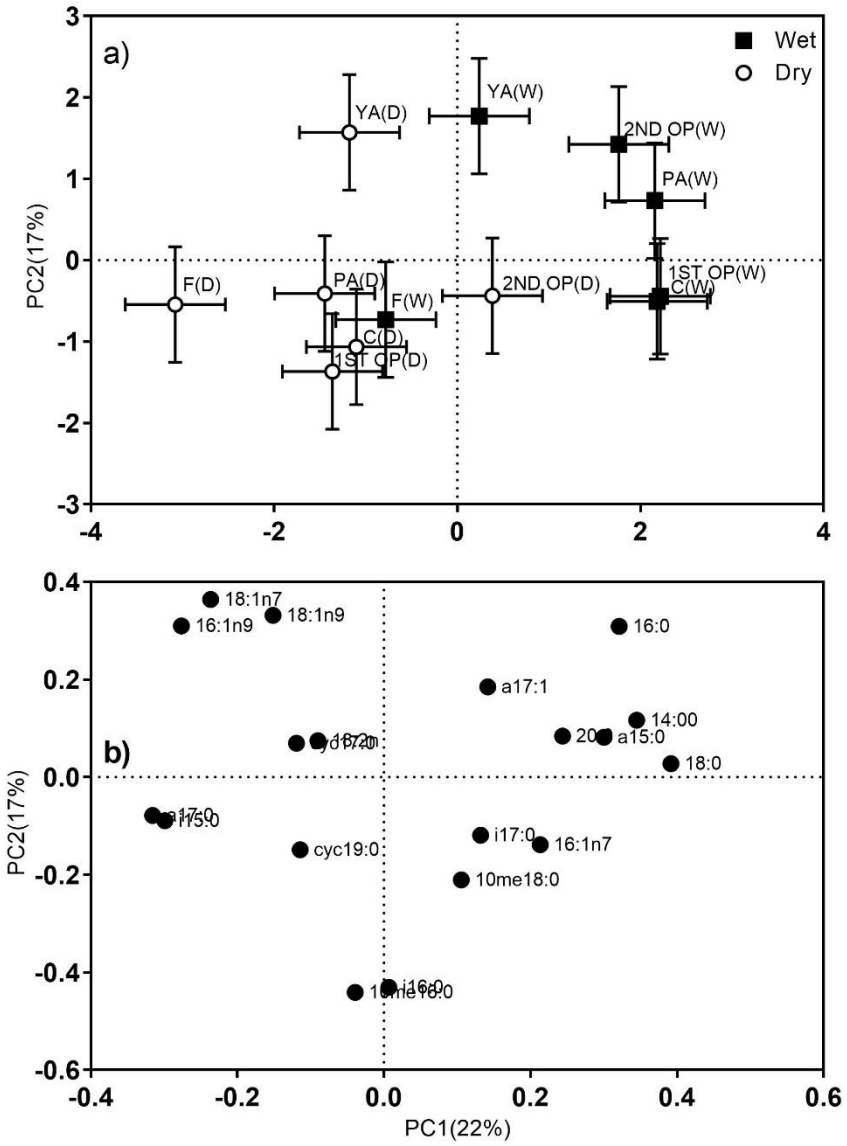



**Figure 3.2:** Effects of site and season upon phenotypic structure of soil microbial communities determined by PLFA analysis, as shown by principal component (PC) analysis. (a) ordination of PC1 and 2 and (b) associated loadings for individual PLFAs. For (a), points denote means (n=5), whiskers denote standard errors. Note F denotes forest site, 1st OP denotes 1st generation oil palm mono-cropping site, C denoted cleared site, yam denotes 2nd generation oil palm and yam intercropping site, PA denotes 2nd generation oil palm and pineapple intercropping sites, and 2nd OP denotes 2nd generation oil palm monocropping site.

### *3.3.3.2 Microbial phenotypic structure*

All of the studied sites were dominated by bacteria, which constituted more than 50% of the microbial relative abundance. Including actinomycetes to Gram-positive bacterial relative abundance, Gram-positive bacteria were the most abundant microbial group at all sites and seasons. The fungal relative abundance was *ca.* 7 times lower than the bacterial relative abundance for most sites.

All the studied microbial groups except Gram-negative bacteria, varied significantly between sites (Table 3.4). Actinomycetes showed increased relative abundance in 1$^{st}$ generation oil palm than in the forest. In the 2$^{nd}$ generation plantations, the actinomycetes relative abundance were lower in both intercropping sites, while the cleared and 2$^{nd}$ generation oil palm maintained similar relative abundance of actinomycetes as the forest site (Fig. 3.3a). Gram-positive relative abundance was highest at the forest, which was reduced in the first generation oil palm. The 2$^{nd}$ generation sites like cleared and pineapple intercropping had similar Gram-positive relative abundance as the first generation oil palm, while the other two second generation plantations such as yam intercropping and 2$^{nd}$ gen oil palm had lower Gram-positive relative abundance, with yam intercropping containing the lowest Gram-positive relative abundance of all sites. The fungal relative abundance were at similar levels (<10%) at all sites except the 2$^{nd}$ generation oil palm and yam intercropping sites that had higher relative abundance, with yam intercropping sites containing the highest fungal relative abundance of all the sites.

At all sites, the relative abundance of all the individual microbial groups were higher in the dry season than in the wet season, except non-specific fatty acid group, which was reduced during the dry season. The interaction between site and season were statistically significant only for Gram-positive microbial group (Table 3.4), as the

Gram-positive relative abundance increased for the forest and the 1st generation oil palm in the dry season from the wet season, but in the 2nd generation cropping systems, Gram-positive relative abundance increased for pineapple intercropping, stayed at the same level for cleared and 2nd generation oil palm and decreased for yam intercropping.

**Table 3.4:** Linear mixed model (REML) for aggregated PLFA data with respect to microbial groups, showing statistical significance of the effects of site, season and the interactions between site and season.

| | **Site** | **Season** | **Site*season** |
|---|---|---|---|
| **Non-specific** | $F_{(5,48)}$=5.34,***p<0.001*** | $F_{(1,48)}$=55.1,***p<0.001*** | $F_{(5,48)}$=2.23,p=0.066 |
| **Fungi** | $F_{(5,48)}$=4.9,***p=0.001*** | $F_{(1,48)}$=11.5,***p=0.001*** | $F_{(5,48)}$=0.79,p=0.561 |
| **Gram-negative** | $F_{(5,48)}$=1.65,p=0.165 | $F_{(1,48)}$=2.90,p=0.095 | $F_{(5,48)}$=1.45,p=0.222 |
| **Gram-positive** | $F_{(5,48)}$=8.37,***p<0.001*** | $F_{(1,48)}$=7.52,***p=0.009*** | $F_{(5,48)}$=3.06,***p=0.018*** |
| **Actinomycetes** | $F_{(5,48)}$=10.4,***p<0.001*** | $F_{(1,48)}$=1.11,p=0.297 | $F_{(5,48)}$=1.47,p=0.218 |
| **F:B** | $F_{(5,48)}$=5.93,***p<0.001*** | $F_{(1,48)}$=4.84,***p=0.033*** | $F_{(5,48)}$=0.87,P=0.509 |
| **G+:G-** | $F_{(5,48)}$=3.85,***p=0.005*** | $F_{(1,48)}$=0.23,p=0.637 | $F_{(5,48)}$=1.41,p=0.238 |

The site and seasonal variations in the ratio between fungi and bacteria (F:B) was driven by the yam intercropping sites, while all the other sites had similar F:B ratio with no seasonal variations (Fig. 3.3b). The F:B ratio was highest at the yam intercropping site and the ratio was higher in the dry season than in the wet season within that site.

The ratio between Gram-positive and Gram-negative (G+:G-) bacterial groups varied significantly between sites (Fig. 3.3c), while variation between seasons and the interaction between site and season were insignificant (Table 3.4). Variation between sites were driven by the two intercropping sites i.e., pineapple intercropping and yam intercropping, which had lower ratio than other sites.

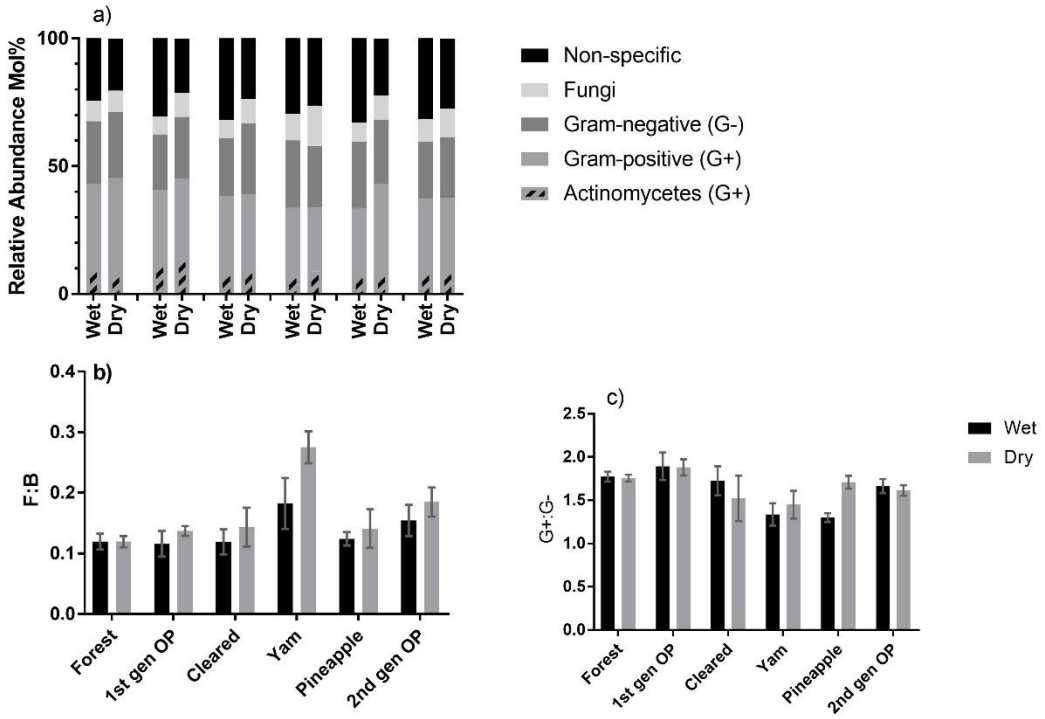



**Figure 3.3:** Effects of site and season upon a) relative abundance of different microbial groups as determined by PLFA analysis, (b) the difference in ratio of the relative abundance of fungi to bacteria (F:B) between different study sites, (c) the difference in ratio of the realtive abundance of Gram-positive to Gram-negative bacteria (G+:G-) between different study sites. Bar denotes mean values (n=5), and whiskers denote standard errors. Note 1st gen OP denotes 1st generation oil palm mono-cropping, yam denotes, 2nd generation oil palm and yam intercropping, pineapple denotes 2nd generation oil palm and pineapple intercropping, 2nd gen OP denotes 2nd generation oil palm mono-cropping

### 3.3.4 Microbial communities and environmental controls

Temperature was significantly related to the changes in both the bacterial and fungal relative abundance, with Gram-positive and Gram-negative groups showing negative correlation while fungal relative abundance was positively correlated with temperature

(Fig. 3.4a, b and Fig. 3.5a). Gram negative microbial group were also positively correlated to pH and organic matter content (Fig.3.5c,d). Actinomycetes exhibited negative correlation with moisture (Fig. 3.4c). Among the ratios, F: B responded positively to temperature, $F_{(1,58)}$=6.52, p=0.013, $R^2$= 0.086; F:B=-0.290+0.01534(temperature).

The ratio G+:G- responded negatively to moisture and pH, $F_{(2,56)}$=3.99, p=0.024, $R^2$=0.093; G+:G-=2.424-0.00428(moisture)-0.1842(pH)

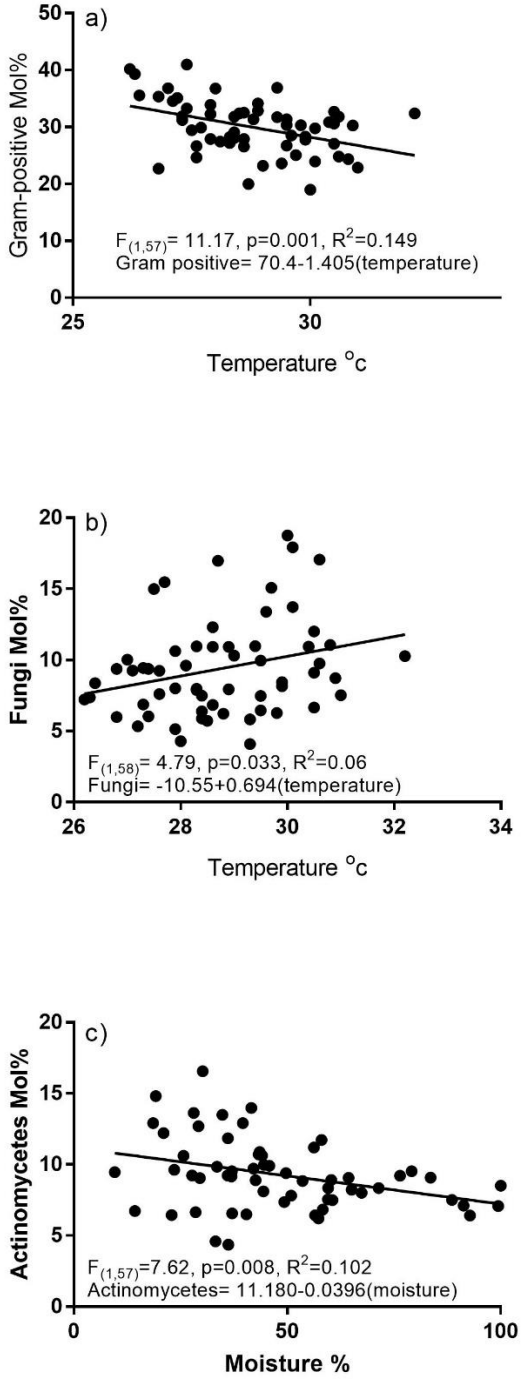



**Figure 3.4:** Relationship between (a) Gram-positive relative abundance (Mol%) and temperature, (b) Fungal relative abundance (Mol%) and temperature, (c) Actinomycetes relative abundance (Mol%) and moisture. Points denote all circmstances of site and season combinations. Significant regression lines and their equations, $R^2$, F and p values are reported in the figures.

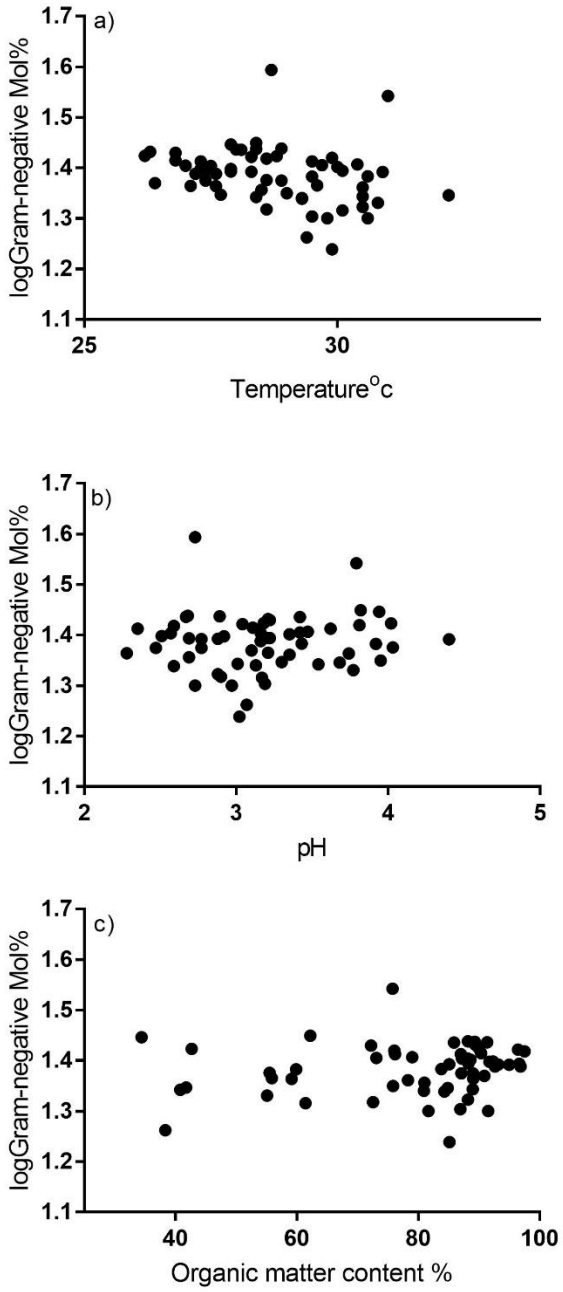



**Figure 3.5:** Multiple regression relationship between Gram-negative relative abundance (Mol%) and (a) temperature, (b) pH, (C) organic matter content. $F_{(3,55)}$=5.48, p+0.002, $R^2$=0.188; log Gram-negative relative abundance (Mol%) = 1.565+0.0530(pH)+0.001455(organic matter content)-0.01611(temperature). Points denote all circumstances of site and season combinations.

### 3.3.5 Greenhouse gas emissions

#### *3.3.5.1 $CO_2$ emissions*

The $CO_2$ emissions were highest at the forest site at both seasons (949 and 971 mg $m^2$ $hr^{-1}$) and this site had the least variation between seasons among all sites (Fig. 3.6a, Table 3.1). The variations between seasons, within the sites, increased with increasing age of conversion from forest. During the wet season, 1st generation oil palm site had the lowest emissions at 603 mg $m^2$ $hr^{-1}$. The cleared site and second generation intercropping sites maintained similar level of $CO_2$ emissions as 1st generation oil palm, while the 2nd generation oil palm mono-cropping had higher emissions closer to the emissions in forest. However, during the dry season the $CO_2$ emissions from the second generation oil palm mono-cropping reduced to less than half of the wet season $CO_2$ emissions and had the lowest value of all sites in the dry season. Reduction in $CO_2$ emissions during dry season was also observed in other 2nd generation agriculture sites, while forest and 1st generation oil palm maintained their respective $CO_2$ emissions on dry season. For dry season, the $CO_2$ emissions decreased with age of conversion from forest with the lowest $CO_2$ emissions from the 2nd generation oil palm mono-cropping.

No statistically significantly relationship was identified between the changes in microbial community structure and $CO_2$ emissions.

#### *3.3.5.3 $CH_4$ emissions*

$CH_4$ emissions were under 1 mg $m^{-2}$ $hr^{-1}$ in all sites during both seasons (Fig. 3.6b), nevertheless $CH_4$ emissions varied significantly between sites, between seasons, with significant interaction between site and season (Table 3.1). Forest site absorbed $CH_4$ during both seasons, while the first generation oil palm mono-cropping emitted similar

low amount of $CH_4$. Among the 2nd generation agricultural systems, cleared and 2nd generation oil palm maintained similar lower level of $CH_4$ emissions as the 1st generation oil palm mono-cropping, while both yam and pineapple intercropping sites emitted higher amount of $CH_4$. Wet season $CH_4$ emissions were higher than dry season $CH_4$ emissions for all sites. The wet season emission for pineapple intercropping site at 497 µg $m^{-2}$ $hr^{-1}$ was considerably higher than the rest of the sites, which were all under 50 µg $m^{-2}$ $hr^{-1}$. However, during the dry season, the $CH_4$ emissions were drastically reduced at pineapple intercropping, with emission values lower than the yam intercropping site at 11.4 µg $m^{-2}$ $hr^{-1}$. The second generation oil palm mono-cropping had the least variations between seasons for $CH_4$ emissions.

No statistically significant relationship was identified between the changes in microbial community structure and $CH_4$ emissions.

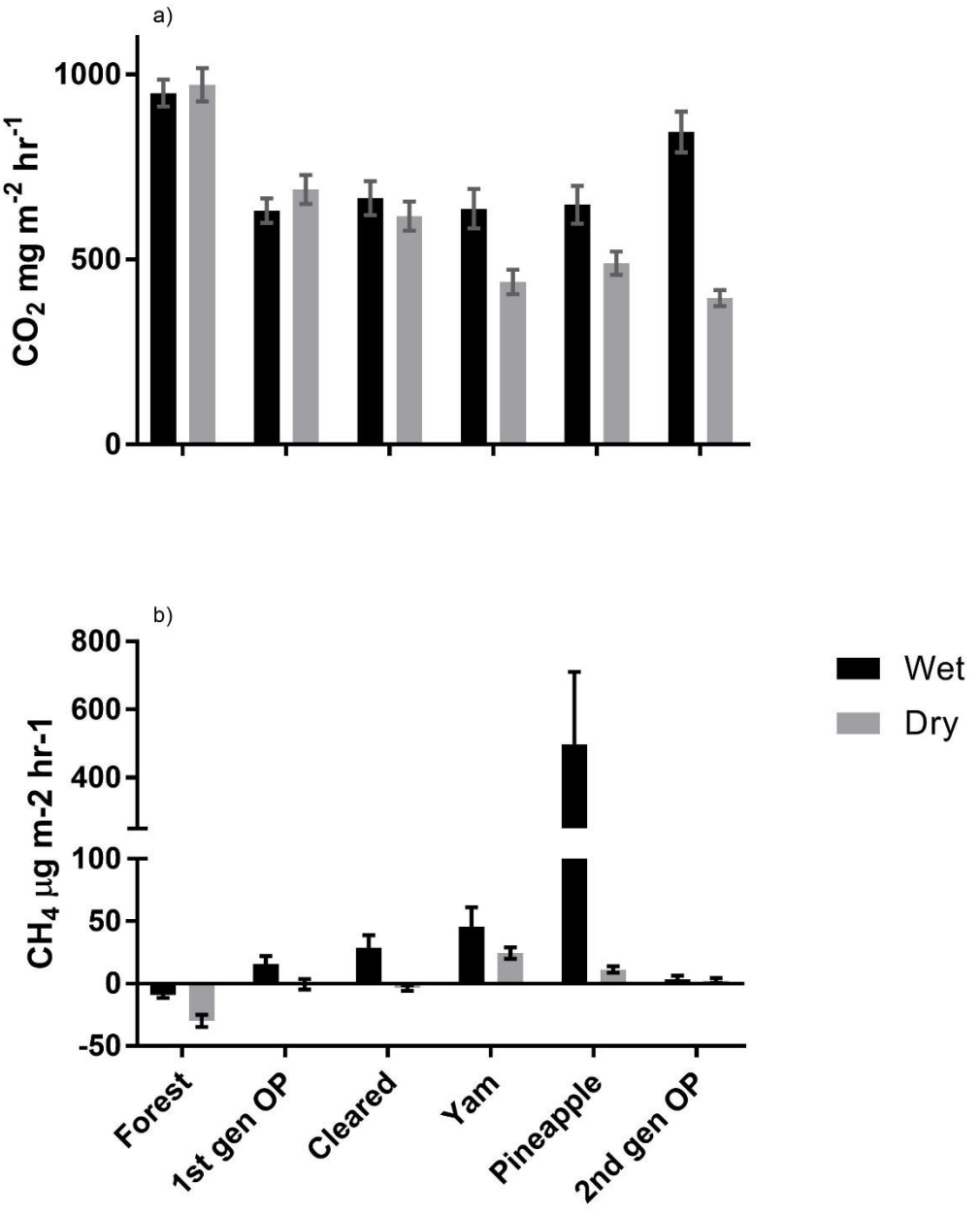



**Figure 3.6:** Effects of site and season upon (a) $CO_2$ emissions, (b) $CH_4$ emissions between different study sites during wet (black) and dry (grey) season. Bars denote mean values (n varies, see text) and whiskers denote standard errors.

### 3.3.6 GHG emissions and environmental controls

#### *3.3.6.1 $CO_2$ emissions and environmental controls*

A multiple linear regression was performed to find a relationship between $CO_2$ emissions and other environmental parameters such as moisture, pH, temperature and organic matter content. A significant regression equation was found ($F_{(4,799)}$= 11.45, $p<0.001$), with an $R^2$ of 0.049 ; $\log CO_2 = 3.538 - 0.001820(\text{moisture}) + 0.0454(\text{pH}) - 0.03645(\text{temperature}) + 0.002386(\text{organic matter content})$

Linear regression between $CO_2$ emissions and moisture for each individual site showed that moisture was a significant predictor of $\log CO_2$ in all sites except yam intercropping site (Fig. 3.7). While for most sites, $\log CO_2$ was negatively correlated with moisture, 2nd generation oil palm plantations exhibited positive correlation between moisture and $\log CO_2$.

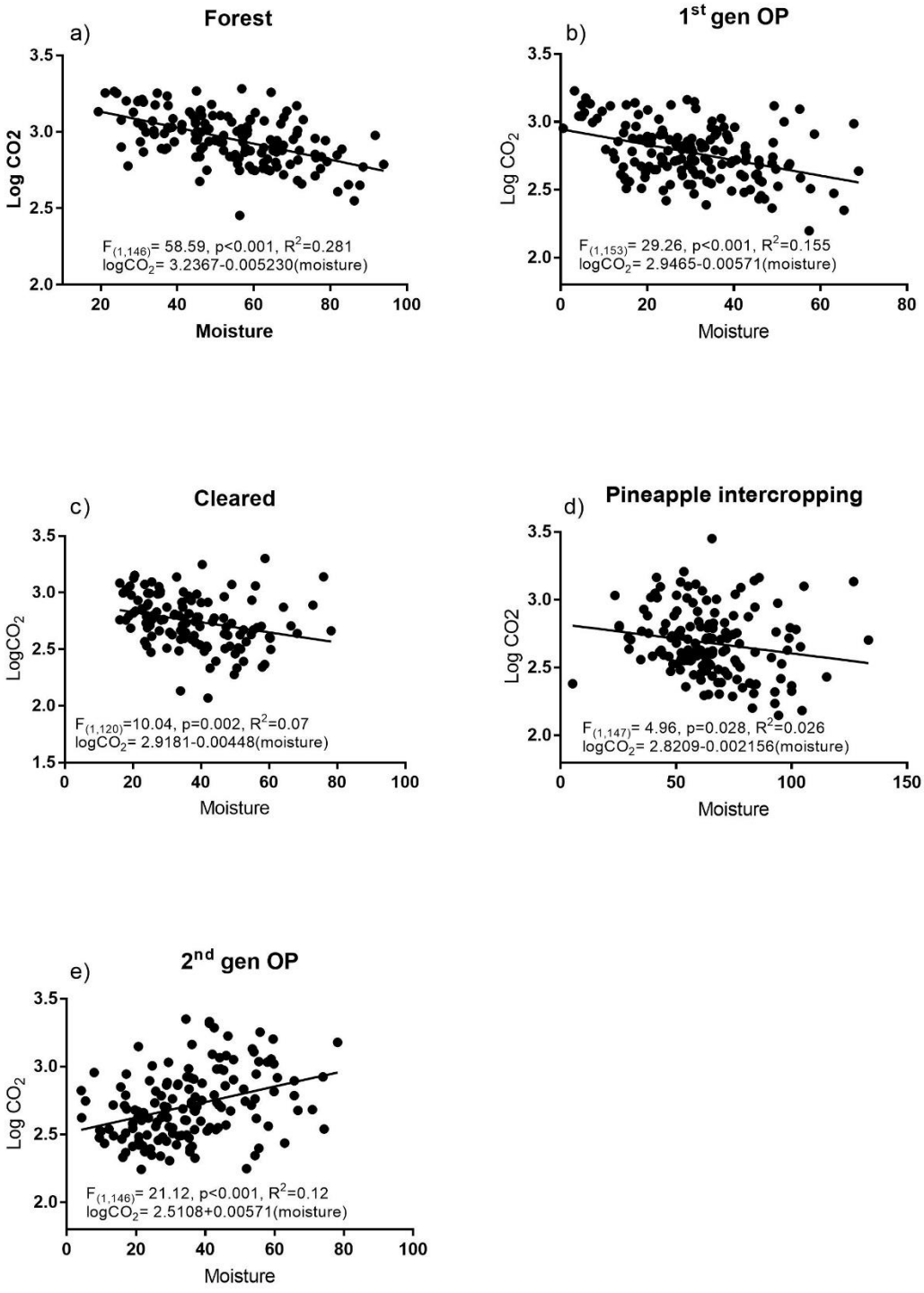



**Figure 3.7:** Relationship between log $CO_2$ and moisture at (a) forest site, (b) 1st generation oil palm mono-cropping site (1st gen OP), (c) cleared site, (d) pineapple intercropping site, (e) 2nd generation oil palm mono-cropping site (2nd gen OP).

*3.3.6.2 $CH_4$ emissions and environmental controls*

A multiple linear regression was calculated to predict $CH_4$ emissions, using the mean values of each visit. Only moisture and temperature were significant predictors of $CH_4$ emissions. So, the rest of the parameters were dropped from the equation. $F_{(2,32)}$=12.39, p<0.001, $R^2$=0.401: $LogCH_4$= -1802 +52.8(moisture) + 7.90 (temperature)

Plotting of $CH_4$ against moisture showed exponential curve, with wet season measurements in pineapple intercropping showing exponentially increased $CH_4$ emissions with increased moisture (Fig. 3.8a). If the wet season pineapple site was removed from the model, moisture was no more a significant predictor of $CH_4$, however $CH_4$ showed a positive linear relationship to temperature (Fig. 3.8b).

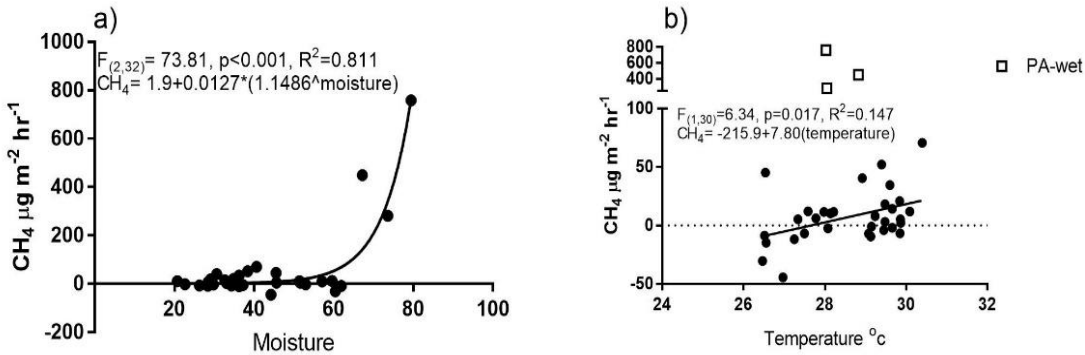



**Figure 3.8:** Relationship between $CH_4$ emissions and (a) moisture, (b) temperature. Points denote mean value of each sampling occasion (n=24-30). Note that for (b) wet season visits for pineapple intercropping site (PA-wet) was left out of the regression equation.

## 3.4 Discussion

### 3.4.1 Peat characteristics at different cropping systems

The conversion of forest to different cropping systems impacted the peat properties in response to dramatic changes in aboveground vegetation and management practices. However, the impact was greatest in progressive generations of oil palm mono-cropping with greatest loss of organic matter and C content, while the other cropping systems maintained similar organic matter content as the forest site and total C content was higher in some 2nd generation agricultural sites, possibly due to the use of fire in the site to clear the previous generation plantations. The N content was highest in the forest resulting in the lowest C:N ratio of all the sites. Increased N deposit through diazotrophy in forest may be due to increased methanotrophy at the site, as the two processes are biologically related in peatlands (Larmola *et al.*, 2014, Vile *et al.*, 2014). The higher N content in forest might be due to N addition through leaf litter in the forest site (Ong *et al.*, 2017).

pH was lowest in the forest compared to plantations, which is consistent with other studies in tropical peatlands (Tonks *et al.*, 2017) and tropical mineral soil systems (Krashevska *et al.*, 2015, Wood *et al.*, 2017), however the effect was greater with prolonged oil palm mono-cropping. Similarly, the difference in temperature between forest and oil palm monoculture were in similar range to the temperature difference observed between forest and oil palm plantation in mineral soil (Lucey and Hill, 2012). Intercropping systems also had similar higher temperature as oil palm mono-cropping, presumably because of open canopy. All the studied non-forest sites except the 1st generation oil palm had more open or no canopy. Though the mature 1st generation oil palm site's canopy was closed, it lacked the structural complexity of multi-layered forest canopy that helps maintain stable microclimate (Sheldon *et al.*, 2010).

Forest ground is usually terraced after clearing above ground vegetation, in preparation for establishing an oil palm plantation (Lucey and Hill, 2012, Luskin and Potts, 2011). This is reflected in our results with plantations showing considerably higher bulk density than forest peat. The 2nd generation oil palm mono-cropping had significantly higher bulk density than rest of the other cropping systems. This might be due to repeated terracing with further generations of palm plantation. However intercropping systems ameliorates this damage by having a more diverse litter input and greater vegetation cover throughout the agricultural land. Confirming the first hypothesis, the peat characteristics were severely altered, while the intercropping systems ameliorated such alterations.

The greater changes in soil volumetric moisture between seasons in older generation of plantations may be due to several factors such as lower water table levels due to prolonged drainage, increased evaporation in dry season due to relatively open canopy, and changes in peat soil characteristics. The surface peat were coarser in older generation plantations with greater bulk density, which may not retain as much water as light, fibrous and organic peat in forest during the times of low rainfall (Campos *et al.*, 2011). Increase in bulk density up to an intermediate level is expected to linearly increase the water retention capacity of soil, but further compression would result in linear reduction in moisture content (Archer and Smith, 1973). It is plausible that peat soil in the 2nd generation mono-cropping had reached the compression threshold and did not retain moisture in dry season that had very low rainfall. However the 2nd generation intercropping systems maintained higher moisture, owing to lower bulk density and management practices that reduce the severity of drainage in agricultural systems.

### 3.4.2 Microbial community structure at different land use types and environmental controls

The study has demonstrated that bacteria overwhelmingly dominate microbial community structure and also plays a functional role in response to disturbances in tropical peatlands. The dominance of Gram-positive bacteria in all the studied sites is in accordance with our previous study, which found all the studied land-use types in Malaysian tropical peatlands, except burnt peatlands, were dominated by Gram-positive bacteria on the surface layers (Chapter 2). This is in contrast with Troxler *et al.*'s (2012) results, which showed that bacterial community in neotropical peatlands were dominated by Gram-negative bacteria by a great margin. This could be because of low moisture level in the studied land-use types in Southeast Asia, as The G+:G- ratio decreased with increasing moisture and the Gram-negative group was found to be dominant in deeper, wetter peat layers below surface at different land-use types in SEA (Chapter 2). Attributing to this hypothesis, the G+:G- ratio was lowest at the wettest site in our study i.e., pineapple intercropping during the wet season. Increased organic matter content favoured Gram-negative relative abundance, however, Gram-negative relative abundance did not vary significantly between sites in line with organic matter content, because of the added effect of temperature and pH changes at different sites (Fig. 3.6).

The microbial community structure was significantly altered by land use change in SEA peatlands, confirming the second hypothesis. The change in community structure with forest conversion was aided by decrease in Gram-positive relative abundance in all agricultural plantations, and increase in fungal relative abundance in some 2$^{nd}$ generation cropping systems. The increased Gram-positive relative abundance may be due to higher nitrogen content in the forest site (Balser, 2001, Liu *et al.*, 2015).

Similarly lower N content also affected higher fungal relative abundance in yam and 2nd gen oil palm, as N limitation affects bacterial communities (Zhang *et al.*, 2016). The difference in C:N ratio also explains the distinction in microbial community structure shown by PCA (Fig. 2a), between the cluster containing 1st generation oil palm, cleared and pineapple intercropping (C:N =25-30, Table 3), and the other two 2nd generation agricultural sites such as yam intercropping and 2nd generation oil palm (C:N>30, Table 1).

The changes in other peat properties with land conversion also influenced the microbial community structure (Fig. 3.4 & 3.5), confirming the second hypothesis. For example, the conversion of forest to agricultural plantations increased temperature, which favoured fungal communities, while reducing both the Gram-negative and Gram-positive bacterial PLFAs. Higher temperature was also observed to favour fungi over bacteria in boreal peatlands (Thormann et al., 2004). The combination of higher temperature, lower N content and relatively drier environment might be the cause behind higher F:B ratio in yam intercropping site than other sites, as bacteria are more dependent on N content and are less favourable to drier conditions (Bossuyt *et al.*, 2001, Fierer *et al.*, 2009, Zhang *et al.*, 2016). Higher pH generally tends to favour bacteria over fungi (Zhang *et al.*, 2016), though the pH was slightly higher in yam intercropping site than at other sites, it was still very acidic (ca. pH 3.5) and did not influence the F:B ratio. The conversion of forest to oil palm plantations was known to increase pH in both peat and mineral soils (Tonks *et al.*, 2017, Tripathi *et al.*, 2012, Wood *et al.*, 2017), however the response of the soil microbial communities to this change in pH was different between these systems, as Gram-negative relative abundance increased with forest conversion and increasing pH in tropical peatlands (Fig. 3.5), while in tropical mineral soil system, Gram-negative

relative abundance decreased with disturbance (Bossio *et al.*, 2005, Krashevska *et al.*, 2015).

The changes in microbial community structure with land-use change has been observed before in different tropical and temperate systems (Bossio *et al.*, 2005, Krashevska *et al.*, 2015, Szoboszlay *et al.*, 2017), however a more intriguing novel finding in this study is that the effect of seasonality on tropical peat microbial community structure was greater than the effect of land-use change. This seasonal change was presumably driven by flooding and the response of microbial PLFAs with higher abundance of straight chain saturated fatty acids (Bossio and Scow, 1998), which are of non-specific origin (Wilkinson *et al.*, 2002). Mono unsaturated (*18:1n7, 18:1n9 & 16:1n9*) fatty acids that are associated with aerobic conditions (Guckert *et al.*, 1985, Parkes and Taylor, 1983, Vestal and White, 1989) were abundant in dry season, adding further evidence to the contribution of flooding to the seasonal variations in microbial community structure.

Although most environmental parameters and greenhouse gas emissions did not vary between seasons for the forest site and 1$^{st}$ generation mono-cropping, the microbial community structure was significantly different between seasons, confirming the microbial part of the third hypothesis. Also, the wet season microbial community structure for forest is similar to dry season agricultural sites. Though flooding explained some of the microbial community changes, it is evident that there are several factors, other than the environmental parameters measured in the current study, which influence the complex mechanisms affecting the peat microbial community structure.

### 3.4.3 Impacts of land-use on GHG emissions

The results show that total $CO_2$ emissions were lower in the agricultural plantations irrespective of the generation of the plantations. However total $CO_2$ comprises both autotrophic root respiration and heterotrophic microbial respiration (Hergoualc'h and Verchot, 2011). The root respiration does not contribute to the C loss as it is part of the plants' photosynthetic cycle, while heterotrophic respiration decomposes peat that is stored over ages (Dariah *et al.*, 2014). The autotrophic contribution from a dense secondary peat forest was observed to be >50% of the total $CO_2$ emissions (Murdiyarso *et al.*, 2017), but the autotrophic component was almost non-existent at a cleared site and about half of the sampling points in other agricultural sites were away from vegetation. Therefore, it is highly likely that heterotrophic contribution from the forest is about 50% of the total emission while for 1$^{st}$ generation oil palm it is >70% and for all the 2$^{nd}$ generation plantations it is >80% of their respective total emissions, owing to the age of the oil palm in each plantations (Comeau *et al.*, 2016, Dariah *et al.*, 2014, Hergoualc'h *et al.*, 2017, Matysek *et al.*, 2017, Murdiyarso *et al.*, 2017). Considering this, C loss through heterotrophic $CO_2$ emissions was in a similar range (*ca.* 400-500 mg $m^{-2}$ $hr^{-1}$) for all the studied sites. However C addition to peatlands through aboveground vegetation is much higher in natural forest than in the agricultural landscapes like oil palm plantations (Guillaume *et al.*, 2016), which results in net C accumulation in forested peatlands (Page *et al.*, 2006). Additionally, it had been found that leaf litter from some natural peatland tree species are more resistant to microbial decomposition, resulting in organic matter accumulation and peat formation (Yule and Gomez, 2009). Whereas, homogenous litter in agricultural land uses are easily degradable (Kerdraon *et al.*, 2017) and presumably lack the chemical and physical properties required for the formation of peat, and with low water levels, the already

stored C is gradually decomposed and lost. The above postulation is supported by our observations and various other observations in oil palm mono-cropping, indicating the complete lack of humus or leaf litter layer (Bruhl and Eltz, 2010, Chung *et al.*, 2000, Fayle *et al.*, 2010). This is also evident in our results showing that organic matter content through loss on ignition and total C, were reduced to half that of the other studied sites in 2$^{nd}$ generation mono-cropping.

The second generation agricultural systems also exhibited distinct seasonal variations which was more pronounced in oil palm mono-cropping, where the $CO_2$ emissions in dry season was reduced to half of that in wet season. Moisture was the only other environmental parameter which showed similar trend of seasonal variations as $CO_2$ emissions. Forest, 1$^{st}$ generation oil palm plantation and both intercropping sites exhibited negative correlation between $CO_2$ emissions and moisture (Fig. 3.7a-d), while $CO_2$ emissions and moisture were negatively correlated with moisture for 2$^{nd}$ generation oil palm mono-cropping (Fig. 3.7e), showing moisture limitation in microbial activity. This is possibly due to severe drainage with progressive generations of mono-cropping. Positive correlation between $CO_2$ and moisture is usually observed in mineral soil systems in tropics (Chen *et al.*, 2002, Huang and Hall, 2017, Werner *et al.*, 2006), while the peatland systems generally exhibit negative correlation between moisture or water table level and $CO_2$ emissions irrespective of the land-use (Couwenberg *et al.*, 2010, Hergoualc'h and Verchot, 2014, Hergoualc'h *et al.*, 2017, Jauhiainen *et al.*, 2005, Sangok *et al.*, 2017, Wakhid *et al.*, 2017). These results infer that prolonged oil palm mono-cropping leads to loss of C in peatlands, leading them to act and respond like mineral soil systems.

$CH_4$ emissions were very low and were under 1 mg $m^{-2}$ $hr^{-1}$ at all the studied sites, which is in the range observed in previous studies showing similar low level emissions

in SEA peatlands (Couwenberg *et al.*, 2010, Inubushi *et al.*, 2003, Melling *et al.*, 2005a). The forest site in Malaysian peatlands absorbed $CH_4$, contradicting the results observed in neotropical peatlands, where high productivity systems emitted higher amount of $CH_4$ (Winton *et al.*, 2017). However, previous studies have shown that $CH_4$ emissions in neotropics were generally higher than that of SEA peatlands (Couwenberg *et al.*, 2010, Girkin *et al.*, 2018, Sjögersten *et al.*, 2014). This might be due to the differences between the neotropical and SEA peatlands in microbial communities and above ground vegetation, that have influence on GHG emissions through root exudation and substrate composition (Girkin *et al.*, 2018, Troxler *et al.*, 2012).

Uniquely higher $CH_4$ emissions in pineapple intercropping site during the wet season directly correlates to the distinctive above surface hydrological condition. The increase in $CH_4$ emissions with increasing temperature in tropical peatlands was consistent with previous observations (Aben *et al.*, 2017, Melling *et al.*, 2005a). This is reflected by higher $CH_4$ emissions in pineapple intercropping site with higher temperature and wetter conditions. The changes in moisture in other sites, where the water table were below surface, did not significantly alter or influence $CH_4$ emissions. Methanogenic archaea were found to be abundant at 30-40 cm below water level (Galand *et al.*, 2002, Lin *et al.*, 2014), leading to a plausible explanation that the water table above surface, made the methanogenic communities become active and abundant closer to the surface and rhizosphere in pineapple intercropping site during the wet season. Conversely, higher methane oxidation in the forest site complements with Jackson *et al.*'s (2009) finding that the methanogenic bacterial communities were completely absent at the top 50 cm of surface peat in North Selangor peat swamp forest. However the same study found phyla containing methanotrophs such as

Proteobacteria and Verrucomicrobia in North Selangor peatlands, plausibly contributing to the methane oxidation in the forest site.

### 3.4.4 Relationship between greenhouse gas emissions and microbial community structure

The microbial community structure along with other environmental parameters in agricultural sites were evidently different from forest. The GHG emissions also considerably varied between forest and agricultural sites, yet in our study we could not identify a statistically significant relationship between microbial community structure and GHG emissions. This might be due to the variations in real abundance of microbes rather than the changes in community structure affecting the GHG emissions. However, it had been identified that microbial communities in oil palm plantations were unable to decompose forest leaf litter after conversion in mineral soil system (Kerdraon *et al.*, 2017), possibly indicating forest conversion to oil palm plantations in mineral soil lead to loss of some key microbial communities that decompose complex forest leaf litter. Therefore, it is possible that some key peat forest microbial groups are lost after conversion into plantations, and it is highly likely that there is a relationship between microbial community structure and GHG emissions in tropical peatlands, which were not detected in our study.

The inability to find a significant relationship between microbial community structure and GHG emissions in this study might also be due to the inclusion of root respiration with heterotrophic respiration. So, the emissions reported in this study are not directly reflective of the microbial respiration, rather total soil GHG emissions from different land use types.

Methanogenic archaea contains ether linked fatty acids in their cell wall (Gattinger *et al.*, 2003). Phospholipid fatty acid analysis extracts only ester linked fatty acids, thus not detecting archaeal communities responsible for methane production. Therefore it was expected that the relationship between PLFA profile and $CH_4$ emissions would be presumably weak or non-existent, which was the case in our results.

## 3.5 Conclusion

The peat properties are altered with conversion of forest to agricultural systems which impacted the microbial community structure and GHG emissions. Bacterial communities dominate the microbial community structure in tropical peatlands irrespective of the land-use types. $CH_4$ emissions were very low and relatively insignificant in comparison to $CO_2$ emissions. Overall the damage to peat properties and loss of C was greatest in the 2$^{nd}$ generation of mono-cropping, which also exhibited unusual moisture limitation to decomposition in peatlands, while the intercropping systems maintained most of the forest peat organic content and caused relatively lesser damage to other peat properties such as pH, moisture and bulk density.

# Chapter 4

## 4. GHG emissions, nutrient content and microbial community structure from a last-remaining pristine tropical peat swamp forest in Peninsular Malaysia

### 4.1 Introduction

Peatlands are globally important ecosystems that support high C storage, unique endemic biodiversity and distinct ecosystem services (Parish *et al.*, 2008, Strack, 2008, Xu *et al.*, 2018). Peatlands are formed as a result of primary production exceeding soil microbial decomposition, due to a unique blend of environmental conditions such as hydrology, topography, climate and microbial ecology (Miettinen *et al.*, 2012, Page *et al.*, 1999, Parish *et al.*, 2008). Owing to the variations in the source of these environmental conditions, diverse range of peatlands exist around the globe covering 423 million hectare or about 2.85% of land surface (Xu *et al.*, 2018).

There is a considerable cover of peatlands in tropics, covering 0.25% of land surface area yet accounting for 3% of global soil C or 18% of the total peat C (Hapsari *et al.*, 2017, Hergoualc'h and Verchot, 2014, Strack, 2008). These are most likely an underestimation due to insufficient information on tropical peatlands in general, along with recent new discovery of tropical peatlands in Africa (Dargie *et al.*, 2017), and other recent estimates showing increased cover in South America (Gumbricht *et al.*, 2017, Xu *et al.*, 2018). Unlike most northern peatlands, tropical peatlands are forested, thus they are C-rich both above- and below-ground (Dargie *et al.*, 2017). Additionally tropical peatlands are biologically active throughout the year and accumulate 200%

more C each year per area than northern peat bogs (Guo and Gifford, 2002, Strack, 2008). Most of these C-rich tropical peatlands are located in South East Asia (SEA), and they here store *ca.* 69 Gt of C and absorb about 2.6 t of $CO_2$ per hectare each year (Dohong *et al.*, 2017, Miettinen and Liew, 2010, Norwana *et al.*, 2011).

In spite of their global importance, tropical peatlands remain relatively poorly understood ecosystems (Posa *et al.*, 2011, Yule, 2010). The interest and importance of tropical peatlands came into the limelight only after most of the peat forests in SEA were degraded for logging and agricultural plantations, creating global attention on endangerment of iconic species such as orang-utans and tigers (Swarna Nantha and Tisdell, 2009), along with persistent smog created by burning of peatlands (McKirdy, 2015). However despite this, anthropogenic disturbance in peatlands continues. Malaysia, which contains a sizeable portion of tropical peatlands has the world's highest deforestation rate in the 21$^{st}$ century (Hansen *et al.*, 2013) and natural undisturbed pristine peatlands are almost extinct from Peninsular Malaysia (Miettinen *et al.*, 2016, Yule, 2010). This has led several researchers to use secondary forest as a yardstick to study natural peat habitats (Melling *et al.*, 2005b, Tonks *et al.*, 2017). Therefore, there is a need to study and understand the last remaining pristine peatlands in peninsular Malaysia, to fully assess the impacts of forest conversion on peat characteristics and their consequent effect on GHG emissions.

Almost all of the remaining peat forests in Peninsular Malaysia are secondary forests, which were either drained or selectively logged (Miettinen *et al.*, 2012, Yule, 2010). Logging often is a pathway for other degrading land-use such as oil palm expansion (Dhandapani, 2015, Koh and Wilcove, 2008, Woodcock *et al.*, 2011). The construction of roads for the transportation of logged timber gives access to the remote forests and has significant indirect effects on other forest degradation and land-use

change (Dohong *et al.*, 2017, Forman and Alexander, 1998, Perz *et al.*, 2008). Illegal logging is also a major concern, threatening the remaining forests in SEA (Dohong *et al.*, 2017, Indrarto *et al.*, 2012, Yule, 2010). The process of logging and associated disturbance result in soil compaction (Chung *et al.*, 2000). Logged forests were also found to have lower leaf litter density, even after 25 years past cessation of timber extraction (Bruhl, 2001, Chung *et al.*, 2000). Logging also reduces the complexity of canopy with some regions of logged forests having open canopy (Floren and Linsenmair, 2005), which might not provide a stable microclimate otherwise provided by a multi-layered canopy in primary forest. All these changes will affect highly sensitive tropical peatlands, where hydrology, canopy cover, leaf litter inputs, above ground vegetation and substrate quality are all inter dependent and very crucial (Yule, 2010).

Timber extractions from peatlands in SEA were often associated with drainage of peatlands, affecting their ecology and function (Dohong *et al.*, 2017, Lo and Parish, 2013). Peatlands in their natural state can hold very high quantities of water, up to 5-10 times the weight of peat (Tonks *et al.*, 2017, Yule, 2010), thus playing an important role in regional flood governance. This high water content is crucial for peatland functioning, as it creates anoxic conditions that prevent aerobic decomposition, resulting in accumulation of dead above-ground vegetation and peat formation. Therefore draining these peatlands will expose the C stored over years to aerobic decomposition, resulting in breakdown and subsidence, also reducing their water holding capacity (Tonks *et al.*, 2017, Yule, 2010). Prolonged drainage may result in the disappearance of peat even in forested land, as higher water table is a key for peat formation (Parish *et al.*, 2008). Drainage also makes peatlands highly susceptible to fire, as dried peat itself is flammable (Posa *et al.*, 2011).

Microbes mineralize nutrients required for high primary production that accumulates as peat, thereby creating C and N cycling (Andersen *et al.*, 2013). Considering the quantity of C stored in tropical peatlands, the activity of peat microbial communities can strongly influence the path of global climate change. As soil microbial communities are responsive to soil moisture status and substrate quality (Bossio and Scow, 1998), it is plausible that the microbial community structure and function differ between the pristine and secondary peat swamp forest.

To understand the difference between the pristine and secondary peat swamp forests and their functions, we tested the hypothesis that peat characteristics, nutrient content and microbial community structure are affected by drainage and logging in the secondary forest and they consequently impact GHG emissions. We anticipated that there are changes in microbial community structure between the primary and secondary forests due to the difference in hydrology, nutrient and oxygen availability, which would result in lower $CH_4$ emissions and higher $CO_2$ emissions in the secondary forest.

## 4.2 Materials and Methods

### 4.2.1 Study sites

#### *4.2.1.1 Terengganu Setiu tropical peatlands - primary forest*

This pristine pocket of tropical peat swamp forest is located in Terengganu state, in the north eastern part of Peninsular Malaysia (Figure 1). The site is roughly 842 hectares and ~11.3 km from the coast, located in Kampung Mat Jintan (5°25'16.2°N 102°55'46.2°E) in the boundary between Kuala Nerus and Setiu districts. This peatland area was previously unrecorded and is yet to be given national protection (WWF Malaysia, pers. comm.). To date, there are no published studies on the site and

many of the site characteristics were unexplored (WWF Malaysia, pers. comm.). There is no known history of disturbance other than the local villagers collecting timber for household uses. There were no large scale oil palm plantations or major roads bordering the peatland area. The forest vegetation was composed of mostly trees up to ~40 m tall and 40-50 cm dbh completely closing the canopy with complex multiple layers. The tree species include *Antisoptera* sp., *Shorea* sp., *Calophyllum sclerophyllum* Vesque, *Calophylum* sp., *Blumeondendron tokbrai* (Blume) Kurz, *Durio carinatus* Mast, *Gonystylus bancanus* (Miq.) Kurz, *Elateriospermum tapos* Blume, *and Syzgium* sp. Trees typical of secondary peat swamp forests *Macaranga pruinosa* (Miq.) Müll.Arg and *Macaranga gigantea* (Rchb.f. & Zoll.) Müll.Arg were present on the forest edges (Yule C., pers. obs.). *Pandanus helicopus* Kurz ex Miq (palms), *Nepenthes ampullaria* Jack (pitcher plants) and *Stenocleaena palustris* (Burm. f.) Bedd. (ferns) were common understory vegetation (Yule C. pers. obs.). The rainfall in the region is high from October to February period where it remains higher than 300 mm, with highest rainfall in November at nearly 1200 mm and low in June to September period remaining well below 200 mm, with lowest recorded rainfall in June at less than 50 mm (Suratman et al., 2017). The peat depth was *ca.* 2 m. The water table is generally above surface throughout the forest all around a year (WWF Malaysia, pers. comm.) The water table was *ca.* 10 cm and 5 cm above surface during wet and dry seasons respectively.

#### *4.2.1.2 North Selangor tropical peatlands – secondary forest*

This historically drained and selectively logged peat swamp forest is the largest area (81,304 ha) of peatlands in the state of Selangor at the central western part of Peninsular Malaysia (Figure 1). The North Selangor peatlands are divided and managed as four natural reserves, which have been protected since 1990 (Tonks et al.,

2017). The sampling site (3°41'39.5"N 101°11'05.4"E) was located in the northern part of the peatlands in Sungai Karang forest reserve and was managed by Kelang forestry office. North Selangor peat forest had undergone drainage for logging and also irrigation for nearby oil palm and paddy fields (Irvine et al., 2013). This site has not been logged since the 1980s and contains old channels for timber extraction, many of which remain blocked. The site is bordered with oil palm plantations that are surrounded by paved roads. The forest vegetation includes of *Macaranga pruinosa* (Miq.) Müll. Arg, *Campnosperma coriaceum* (Jack) Hallier f., *Blumeodendron tokbrai* (Blume) Kurz, *Shorea platycarpa* F.Heim, *Parartocarpus venenosus* Becc., *Ixora grandiflora* Ker Gawl, *Pternandra galeata* Ridl., *Stenoclaena palustris* (Burm. f.) Bedd., *Asplenium longissimum* Baker, *Nephrolepsis biserrata* (Sw.) Schott, *Crytostachys* sp., *Cyperus rotundus* L., and *Pandanus atrocarpus* Griff. (Yule and Gomez, 2009). Above ground biomass in North Selangor peat swamp forests ranged between 126.96 – 443.27 mg $ha^{-1}$ with an average of 319.52 mg $ha^{-1}$, while the breast height dimeter ranged from 17 to 375 cm with an average of 46.39 cm (Brown et al., 2018). The observed ground vegetation was generally less dense in comparison to the Terengganu primary forest. The rainfall patterns around the year in North Selangor peatlands have two distinct peaks in March-April period and October-November period with rainfall greater than 200 mm, while the rainfall is low in the months between May and August at just under 125 mm, with lowest rainfall in June (Global Environmental Centre, 2014). The peat depth was roughly 2 m at the sampling region. The water table was below the surface on both the wet and dry season sampling periods. The maximum water table drawdown during the drought period is *ca.* 50-60 cm below surface and the water table is close to the surface for most of the year (Tonks

et al., 2017). More information on North Selangor peatlands is given by Tonks et al. (2017).

### 4.2.2 Sampling strategy

Sampling were carried out during both the wet and dry seasons. The wet season sampling were carried out during December 2016 and October 2017 for secondary and primary forest respectively, while the dry season sampling was done during July 2017 for both forest types. Secondary forest was visited three times during each season, while the primary forest was visited just once on both seasons. At each time, samples were collected from 25 random points distributed over an area of *ca.* 100×100 m. At each sampling point, greenhouse gas measurements were taken and soil samples were collected for laboratory analyses. This resulted in 150 independent sampling points in secondary forest, with 75 samples from each season. For primary forest a total of 50 samples, with 25 samples from each season were taken. Of these samples, 5 random samples were taken from each visit for PLFA analysis and a different 10 random samples from each visit were used for nutrient analysis.

### 4.2.3 Greenhouse gas measurement

$CO_2$ and $CH_4$ emissions from soil surface were measured using a Los Gatos (San Jose, California, USA) ultraportable greenhouse gas analyser. The gas analyser works on the principle of laser absorption spectroscopy and gives readings of $CH_4$ and $CO_2$ ppm as well as gas temperature. The measurements were made using closed chamber method using a chamber with a height of 15 cm and the inner diameter of 13.5 cm. The chamber had an inlet and an outlet port that were connected to the gas analyser, using a quarter inch outer diameter polytetrafluoroethylene (PTFE) tube. During each measurement about 1 cm of the chamber was carefully inserted into the ground until it was sealed to the ground surface, and gas measurements were taken for 5 minutes.

There was no surface vegetation in any of the measurement points. The gas analyser was set to record gas flux every 20 seconds, resulting in at least 12 recorded measurement points for each plot. The first minute of each measurement was ignored allowing the gas flux to settle down after initial disturbance of placing the chambers. The gas measurements in ppm were converted to mg $CO_2$ $m^{-2}$ $hr^{-1}$ and µg $CH_4$ $m^{-2}$ $hr^{-1}$ for $CO_2$ and $CH_4$ respectively, as described in (Samuel and Evers, 2016), using the ideal gas law PV=nRT. Where: P = atmospheric pressure; V = volume of headspace; n = number of moles (mol); R = universal Gas Constant law (8.314J. $K^{-1}mol^{-1}$) and T = temperature in kelvin (K), with conversion factor, 1 mol of $CO_2$ = 44.01g and 1 mol $CH_4$ = 16.02g.

### 4.2.4 Soil properties

Soil temperature and moisture were measured *in situ*, using a digital thermometer from Fischer Scientific (Loughborough, UK) and a theta probe® (Delta-T Devices, Cambridge, UK) digital volumetric moisture meter, respectively.

For pH measurements, about 5 ml volume of peat sample was diluted in 10 ml deionised water in a centrifuge tube and shaken in a rotary shaker for 30 minutes. The pH of the supernatant was then measured using pH meter supplied by Mettler Toledo (Leicester, UK).

Oven dried peat samples were used to calculate the organic matter content. Dried peat samples were placed in silica crucibles and then transferred to a muffle furnace and maintained at 550$^{o}$C for 4 hours. The organic matter content was then calculating the loss on ignition as follows, organic matter content (%) = [(weight of oven dried soil – weight of ash) / weight of oven dried soil] ×100.

For analysing total C and N content, all samples were oven dried and finely ground using a ball mill. Approximately 10mg of sample was weighed into a tin cap and the exact weight was recorded. The samples were then transferred to an auto sampler on Flash 2000 CHNS-O elemental analyser supplied by Thermo Scientific (Loughborough, UK) to measure total C and N. The analyser was set at 55$^{o}$C oven temperature, with helium as the carrier gas at the flow rate of 140 ml min$^{-1}$. L-aspartic acid supplied by Sigma Aldrich (St Louis, USA) was used as quality control and peaty soil standard supplied by Elemental Microanalysis (Okeham, UK) was used as a standard.

The soil nutrient content were analysed using inductively coupled plasma mass spectroscopy (ICP-MS). For this, approximately 0.1g of oven dried and ball milled peat were weighed in digitubes and the exact weights were noted down. The digitubes were then placed in the heating blocks and 8 ml of nitric acid was added to each sample. The samples were left overnight and then 2 ml of hydrogen peroxide was added, the tubes were closed with watch glasses. Samples were then heated at 95$^{o}$C for 2 hours. After the heat block digestion, the samples were diluted by filling milliQ water up to 50 ml, 1 ml of each sample was transferred in to 10ml tube and further diluted with 9ml of miliQ water. The samples were then analysed using 'Thermo Scientific (Loughborough, UK) ICAP Q' ICP-MS fitted with 'CETAC™ A5X- 520' auto sampler.

### 4.2.5 Phospholipid fatty acid analysis

#### *4.2.5.1 PLFA extraction*

Microbial community phenotypic structure was determined by phospholipid fatty acid (PLFA) analysis. PLFAs were extracted from replicate 1 g freeze-dried tropical peat samples using a modification of the method described by (Frostegard et al., 1991). The

lipids from peat were extracted using Bligh & Dyer extraction (Bligh & Dyer, 1959). The extracted lipids were then separated into neutral lipids, glycol lipids and polar lipids (containing phospholipids) fractions using Megabond Elut® silica gel column supplied by Agilent (Santa Clara, USA). The extracted polar lipids were then methylated by mild alkaline methanolysis and converted into fatty acid methyl esters, which were then analysed using gas chromatography.

#### *4.2.5.2 Gas chromatography and peak identification*

The dried fatty acid methyl esters were suspended in 200 µl of hexane, ready for GC injection. One µl of each sample was injected into the GC in split-less mode. The column used in the GC for phospholipid analysis was 'ZB-FFAP' column, supplied by Phenomenex (Torrance, USA). The column was 30 m length x 0.25 mm inner diameter x 0.25 µm film thickness. The carrier gas was helium with the constant pressure of 18 psi. The initial oven temperature in GC was 120°C; this was maintained for 1 min and then programmed to 250°C at the rate of 5°C $min^{-1}$. The constant temperature of 250$^{o}$C was maintained throughout the run. The results were displayed as a chromatogram of retention times of the compounds and the mass spectroscopy provides the ion profile of each compounds.

The fatty acids *i15:0, a15:0, i16:0, i17:0, a17:0* were considered as Gram-positive biomarkers (Wilkinson et al., 2002). The fatty acids *10me16:0* and *10me18:0* were described as the biomarkers for actinomycetes (Wilkinson et al., 2002, Moore-Kucera & Dick, 2008), a group that belongs to Gram-positive bacteria. The relative abundance of Gram-negative bacteria were calculated using *16:1n9, 16:1n7, cyc17:0, 18:1n7* and *cyc19:0* as biomarkers (Wilkinson et al., 2002, Kaiser et al., 2010). *18:2n6* and *18:1n9* were used as fungal biomarkers (Vestal & White, 1989, Wilkinson et al., 2002, Kaiser et al., 2010). *14:0, 16:0, 18:0, a17:1* and *20:0* were non-specific fatty acids (Wilkinson

et al., 2002). The fatty acids with similar mass spectrum *18:1n9* and *18:1n7* were differentiated with the help of neutral lipid fatty acid analysis, by the findings that fungal biomarker *18:1n9* should have much higher NLFA/PLFA ratio that the Gram-negative biomarker *18:1n7* (Baath, 2003). The ratio of cyclopropane fatty acids (*cyc17:0* & *cyc19:0*) to their monoeionic precursors (*16:1n7* & *18:1n7*) and the ratio of total saturated fatty acids (*14:0, 16:0, 18:0, 20:0*) to mono-unsaturated fatty acids (*16:1n9, 16:1n7, a17:1n, 18:1n9, 18:1n7*) were used indicators of stress and other ecological conditions (Bossio & Scow, 1998).

### 4.2.6 Statistical analysis

All the statistical analyses were carried out using Genstat® 17$^{th}$ edition (VSN international, 2017). The significance of differences between sites for greenhouse gas emissions and other environmental parameters were evaluated using linear mixed models with restricted maximum likelihood (REML) incorporating seasons and sites as fixed affects. For the data sets that were not normally distributed, the data were log transformed. For data that did not meet normality assumption after log transformation, non-parametric Kruskal- Wallis test was performed.

Principal component analysis (PCA) was performed on PLFA data using Mol% normalised spectra. Relative abundance of individual microbial groups, and ratios between groups, were calculated and were subjected to statistical analysis using restricted maximum likelihood (REML) models, to identify the interactions of individual microbial groups with forest type, season and combination of forest type and season. Similar REML were also performed for PCs. REML was carried out using 'forest type' and 'season' as fixed model.

Backward stepwise multiple regression was performed with $CO_2$ and $CH_4$ as response variate and other environmental parameters as fitted terms. Similar backward stepwise multiple regression was also performed with macronutrient and micronutrients separately as fitted terms. To meet the normality assumptions considering the dramatic difference between the primary forest and secondary forest in terms of $CH_4$ emissions, each individual data was divided by the calculated variance of $CH_4$ for each site, and the variance adjusted data was used for $CH_4$ multiple regression with environmental parameters. The variance adjusted $CH_4$ was used to find correlations between $CH_4$ and other environmental parameters. Backward stepwise multiple regression was also carried out to determine the relationship for $CO_2$ and $CH_4$ emissions with relative abundance of different microbial groups.

## 4.3 Results

### 4.3.1 Greenhouse gas emissions

$CO_2$ emissions in North Selangor secondary forest were twice as high as the $CO_2$ emissions from the Terengganu primary peat swamp forest ($F_{(1,195)}$= 93.65, P<0.001) and there was no significant seasonal variation in $CO_2$ emissions in either of the forest types ($F_{(1,195)}$= 0.02, P=0.9; Fig. 4.1a).

$CH_4$ emissions also varied significantly between the forest types (Kruskal Wallis H=80.08, p<0.001). The secondary forest absorbed methane at -10 and -30 μg $m^{-2}hr^{-1}$ during wet and dry season respectively, showing statistical difference between seasons (Kruskal Wallis H=21.15, p<0.001), while the primary forest emitted about 2 mg $m^{-2}$ $hr^{-1}$ and showed no significant variations between seasons (Kruskall Wallis H=0.0036, p=0.952) (Fig. 4.1b).

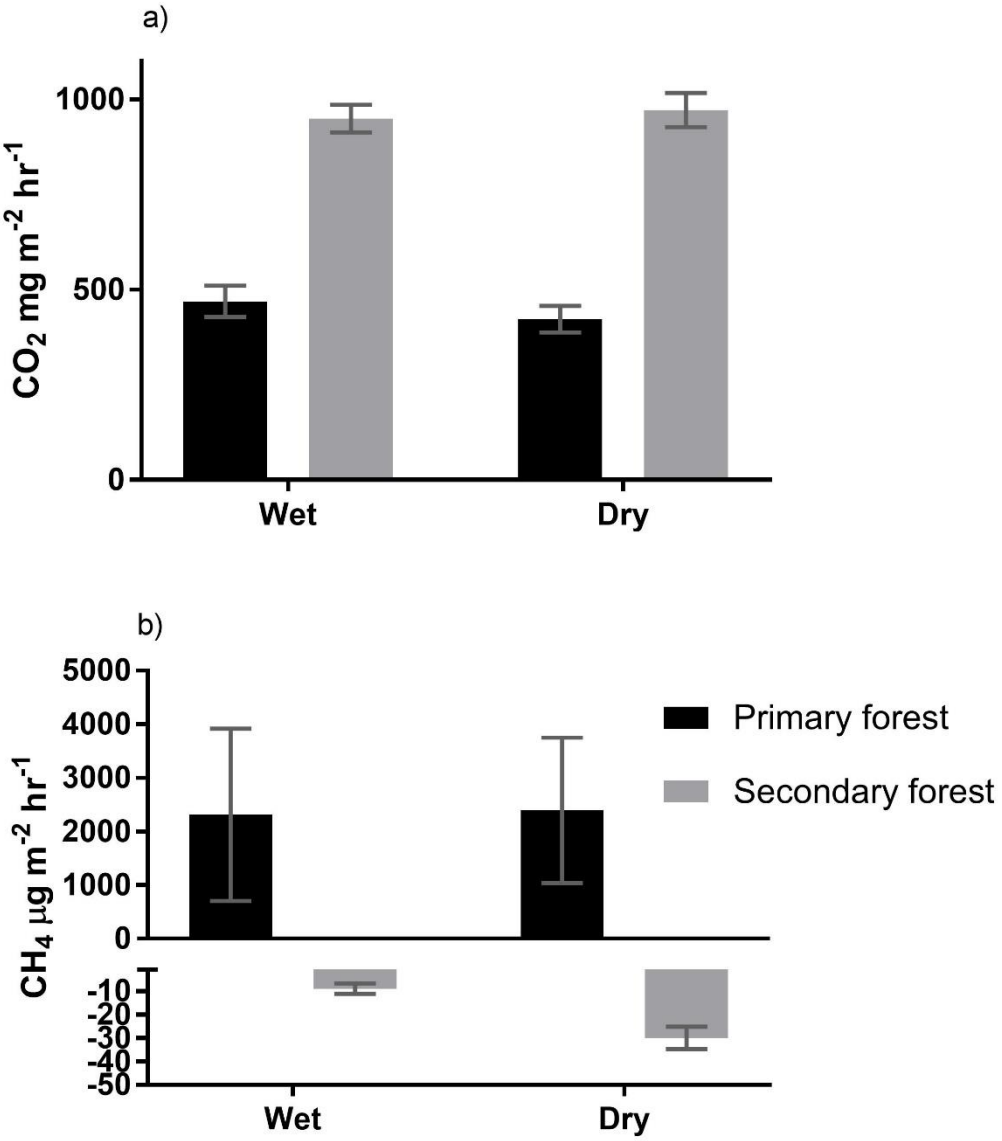



**Figure 4.1:** Effects of forest type and season upon (a) $CO_2$ emissions, (b) $CH_4$ emissions between the forest types (Black- primary forest, grey- secondary forest) during wet and dry season. Bars denote mean values (n varies, see text) and whiskers denote standard errors.

### 4.3.2 Peat properties

Peat organic matter content was >90% in both forests, with primary forest showing significantly higher percentage of organic matter content ($F_{(1,164)}$=4.03,p=0.046; Table

4.1). Volumetric moisture was significantly lower in the secondary forest at <55% during both seasons ($F_{(1,190)}$=344.25,p<0.001) with no statistically significant differences between seasons ($F_{(1,190)}$=1,p=0.318). The primary forest had water table above the surface during both seasons. pH did not vary significantly between primary and secondary forest ($F_{(1,167)}$=0.01,p=0.926). The secondary forest had significantly higher temperature than the primary forest ($F_{(1,196)}$=527, p<0.001). Secondary forest was >1$^{o}$ C higher than the primary forest. Both forest types had significantly lower temperature in the dry than in the wet season ($F_{(1,196)}$=58.33, p<0.001).

**Table 4.1:** Peat properties under different forest types

| **Forest type** | **Primary forest** | | **Secondary forest** | |
|---|---|---|---|---|
| **Season** | **Wet** | **Dry** | **Wet** | **Dry** |
| **Organic matter content %** | | 93.8 ±0.34 | 90.7 ±0.26 | 91.1 ±0.97 |
| **Moisture%** | Water table above surface | | 55.2 ±1.99 | 52.4 ±1.86 |
| **pH** | | 3.1 ±0.03 | 3.0±0.07 | 3.2 ±0.02 |
| **Temperature (°C)** | 25.9 ±0.16 | 25.5 ±0.04 | 27.1 ±0.05 | 26.7 ±0.04 |

### 4.3.3 Nutrient content

Total C and N were at *ca.* 50%, and 2.5% respectively, on both forest types (Fig. 4.2a). All the other studied macronutrients were substantially higher in the primary forest than in the secondary forest, with P being a notable exception, being present at similar levels in both forest types (Fig. 4.2b). Similarly all the micronutrients and trace elements, except molybdenum were substantially higher in the primary forest (Fig 4.2c).

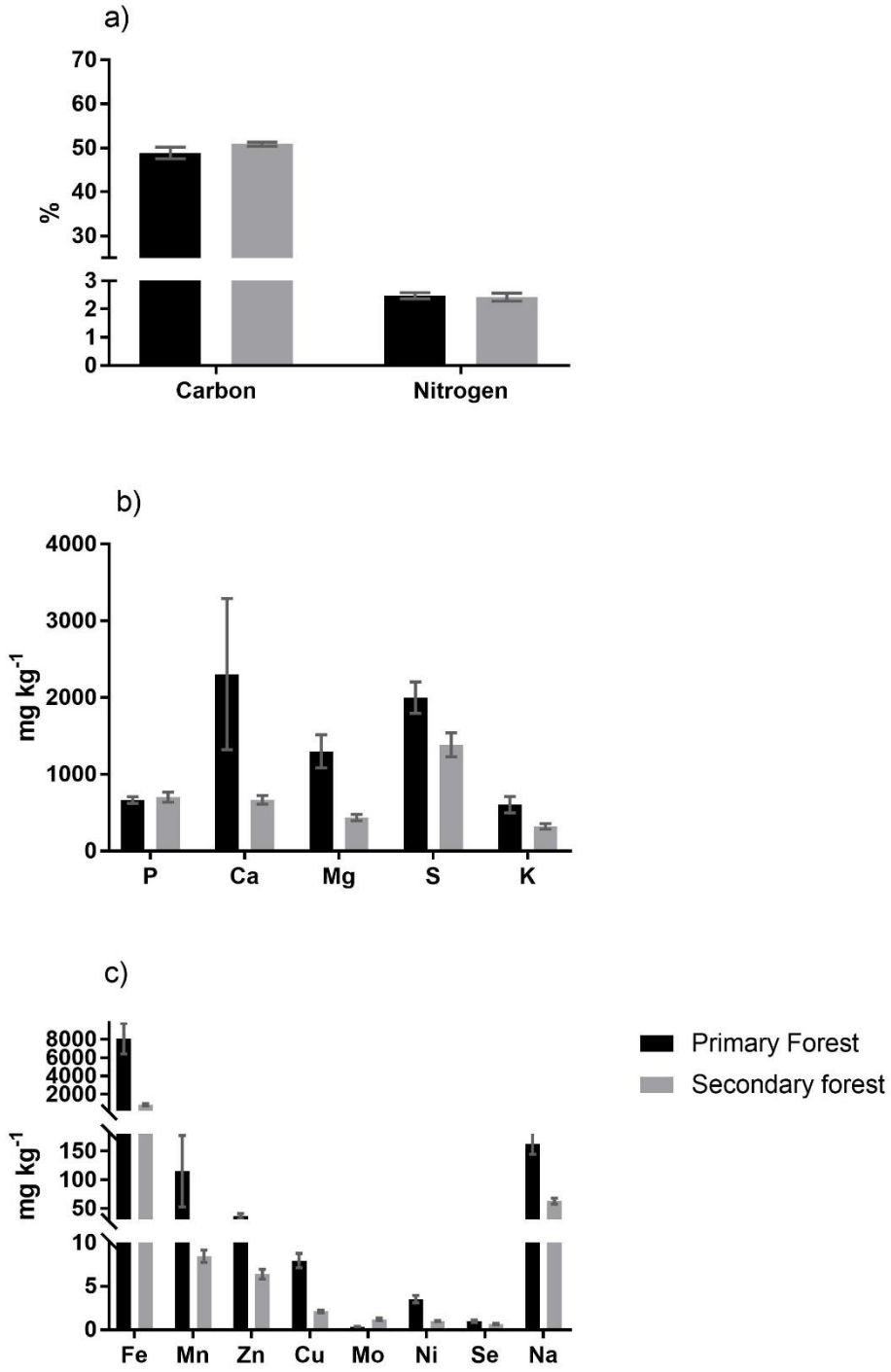



**Figure 4.2:** Effect of forest type upon (a) C and N content, (b) essential macronutrients, (c) essential micronutrients and trace elements, between the primary forest (black) and the secondary forest (grey), Bars denote mean values (n=10) and whiskers denote standard errors.

### 4.3.4 GHG emissions and environmental controls

Backward step-wise multiple regression analysis showed that $CO_2$ emissions were significantly predicted by peat moisture level (Fig. 4.3a), while none of the other measured environmental parameters was a significant predictor. For $CH_4$ emissions: moisture, pH and temperature were significant predictors of $CH_4$ emissions, $F_{(3,160)}$=21.90, p<0.001, $R^2$=0.28: $CH_4$= 0.697 +0.01153(pH) +0.02167(Temperature) +0.001148(Moisture)

The C:N ratio was positively correlated with $LogCH_4$ (Fig. 4.3b). Cu was negatively related to $LogCO_2$ (Fig 4.3c), and Mo was negatively related to $LogCH_4$ (Fig 4.3d).

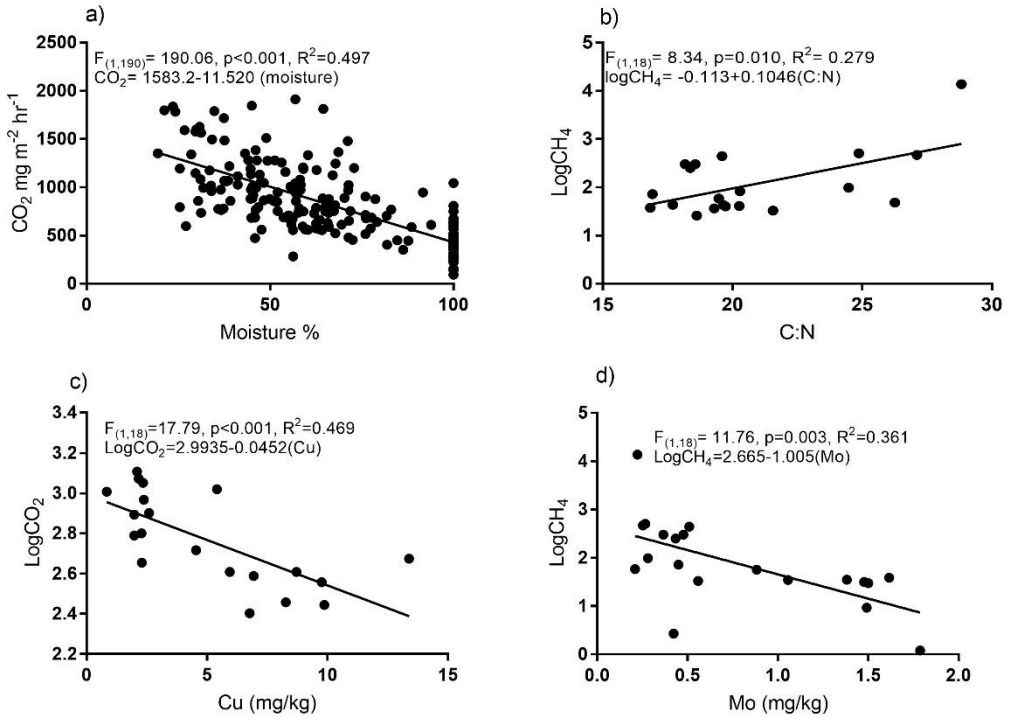



**Figure 4.3:** Relationship between (a) $CO_2$ and moisture, (b) $LogCH_4$ and C:N, (c) $LogCO_2$ and Cu concentration, (d) $LogCH_4$ and Mo concentration. Significant regression lines and their equations, $R^2$, F and p values are reported in the figures.

### 4.3.5 Peat microbial communities

#### *4.3.5.1 Effect of forest type and season*

PC1&2 collectively accounted for 64% of the total variations. PC1 significantly discriminated the two forest types from each other, while PC2 significantly discriminated the two seasons from each other (Fig. 4.5a). Both PC1&2 showed significant interaction between forest type and season (Table 4.2). PC1 significantly discriminated seasons in secondary forest, but not in the primary forest, resulting in significant interactions between site and season. Similarly for PC2, the difference between the seasons were greater in primary forest than in secondary forest, resulting in significant interactions. The loadings for individual PLFAs associated with PC1 showed two groupings (Fig. 4.5b). All the fungal biomarkers (*18:2n6, 18:1n9*) and all the monenoic Gram-negative biomarkers (*16:1n9, 16:1n7, 18:1n7*) were grouped together and were associated with separation of primary forest with respect to PC1. The separation of secondary forest with PC1 was aided by the grouping of all actinomycetes biomarker (*10me18:0, 10me16:0*) and most of the Gram-positive biomarkers (*i16:0, i17:0, i15:0*). Non-specific biomarkers particularly *a17:1* and *18:0* were associated with distinctness of wet season for both forest types with respect to PC2. Likewise, Gram-positive biomarkers *a17:0* and *a15:0* were strongly associated with distinctness of dry season for both forest types with respect to PC2.

**Table 4.2:** Linear mixed model (REML) for prinicipal component scores, showing statistical significance of the effects of forest type, season and the interaction between forest type and season.

| | **PC1** | **PC2** |
|---|---|---|
| **Forest type** | $F_{(1,16)}$=445, ***p<0.001*** | $F_{(1,16)}$=0.21, p=0.651 |
| **Season** | $F_{(1,16)}$=2.97, p=0.104 | $F_{(1,16)}$=127, ***p<0.001*** |
| **Season*Forest type** | $F_{(1,16)}$=11.4, ***p=0.004*** | $F_{(1,16)}$=10.6, ***p=0.005*** |

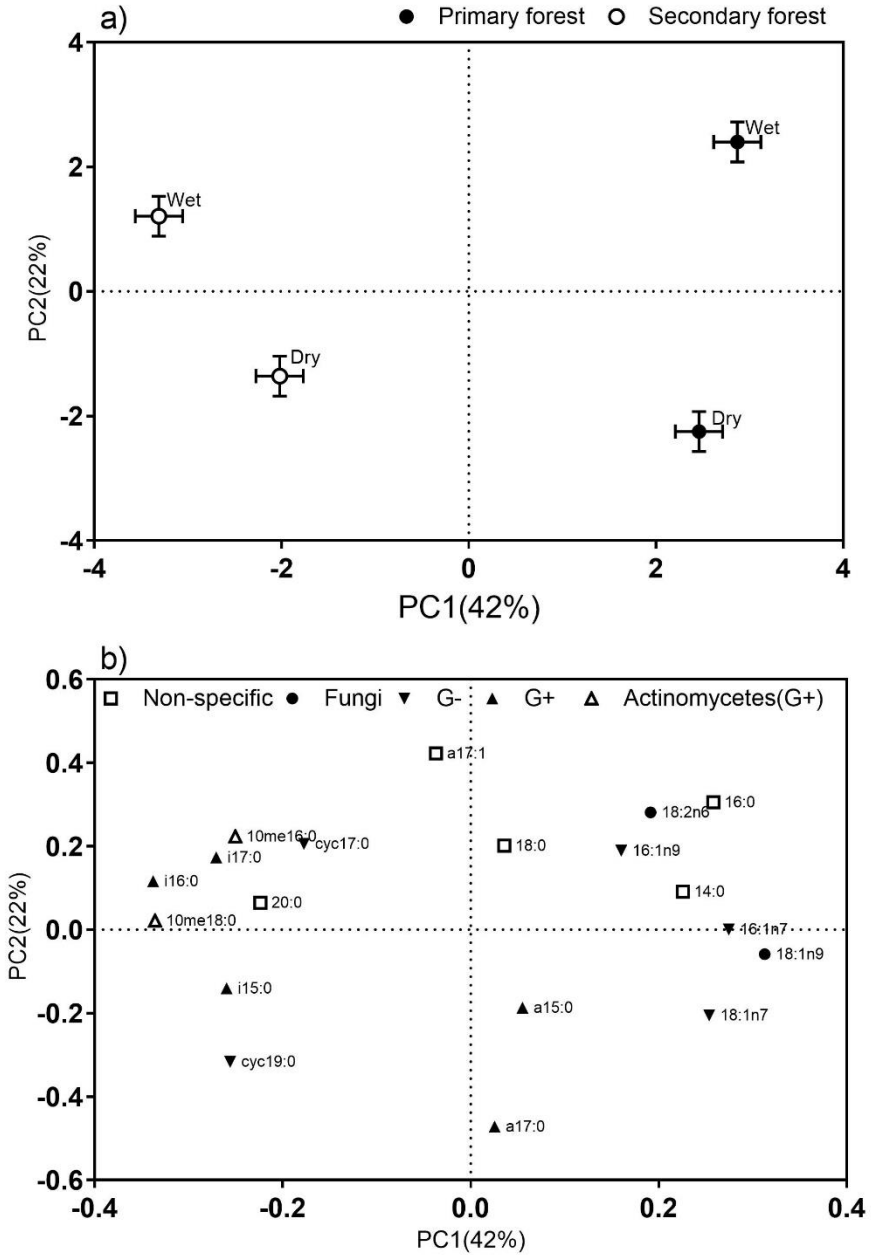



**Figure 4.4:** Effects of forest type and season upon phenotypic structure of soil microbial communities determined by PLFA analysis, as shown by principal component (PC) analysis. (a) ordination of PC1 and 2 and (b) associated loadings for individual PLFAs. For (a) points denote means (n=5), whiskers denote standard errors.

*4.3.5.2 Relative abundance and microbial community structure*

Both forest types were overwhelmingly dominated by bacteria over fungi (Fig. 4.5). Among bacterial groups, Gram-positive was the most dominant group in both seasons and forest types, except the pristine forest in dry season, which was dominated by Gram-negative bacterial group. The relative abundance of all the microbial groups significantly varied between the forest types. All the microbial groups, except fungi, significantly varied between seasons, and the interactions between site and season was significant only for Gram-negative and actinomycetes microbial groups (Table 4.3). The relative abundance of Gram-negative, fungi and non-specific fatty acids were greater in the pristine forest than the secondary forest. Gram-positive and actinomycetes relative abundances were greater in the secondary forest than the primary forest. The respective relative abundances of both Gram-positive and Gram-negative groups were greater in the dry than the wet season. Relative abundance of non-specific fatty acids were greater in wet season. Relative abundance of actinomycetes was greater in wet season for secondary forest, but did not differ between seasons at the primary forest site.

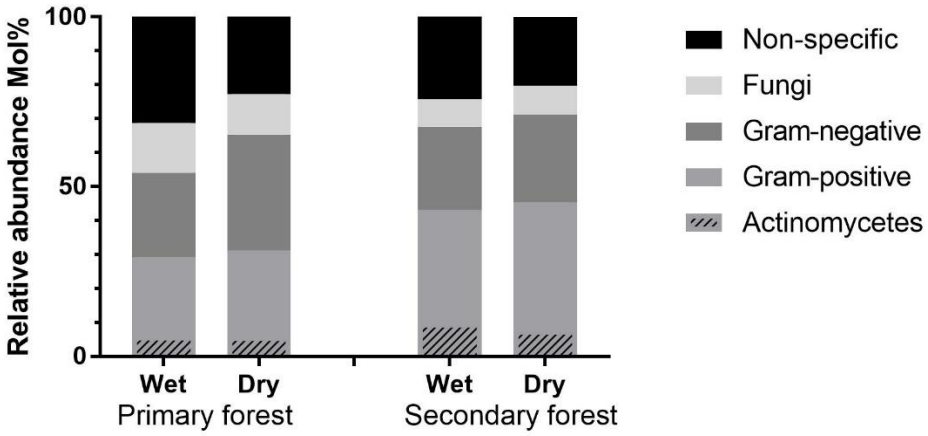



**Figure 5:** Relative abundance of different microbial groups as determined by PLFA analysis. Mean values are presented (n=5).

All the studied ratios namely, G+:G-, F:B, cyc:pre and mon:sat, were significantly different between the forest types (Fig 4.6 and Table 4.3). All the ratios except cyc:pre, were significantly different between seasons and also exhibited significant interactions between season and forest type. All the ratios except F:B, were greater in secondary forest than primary forest in both seasons (Fig 4.6 and Table 4.3). Secondary forest did not exhibit seasonal variations in any of the ratios, while in primary forest, all the ratios were greater in wet season than dry season, resulting in statistically significant interactions between season and forest type.

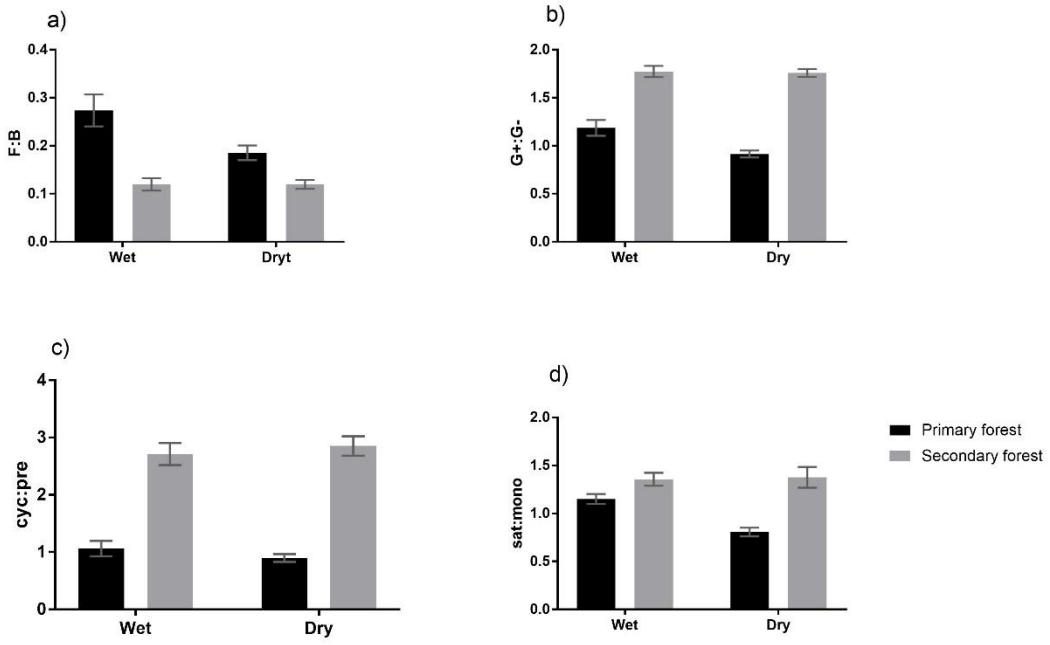



**Figure 4.6:** The difference in (a) fungi to bacteria relative abundance ratio (F:B), (b) Gram-positive to Gram-negative relative abundance ratio (G+:G-), (c) cyclopropane fatty acids (*cyc17:0, cyc19:0*) to their monenoic precursors (*16:1n7 & 18:1n7*) ratio (cyc:pre), and (d) saturated fatty acids (*14:0, 16:0, 18:0, 20:0*) to mono-unsaturated fatty acids (*16:1n9, 16:1n7, a17:1n, 18:1n9, 18:1n7*) ratio (sat:mono) (Black- primary forest, grey- secondary forest) during wet and dry season. Bars denote mean values (n=5) and whiskers denote standard errors.

**Table 4.3:** Linear mixed model (REML) for aggregated PLFA data with respect to microbial groups, showing statistical significance of the effects of forest type, season and the interactions between forest type and season.

| | Forest type | Season | Season*Forest type |
|---|---|---|---|
| **Non-specific** | $F_{(1,16)}$=27.5, ***p<0.001*** | $F_{(1,16)}$=52.5, ***p<0.001***` | $F_{(1,16)}$=3.63, p=0.075 |
| **Fungi** | $F_{(1,16)}$=28.1, ***p<0.001*** | $F_{(1,16)}$=1.36, p=0.260 | $F_{(1,16)}$=2.57, p=0.128 |
| **Gram-negative** | $F_{(1,16)}$=26.6, ***p<0.001*** | $F_{(1,16)}$=38.8, ***p<0.001*** | $F_{(1,16)}$=20.8, ***p<0.001*** |
| **Gram-positive** | $F_{(1,16)}$=150, ***p<0.001*** | $F_{(1,16)}$=12, ***p=0.003*** | $F_{(1,16)}$=1.30, p=0.271 |
| **Actinomycetes** | $F_{(1,16)}$=70.7, ***p<0.001*** | $F_{(1,16)}$=8.8, ***p=0.009*** | $F_{(1,16)}$= 4.7, ***p=0.046*** |
| **F:B** | $F_{(1,16)}$=30.2, ***p<0.001*** | $F_{(1,16)}$= 4.9, ***p=0.042*** | $F_{(1,16)}$=4.9, ***p=0.042*** |
| **G+:G-** | $F_{(1,16)}$=155, ***p=0.001*** | $F_{(1,16)}$=6.3, ***p=0.024*** | $F_{(1,16)}$=5, ***p=0.041*** |
| **Cyc:pre** | $F_{(1,16)}$=145, ***p<0.001*** | $F_{(1,16)}$=0.01, p=0.928 | $F_{(1,16)}$=1.03, p=0.324 |
| **sat:mono** | $F_{(1,16)}$=38, ***p<0.001*** | $F_{(1,16)}$=9.66, ***p=0.007*** | $F_{(1,16)}$=10.7, ***p=0.005*** |

### 4.3.6 Microbial communities and GHG emissions

Gram-positive and Gram-negative relative abundance showed a positive relationship with $CO_2$ emissions and Log$CH_4$, respectively (Fig 4.7a & b). Among ratios, cyc:pre was positively correlated with $CO_2$, and sat:mono ratios was negatively correlated with log$CH_4$ (Fig 4.7c & d). None of the other microbial groups or ratios, was a statistically significant predictor of GHG emissions.

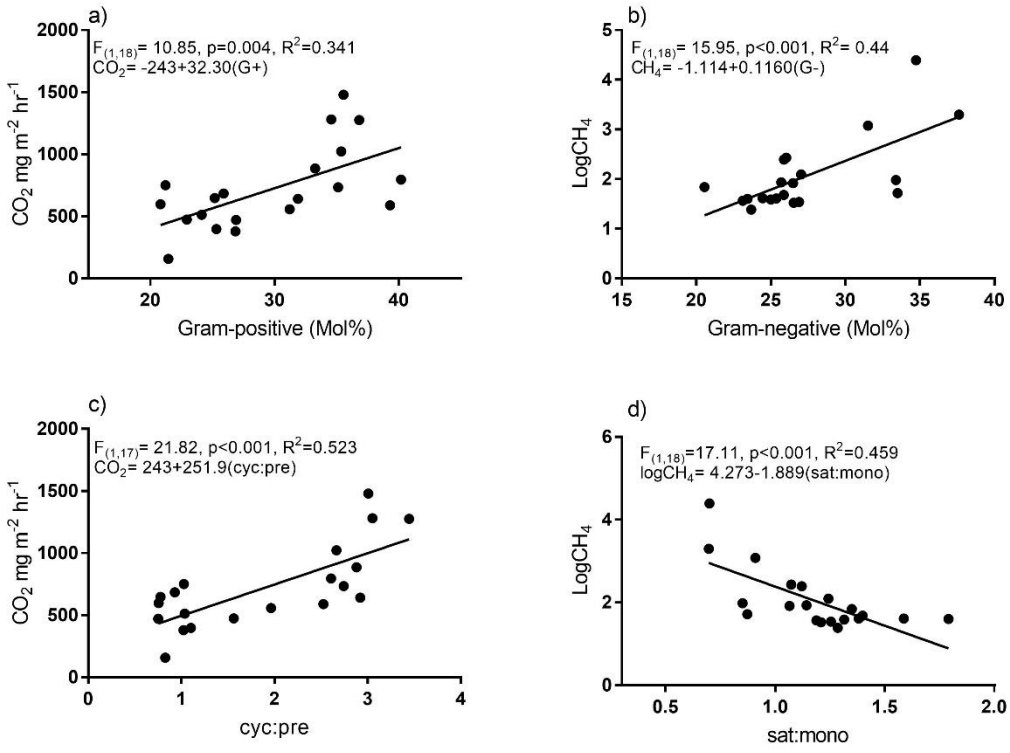



**Figure 4.7:** Relationship between (a) $CO_2$ emissions and Gram-positive (G+) relative abundance, (b) $LogCH_4$ and Gram-negative (G-) relative abundance, (c) $CO_2$ emissions and cyclopropane fatty acids (*cyc17:0, cyc19:0*) to their monenoic precursors (*16:1n7 & 18:1n7*) ratio (cyc:pre), (d) $LogCH_4$ and saturated fatty acids (*14:0, 16:0, 18:0, 20:0*) to mono-unsaturated fatty acids (*16:1n9, 16:1n7, a17:1n, 18:1n9, 18:1n7*) ratio (sat:mono).

## 4.4 Discussion

### 4.4.1 Lower total GHG emissions in primary forest

The peat properties such as loss on ignition, pH and temperature showed minimal changes between the forest types; however, there was a stark difference in $CO_2$ emissions. High $CO_2$ emissions in the drained forest was expected as the water level in the North Selangor peat swamp forests were reduced to below ground level during the times of logging and the drainage ditches still run through the forest. This drainage

exposes the surface peat to aerobic decomposition, unlike the primary forest in Terengganu which remained water logged throughout the seasons, with no history of drainage. Water level was the significant predictor of $CO_2$ emissions, showing a strong negative correlation, which is in agreement with previous studies (Couwenberg *et al.*, 2010, Hergoualc'h *et al.*, 2017, Jauhiainen *et al.*, 2005, Sangok *et al.*, 2017, Wakhid *et al.*, 2017). In mineral soil systems, secondary forests were found to have greater heterotrophic respiration than primary forests (Shi *et al.*, 2015). In tropical peat forest systems, Murdiyarso *et al.* (2010) found that heterotrophic respiration contributed to >50% of the total respiration in restored secondary forest, while Hergoualc'h *et al.* (2017) found *Ca.* 55% of the total $CO_2$ emissions were heterotrophic emissions in a primary forest with water table 25 cm below surface. Indeed, $CO_2$ emissions in the primary forest were 50.6% and 56.5% lower than that in the secondary forest during wet and dry season respectively, equivalent to the heterotrophic contribution reported by Hergoualc'h *et al.* (2017)in drier peat forest systems in SEA.

$CH_4$ emissions in the primary forest was at 2 mg $m^{-2}$ $hr^{-1}$, which is within the previously observed range for peat swamp forests in South East Asia (Couwenberg *et al.*, 2010, Sjögersten *et al.*, 2014). $CH_4$ emissions in the pristine peat swamp forest in SEA is markedly lower than what is observed in peatland ecosystems in other regions, including neotropics (up to 143 mg $m^{-2}$ $hr^{-1}$) (Sjögersten *et al.*, 2014). This stark difference in $CH_4$ emissions between climatically similar SEA peatlands and neotropical peatlands is possibly due to the difference in above ground vegetation and their influence on $CH_4$ emissions through species specific root exudation (Girkin *et al.*, 2018). However, the secondary forest in North Selangor peatlands oxidised methane due to far lower moisture level that forsake the prerequisite anoxic conditions for anaerobic decomposition. Moreover $CH_4$ oxidation in North Selangor peatlands

complements Jackson *et al.* (2009)'s finding that methanogenic bacteria were absent in the top 50 cm. The same study found Proteobacteria and Verrucomicrobia, the phyla that contain methanotrophic bacteria, in North Selangor peatland, plausibly explaining higher methane oxidation than production in the secondary forest. Our study also showed positive correlation of $CH_4$ emissions with moisture, temperature and pH. These positive correlations with $CH_4$ was observed in several other studies for moisture (Inubushi *et al.*, 2005, Melling *et al.*, 2005a), pH (Inubushi *et al.*, 2005), temperature (Aben *et al.*, 2017, Melling *et al.*, 2005a). The secondary forest had higher temperature and similar pH to the primary forest yet it oxidised methane due to moisture limitation. For peatland restoration, just restoring the water table level ignoring the canopy cover and vegetation that have impact on other peat properties such as temperature, pH and carbon input would possibly result in higher $CH_4$ emissions in secondary forest than what is observed in primary forest. Confirming to the first hypothesis, though the change in peat characteristics were minimal, greenhouse gas emissions were significantly different between the pristine and secondary peat swamp forest, potentially due to the difference in water table level.

### 4.4.2 Higher nutrient content in primary forest and their impact on GHG emissions

North Selangor peat swamp forests had undergone severe drainage in the past decades (Irvine *et al.*, 2013), which possibly have resulted in leaching and reduction of the nutrients (Kløve *et al.*, 2010) in contrast to the primary forest in Terengganu which had higher nutrient content. Loss of nutrients with drainage were observed in northern peatlands, where the nutrients further decreased with increasing age of drainage (Laiho and Laine, 1995, Sallantaus, 1992). Although most macronutrients were about 50% lower in the secondary forest, P was a notable exception, due to leaf litter addition, as P was found to be more rapidly released into the environment from leaf litter than

other nutrients at the secondary forest (Ong *et al.*, 2017). Micronutrients and trace elements except Mo and Se showed significant reduction in concentration in secondary forest in comparison to the primary forest. Some of these elements such as Na are known to have strong antimicrobial property that decrease aerobic activity (Macalady *et al.*, 1998, Rietz and Haynes, 2003, Sederholm, 2016, Wilke, 1987), while sometimes being favourable to anaerobic microbes (Lassiter *et al.*, 1963).

Cu has long been considered and proven to have antimicrobial property (Berg *et al.*, 2005, Gajjar *et al.*, 2009, Wheeldon *et al.*, 2008), and was also found to alter soil microbial communities (Sauvé *et al.*, 1998, Smit *et al.*, 1997), which could have very likely impacted the microbial communities involved in aerobic decomposition, resulting in reduced $CO_2$ emissions with increased concentration of Cu. Cu was also similarly known to affect anaerobic microbial communities, however the effect of Cu on methanotrophs are greater than their effect on methanogens (Mao *et al.*, 2015), and in environment with high C content, Cu concentrations were also found to favour $CH_4$ emissions (Jiao *et al.*, 2005). Therefore higher Cu concentration significantly impacted $CO_2$ emissions but not $CH_4$ emissions in our study.

However $CH_4$ emissions were negatively correlated with Mo concentrations. Mo is an essential element that influence $N_2$ fixation in peatlands (Warren *et al.*, 2017), In peatlands, methanotrophs are considered to play a major part in $N_2$ fixation (Larmola *et al.*, 2014), which is dependent on Mo for nitrogenase activity (Kaiser *et al.*, 2005). It is plausible that increased presence of Mo supports $N_2$ fixing bacteria that are predominantly also methanotrophs in peatlands (Vile *et al.*, 2014). Mo limitation may act as a mechanistic control on biological $N_2$ fixation, which likely affected the peatland methonotrophic communities (Vile *et al.*, 2014, Warren *et al.*, 2017). Mo is also an essential element in the activity of sulphite oxidase, the enzyme that reduces

sulphite to sulphate (Kaiser *et al.*, 2005). Such increase in sulphate concentrations could stimulate suphate-reducing bacteria that compete with methanogenic archaea for substrate (Dowrick *et al.*, 2006). Attributing to this, increase in sulphate concentrations were known to suppress $CH_4$ emissions in peatlands (Fowler *et al.*, 1995). Therefore, higher Mo concentrations may have benefited the methanotrophic community and suppressed methanogenic archaea in the secondary forest, resulting in higher methane oxidation in that site.

### 4.4.3 Tropical peatland microbial community structures and their relationship with GHG emissions

This study has demonstrated that microbial communities in natural tropical peat swamp forests were dominated by bacteria, regardless of hydrology and level of disturbance. Higher abundance of Gram-negative forms in primary forest was in accordance with Troxler *et al.* (2012)'s finding in neotropical peatlands and Krashevska *et al.* (2015)'s finding in tropical mineral soil systems, who also found that Gram-positive relative abundance increased with disturbance (Krashevska *et al.*, 2015). Fungi were generally observed to be more tolerant to acidic conditions than bacteria, and found to be more dominant in afforested systems, with low N inputs, as bacteria require more N per C biomass than fungi for substrate utilization (Bardgett, 2005, Bossuyt *et al.*, 2001, Fierer *et al.*, 2009). However, this did not hold true for tropical peat forest systems, even though the C:N ratio was low and the conditions were highly acidic. Bacteria often depend on fungal decomposition products in ecosystems with complex substrate input (Moore-Kucera and Dick, 2008), the bacterial dominance despite the complexity of SEA peat swamp forests, might be due to the availability of more labile C through leaf litter and dissolved organic C in these

ecosystems (Yule, 2010), and may also be due to fungal decomposition activity in leaf litter and plant parts before they are incorporated into peat soil.

The wetter conditions in undrained primary forest and in deeper layers in other disturbed ecosystems in SEA peatlands (Jackson *et al.*, 2009) (Chapter 2), might be the factor favouring Gram-negative over Gram-positive bacteria. This is further supported by the observation in this study that Gram-negative relative abundance was positively correlated with $CH_4$ emissions, which is a by-product of submerged anaerobic decomposition (Aben *et al.*, 2017), and Gram-positive relative abundance was positively correlated with $CO_2$ emissions, which is a by-product of aerobic decomposition. Indeed, results from (Jackson *et al.*, 2009) show both extracellular microbial activity and Gram positive relative abundance decreased with depth in North Selangor secondary forest. In addition, all the aerobic surface peat layers in different agricultural and forest systems were dominated by gram-positive bacteria (Chapter 2 and 3), strongly suggesting that Gram positive bacteria are an important contributor in aerobic decomposition in SEA tropical peatlands.

The cyc:pre ratio that were used as stress indicators were substantially higher in the secondary forest. The fatty acids *16:1n7* and *18:1n7* are transformed into *cyc17:0* and *cyc19:0* in stressful conditions as this adaptation helps maintaining functional living membrane under stressful conditions, and the cyclopropane fatty acids are more stable than their monoenoic precursors (Kaur *et al.*, 2005). This likely indicates that the secondary forest conditions were more stressful for the peat microbial communities.

Even though the changes in environmental parameters were minimal between seasons, the microbial community structure was significantly different between the seasons for both forest types. The seasonal changes in secondary forest that had water table below

surface may be due to sudden heavy downpour during the wet season, as the response of microbial communities to sudden flooding by rainfall can be very quick ranging from minutes to days (Bardgett and van der Putten, 2014). However the water table was above surface on both seasons for primary forest, yet the microbial community structure was significantly different between seasons, indicating the possibility that there are more factors influencing the seasonal variations other than flooding. Nevertheless, the sat:mono ratio that responded to flooding were higher in wet season than in dry season at the primary forest (Bossio and Scow, 1998), indicates increased physiological stress in the wet season (Willers *et al.*, 2015). Most of the saturated fatty acids were non-specific biomarkers in our study, also explaining the increased relative abundance of non-specific PLFAs in wet season for both forest types, and also explaining the higher proportion of non-specific fatty acids in submerged primary forest peat. Higher sat:mono ratio is used as an indication of nutritional stress (Kieft *et al.*, 1994, Moore-Kucera and Dick, 2008), and the ratio was also known to increase with flooding (Bossio and Scow, 1998). The ratio was higher in secondary forest, despite the flooded conditions in the primary forest. This might be due to greater nutrient content and greater C input, resulting in higher substrate availability in the primary forest (Bossio and Scow, 1998). Similarly, greater nutrient availability may be the factor that influenced greater fungal abundance in primary forest than the secondary forest, resulting in higher F:B ratio in primary forest. Confirming the second hypothesis, the microbial community structure was significantly different between pristine and secondary forest due to the differences in hydrology and nutrient content, and as anticipated all these changes exhibited some significant correlations with greenhouse gas emissions, resulting in lower CH4 emissions and higher CO2 emissions in the secondary forest.

## 4.5 Conclusion

Drainage and selective logging of peat swamp forests severely affect peatlands' ability to function as a carbon sink. The drier conditions in the secondary forest favoured Gram-positive bacteria over Gram-negative bacteria, thereby increasing aerobic microbial activity and $CO_2$ emissions in secondary peat swamp forests. Drainage in secondary forest also resulted in loss of essential nutrients including some containing antimicrobial properties that inhibit aerobic activity. Though the secondary forest oxidised methane contrasting $CH_4$ emissions in the primary forest, it was insignificant in comparison to the scale of increase in $CO_2$ emissions in the secondary forest from the primary forest. It is important to conserve the last few remaining unprotected pristine peatlands under national law, irrespective of their area.

# Chapter 5

## 5. Effect of proximity to plants on peat characteristics and GHG emissions under different cropping systems in tropical peatlands

### 5.1 Introduction

Peatlands are formed because of primary production exceeding microbial decomposition of such produced material, resulting in accumulation of partially-decomposed plant organic matter (Daf, 2009, Miettinen *et al.*, 2012, Parish *et al.*, 2008). Peatlands cover about 2.85% of the global land surface area (Xu *et al.*, 2018), yet store one third of the total global carbon pool (Hapsari *et al.*, 2017). Though most peatlands are located in the northern hemisphere, there is a considerable cover of these carbon rich ecosystems in the tropics, which store 18% of total global peat carbon (Hapsari *et al.*, 2017, Strack, 2008), most of which are in South East Asia (Dohong *et al.*, 2017). Tropical peatlands are dramatically different from boreal and temperate peatlands in terms of above-ground vegetation and the biodiversity they support (Yule,

2010). Unlike the northern temperate and boreal peatlands, tropical peatlands are carbon rich both above- and below-ground, and are all active throughout the year, building up 200% more carbon every year per unit area than northern peat bogs (Guo and Gifford, 2002, Strack, 2008, Yule, 2010). Hydrology is a major driving factor in the formation of tropical peatlands, inhibiting aerobic decomposition by reducing oxygen availability (Parish *et al.*, 2008, Wösten *et al.*, 2008). Drainage of peatlands for agriculture and logging have severely affected the South East Asian peatlands and their functions in recent decades (Couwenberg *et al.*, 2010).

Agricultural expansion, especially oil palm plantation remains as one of the biggest threats to Malaysian forests including peatlands (Lo and Parish, 2013, Tan *et al.*, 2009). Oil palm is native to west Africa and first introduced to Malaysia as an ornamental plant in 1875 (Abdullah *et al.*, 2009). The commercial use of the plantations started at early 20$^{th}$ century on a smaller scale, which had grown extensively in industrial scale in recent decades to an extent that Malaysia is currently the second largest producers of palm oil in the world and has the highest deforestation rate in the 21$^{st}$ century of all the countries in the world (Abdullah *et al.*, 2009, Hansen *et al.*, 2013, Strack, 2008). The peatlands are usually drained and completely cleared of forest vegetation to establish oil palm plantation, effecting carbon loss above-ground with loss of dense and large vegetation structures (Bruhl and Eltz, 2010, Luskin and Potts, 2011).

Though most of the rapid expansion in last few decades were industrial plantations, there is considerable cover of small holder plantations in Malaysia (Miettinen *et al.*, 2016). Particularly in Peninsular Malaysia, about half of the managed peatlands were small holder plantations (Miettinen *et al.*, 2016). Small holder plantations follow different cropping systems depending on local needs, and vary greatly in intensity

compared to industrial plantations (Azhar *et al.*, 2011) . Furthermore, small holders often practice intercropping and multiple cropping with oil palm. However, the GHG emissions, environmental conditions and microbial communities on these cropping systems in tropical peatlands are virtually unknown.

Varied cropping systems brings in an increased diversity in above ground vegetation, which were known to impact temporal and spatial variations of soil respiration (Han *et al.*, 2014, Johnson *et al.*, 2008). Plants also add rhizosphere microbial communities to a habitat, often with cell densities that are greater than the cell density of the root system itself (Mendes *et al.*, 2013), which could possibly impact GHG emissions from soil. Increased diversity in vegetation above ground can also diversify the carbon source that's available for microbial decomposition, thus potentially affecting both microbial community composition and activity (Blagodatskaya and Anderson, 1998), which could in turn effect GHG emissions. Multiple cropping systems generate varied patches of microclimate, with high habitat heterogeneity that creates uncertainty in the estimation of GHG emissions from a habitat (Azhar *et al.*, 2015, Han *et al.*, 2014). Therefore, understanding the rhizosphere influence of different plants on total GHG emissions is crucial to evaluate the environmental impacts of these different cropping systems followed in tropical peatlands.

Total soil respiration is composed of autotrophic root respiration, and microbial decomposition through heterotrophic activity (Hergoualc'h *et al.*, 2017). The root respiration does not contribute to C loss, and often contributes to a larger portion of total emissions measured in ecosystems with dense and large trees such as tropical peat swamp forests (Hergoualc'h and Verchot, 2011, Murdiyarso *et al.*, 2010). Oil palm has a fibrous root systems that is divided into primary, secondary, tertiary and quaternary roots (Dariah *et al.*, 2014). The root density decreasing with increasing

distance from the stem (Dariah *et al.*, 2014) and the majority of roots are present within the first 60 cm from the surface (Mutert, 1999). The contribution of oil palm rhizosphere to total soil respiration was observed to be dependent on the age of the oil palm plants with increased autotrophic contribution with higher age of oil palm (Dariah *et al.*, 2014). There are a few, yet limited number of studies on autotrophic and heterotrophic component of GHG emissions from oil palm plantations, but the microbial communities associated with those difference in GHG emissions between bulk soil and rhizosphere remain unexplored.

To address this knowledge gap, we studied two intercropping systems, and two mono-cropping systems of two different ages and generations to evaluate the influence of the proximity of plants on GHG emissions. We hypothesised that the environmental parameters and GHG emissions are contingent on the nearby vegetation and vegetation age in a cropping system, due to the influence of such vegetation on microclimate, carbon substrate supply, and associated rhizosphere microbial communities.

## 5.2 Materials and Methods

### 5.2.1 Study sites

#### *5.2.1.1 First generation oil palm mono-cropping site*

The oil palm mono-cropping site (3°25'25.8"N 101°20'12.9"E) was located on a border adjacent to Raja Musa natural reserve on the southern edge of the North Selangor peatlands. The oil palm plantations were about 15 years old. The site borders oil palm plantations which were left for forest regeneration inside Raja Musa protected forest reserve. A drainage ditch, running in the middle, divides the site into two halves. The site had a sizeable cover of understorey vegetation, predominantly ferns. There

was abundant decaying dead wood of non-oil palm plants on the site. There were some dead cut stems of other plants and trees of previous land use, visibly protruding the surface.

There was no observable change in the physical environment such as vegetation, surface flooding etc., at this site between wet season and dry season sampling periods. This site was denoted as '1st gen OP'.

#### *5.2.1.2 Second generation oil palm mono-cropping site*

This young oil palm site (3°24'51.3"N 101°19'42.7"E) was located in Kampung Raja Musa village. The plantation itself is second generations with the age of 3-5 years old. The first generation oil palm trees were killed off chemically and were still standing on the site. Most of the site surface was covered with dead, dried and brown grass. During the wet season, there were green algal growths on the surface under the shades of young oil palm trees.

During the dry season measurements, most of the grass on surface were cleared and there were no algal growth on the surface. This site is denoted as '2$^{nd}$ gen OP'

#### *5.2.1.3 Second generation oil palm and pineapple intercropping*

The oil palm and pineapple intercropping site (3°25'20.6"N 101°19'56.6"E) in Kampung Raja Musa consisted of *circa* one year-old oil palm plants in rows with pineapple planted densely between the oil palm rows. There were two drainage ditches in the border on the either side of the site, but none within the site. There were stagnant water on most part of the site during the wet season measurements. Some open regions without any stagnant water were covered with grass.

During dry season measurements, the pineapple plants were fully grown and covered any remaining open spaces between pineapple rows and there was no stagnant water at the surface. The site is denoted as ‘pineapple intercropping’.

#### *5.2.1.4 Second generation oil palm and yam intercropping site*

The oil palm and yam intercropping site (3°25'22.7"N 101°18'46.7"E) was a site containing one year-old oil palms planted in rows with ample space in between, where four to six rows of yam were planted. There was a cleared open space between each paired yam and oil palm rows. The surface of the site was relatively dry and flat with numerous dead oil palm roots of the previous generation of plantation. The edge of the site were cropped with pineapple plants on the available spaces in between yam and oil palm rows.

During the dry season, yam crops were harvested and a new pineapple crop were planted in the open areas. The pineapple crop newly planted and presumably the root structures were in their preliminary stage. There were smaller yam saplings in place of the older harvested yam. The site is denoted as ‘yam intercropping’ for both wet and dry season results.

### 5.2.2 Sampling strategy

Sampling was carried out during both the wet and dry seasons. The wet season sampling was carried out during November 2016 to January 2017 and the dry season sampling was done during July 2017. Each site was visited three times during each season. At each time, samples were collected from 25 random points distributed over the site. At each sampling point, greenhouse gas measurements were taken and soil samples were collected for laboratory analyses.

The sampling types within a site were represented depending on whether they were proximal or distant from a plant. The notation used here is 'P' for proximal with subscript explaining the neighbouring plant, followed by the distance from the plant, and 'D' for sampling type distant from plants. Hence oil palm, yam and pineapple were denoted in subscripts as $P_{OP}$, $P_{YA}$, $P_{PA,}$ respectively. The distances are denoted as 'm' and 'cm' for metres and centimetres and respectively. For second generation oil palm site, some of the measurements were taken near (<1 m from) the dead standing oil palm tree trunks and were denoted as '$P_{OPD<1m}$'.

### 5.2.3 Greenhouse gas measurements

$CO_2$ and $CH_4$ emissions from soil surface were measured using a Los Gatos (San Jose, California, USA) ultraportable greenhouse gas analyser. The gas analyser works on the principle of laser absorption spectroscopy and gives readings of $CH_4$ and $CO_2$ ppm as well as gas temperature. The measurements were made using closed chamber method using a chamber with a height of 15 cm and the inner diameter of 13.5 cm. The chamber had an inlet and an outlet port that were connected to the gas analyser, using a quarter inch outer diameter polytetrafluoroethylene (PTFE) tube. During each measurement about 1 cm of the chamber was carefully inserted into the ground until it was sealed to the ground surface, and gas measurements were taken for 5 minutes. There was no surface vegetation in any of the measurement points. The gas analyser was set to record gas flux every 20 seconds, resulting in at least 12 recorded measurement points for each plot. The first minute of each measurement was ignored allowing the gas flux to settle down after initial disturbance of placing the chambers. The gas measurements in ppm were converted to mg $CO_2$ $m^{-2}hr^{-1}$ and µg $CH_4$ $m^{-2}hr^{-1}$ for $CO_2$ and $CH_4$ respectively, as described in (Samuel and Evers, 2016), using the ideal gas law PV=nRT. Where: P = atmospheric pressure; V = volume of headspace;

n = number of moles (mol); R = universal Gas Constant law (8.314J. $K^{-1}mol^{-1}$) and T = temperature in kelvin (K), with conversion factor, 1 mol of $CO_2$ = 44.01g and 1 mol $CH_4$ = 16.02g.

### 5.2.4 Soil properties

Soil temperature and moisture were measured *in situ*, using a digital thermometer from Fischer Scientific® and a theta probe® digital volumetric moisture meter, respectively.

For pH measurements, about 5 ml volume of peat sample was diluted in 10 ml deionised water in a centrifuge tube and shaken in a rotary shaker for 30 minutes. The pH of the supernatant was then measured using Mettler Toledo® pH meter.

Oven dried peat samples were used to calculate the organic matter content. Dried peat samples were placed in silica crucibles and then transferred to a muffle furnace and maintained at 550$^{o}$C for 4 hours. The organic matter content was then calculating the loss on ignition as follows, organic matter content (%) = [(weight of oven dried soil – weight of ash) / weight of oven dried soil] ×100.

### 5.2.5 Statistical analyses

All the statistical analyses were carried out using Genstat® 17$^{th}$ edition (VSN international, 2017). The significance of differences between sites for greenhouse gas emissions and other environmental parameters were evaluated using linear mixed models with restricted maximum likelihood (REML). To meet normality assumptions for the data that were not normally distributed, log transformation was used. If the log transformed data was not normally distributed, Boxcox transformation was used.

## 5.3 Results

### 5.3.1 $CO_2$ emissions

The difference between $CO_2$ emissions from different sampling types within a site was significant only at the 1st generation oil palm mono-cropping and yam intercropping sites. In 1st generation oil palm site, $CO_2$ emissions at $P_{OP<2.5m}$ was higher than the $CO_2$ emissions at D (Fig 5.1 & Table 5.1). In the yam intercropping site, $CO_2$ emission was highest in $P_{YA<30cm}$ and lowest in $P_{PA<30cm}$, while the $CO_2$ emissions did not significantly vary between $P_{OP<1m}$ and D in that site.

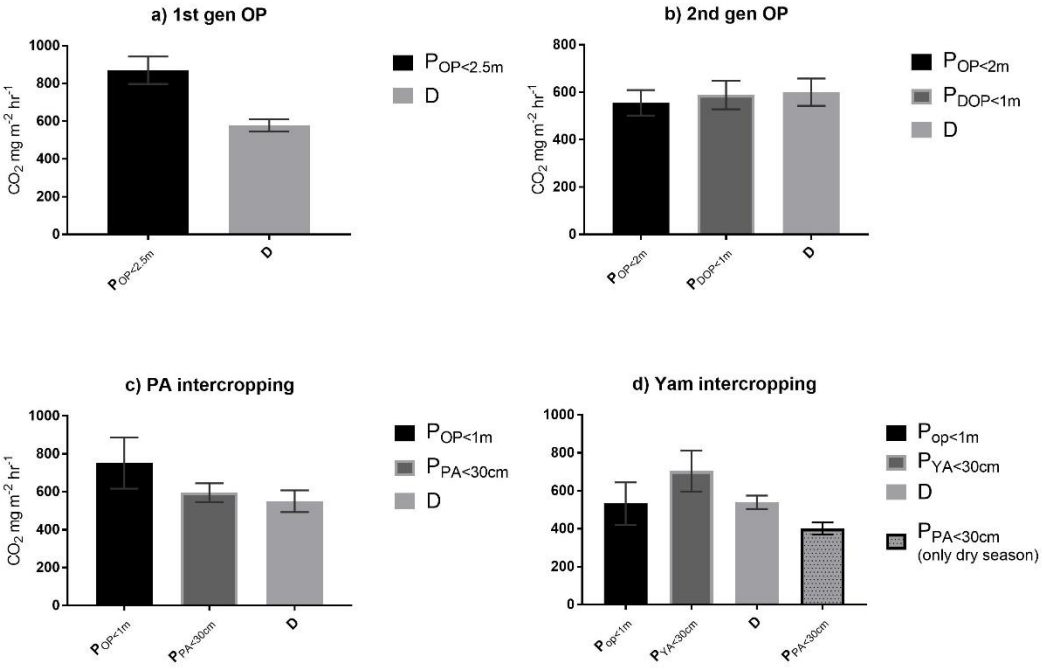



**Figure 5.1:** Effect of sampling type upon $CO_2$ emissions under different oil palm cropping systems. Bars denote mean values (n varies, see text) and whiskers denote standard errors. Note 1st gen OP denotes 1st generation oil palm mono-cropping, 2nd gen OP denotes 2nd generation oil palm mono-cropping, pineapple denotes 2nd generation oil palm and pineapple intercropping, yam denotes 2nd generation oil palm

and yam intercropping, pineapple denotes 2nd generation oil palm and pineapple intercropping.

### 5.3.2 $CH_4$ emissions

$CH_4$ emissions differed between sampling types within a site for all sites except 1st generation oil palm site (Fig 5.2 and Table 5.1). For 2nd generation oil palm site, $P_{OP<1m}$ absorbed $CH_4$ on both seasons, while the other respiration types emitted similar lower levels of $CH_4$.

$CH_4$ emissions within pineapple intercropping was highest at $P_{PA<30cm}$ followed by $P_{OP<1m}$, while $CH_4$ emissions at D was near zero. $CH_4$ emissions within yam intercropping site was highest at $P_{YA<30cm}$, and the variations between other sampling types were insignificant Similar to $CO_2$ emissions, planting of new pineapple crop during dry season did not affect $CH_4$ emissions in a short term.

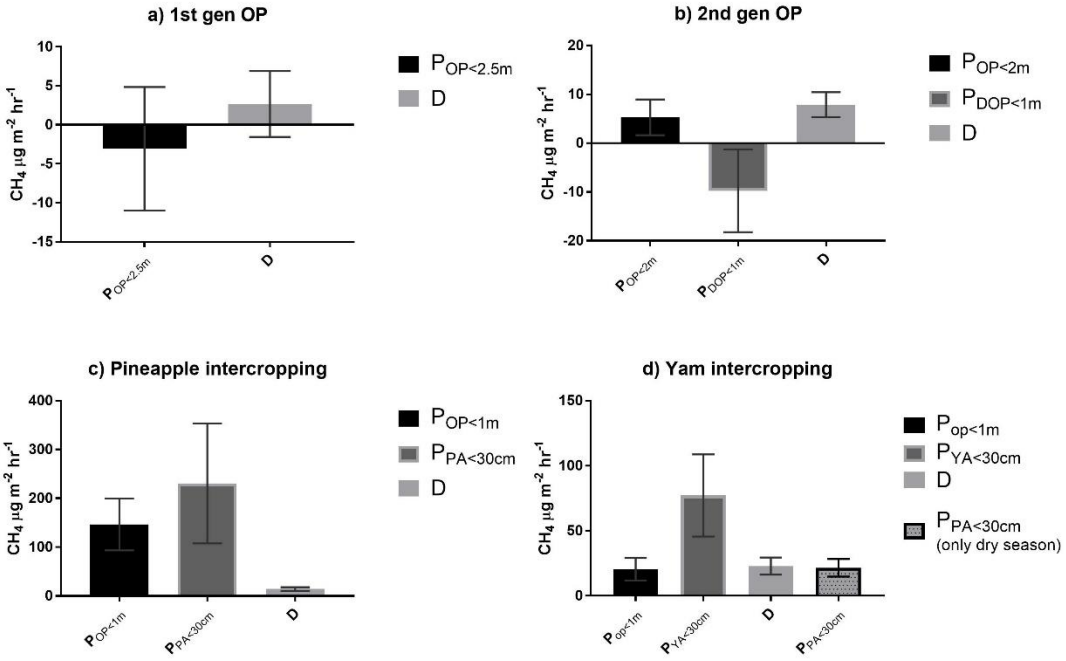



**Figure 5.2:** Effect of sampling type upon $CH_4$ emissions under different oil palm cropping systems. Bars denote mean values (n varies, see text) and whiskers denote standard errors. Note 1st gen OP denotes 1st generation oil palm mono-cropping, 2nd

gen OP denotes 2nd generation oil palm mono-cropping, pineapple denotes 2nd generation oil palm and pineapple intercropping yam denotes, 2nd generation oil palm and yam intercropping, pineapple denotes 2nd generation oil palm and pineapple intercropping.

**Table 5.2:** Linear mixed model (REML) for GHG emissions and environmental parameters, showing statistical significance of the effects of sampling type.

| | 1st gen OP | 2nd gen OP | Pineapple | Yam |
|---|---|---|---|---|
| $CO_2$ | $F_{(1,88)}$=16.4, ***P<0.001*** | $F_{(2,107)}$=0.40, p=0.670 | $F_{(2,103)}$=1.11, p=0.333 | $F_{(3,146)}$=5.93, ***p<0.001*** |
| $CH_4$ | $F_{(1,88)}$=0.85, p=0.358 | $F_{(2,107)}$=3.47, **p=0.035** | $F_{(2,103)}$=5.17, **p=0.007** | $F_{(3,146)}$=3.52, **p=0.017** |
| Organic matter % | $F_{(1,86)}$=4.19, ***p=0.044*** | $F_{(2,107)}$=6.29, **p=0.003** | $F_{(2,104)}$=3.71, ***p=0.028*** | $F_{(3,145)}$=3.25, ***p=0.024*** |
| Moisture | $F_{(1,88)}$=6.76, ***p=0.011*** | $F_{(2,107)}$=2, p=0.141 | $F_{(2,104)}$=0.26, p=0.775 | $F_{(3,146)}$=0.80, p=0.495 |
| pH | $F_{(1,86)}$=3.15, p=0.079 | $F_{(2,107)}$=4.13, **p=0.019** | $F_{(2,104)}$=0.72, p=0.489 | $F_{(3,145)}$=16.30, ***p<0.001*** |
| Temperature | $F_{(1,88)}$=2.13, p=0.148 | $F_{(2,107)}$=2.24. p=0.111 | $F_{(2,100)}$=0.88, p=0.419 | $F_{(3,146)}$=4.04, ***p=0.009*** |

### 5.3.3 Peat characteristics

Organic matter content varied only slightly yet significantly between the sampling types in all studied sites (Tables 5.1 & 5.2). For 1st generation oil palm $P_{OP}$ had lower organic matter content than D. For 2nd generation oil palm, organic matter content was highest at $P_{OPD<1m,}$ followed by $P_{OP<2m}$ and then D. For pineapple intercropping, organic matter content lower in $P_{PA<30cm}$ than at other sampling types. For yam intercropping, organic matter content was highest at $P_{OP<1m}$ and lowest at $P_{YA<30cm}$.

The changes in moisture between the sampling types was significant only for the 1st generation oil palm mono-cropping, here $P_{OP<2.5m}$ had lower moisture than D.

The variations in pH between the sampling types were significant for all sites except pineapple intercropping site, where pH was about 3 at all respiration types. For 1st generation oil palm, pH was higher in $P_{OP<2.5m}$ than D. For 2nd generation oil palm, pH was higher near the dead oil palm at $P_{OPD<1m}$, while the other sampling types had a similar pH. For the yam intercropping site, the significant difference was because of higher pH at $P_{YA<30cm}$, while all the other sampling types had a similar pH. Temperature was significantly different between the sampling types only for yam intercropping site, due to higher temperature in D and $P_{PA<30cm}$ region.

**Table 5.2:** Environmental parameters at different sampling types under different sites. Note 1st gen OP denotes 1st generation oil palm mono-cropping, 2nd gen OP denotes 2nd generation oil palm mono-cropping, pineapple denotes 2nd generation oil palm and pineapple intercropping yam denotes, 2nd generation oil palm and yam intercropping, pineapple denotes 2nd generation oil palm and pineapple intercropping

| Site | Position | LOI | pH | Moisture | Temperature |
|---|---|---|---|---|---|
| 1st gen OP | $P_{op<2.5m}$ | 81.5 ±0.88 | 3.29 ±0.07 | 22.7 ±2.03 | 29.6 ±0.18 |
| | D | 83.5 ±0.53 | 3.16 ±0.04 | 30.4 ±1.74 | 29.3 ±0.12 |
| 2nd gen OP | $P_{OP<2m}$ | 53.9 ±1.83 | 3.5 ±0.09 | 28.4 ±2.88 | 27.8 ±0.17 |
| | $P_{OPD<1m}$ | 59.1 ±2.76 | 3.8 ±0.1 | 32.4 ±4.63 | 28.3 ±0.28 |
| | D | 49.7 ±1.25 | 3.47 ±0.05 | 34.7 ±1.47 | 28.2 ±0.12 |
| Pineapple | $P_{OP<1m}$ | 88.6 ±1.36 | 2.94 ±0.07 | 59.7 ±9.47 | 28 ±0.28 |
| | $P_{PA<30cm}$ | 86.6 ±0.84 | 3.08 ±0.04 | 64.1 ±2.16 | 28.1 ±0.10 |
| | D | 90.4 ±0.85 | 3.05 ±0.07 | 61.9 ±4.06 | 28.3 ±0.17 |
| Yam | $P_{op<1m}$ | 83.8 ±2.37 | 3.25 ±0.12 | 34.7 ±7.27 | 28.5 ±0.33 |
| | $P_{YA<30cm}$ | 74.2 ±2.08 | 4.07 ±0.10 | 39.6 ±2.41 | 28.8 ±0.23 |
| | D | 79.3 ±1.38 | 3.24 ±0.08 | 36.4 ±1.39 | 29.3 ±0.22 |
| | $P_{PA<30cm}$ | 81.2 ±1.80 | 3.4 ±0.08 | 35.3 ±1.88 | 29.7 ±0.07 |

## 5.4 Discussion

### 5.4.1 Effect of plant proximity on $CO_2$ emissions

$CO_2$ emissions were significantly influenced by proximity to oil palm only for the cropping system that had mature oil palm trees. The $CO_2$ emissions at $P_{OP<2.5m}$ was 33.5% higher than the $CO_2$ emission at D, which is in the similar range to the 32.5% autotrophic contribution reported in 14 year old 1st generation oil palm plantations in Peninsular Malaysia (Matysek *et al.*, 2017). However, it was slightly higher than the 29% autotrophic contribution reported by Dariah *et al.* (2014) at 15 year old oil palm plantations in Indonesia. It should be noted that there was no difference in $CO_2$ emissions between the measurements made near recently dead oil palm tree, $P_{OPD<2m}$ and other areas in 2nd generation oil palm mono-cropping site, showing the significant contribution of autotrophic root respiration from mature oil palm trees is instantaneously reduced after the death of mature oil palm. Nonetheless, the second generation mono-cropping site also had oil palm plants about 5 years old, and their contribution to the total soil respiration was not significant enough to make a difference in total $CO_2$ emissions near the young palm trees. Indeed, Dariah et al. (2014) concluded that autotrophic respiration from 6 year-old oil palm plants contributed only about 14% of the total respiration. This is strikingly different from the results of Hergoualc'h *et al.* (2017) which showed autotrophic emissions from a 6 year old plantation contributed 39% to the total $CO_2$ emissions. For any future studies of plantations with older oil palm trees, GHG emission measurements can be stratified based on the distance from the nearby tree and area weighted calculation can be used to get total emissions from a habitat.

Yam is tuber crop with adventitious rooting system preceded by temporary seminal or tubercular rooting system (Charles-Dominique *et al.*, 2009). The yam plants in our

study were mature during wet season measurements, and the dry season measurements were made when the older yam plants were harvested and replaced with new younger plants, possibly containing the temporary seminal or tubercular rooting system. The root length density of yam plants are usually low with a maximum of 0.25 cm $cm^{-3}$. The root system can grow as far as 5 m horizontally and up to 40 cm depth (O'Sullivan, 2008), however (Hgaza *et al.*, 2012) did not find any roots at 15-30 cm depth at the distance of 50 cm from yam plant. The contribution of yam root respiration to total soil respiration has not been studied before, but our study has demonstrated that they make a significant contribution to total $CO_2$ emissions, with about 23.4% increase in $CO_2$ emissions than what is observed in D.

Pineapple root systems are adventitious, shallow and can grow well beyond the length of the associated plant's leaves (DHA, 2008). A single plant can produce about 450 main roots, which do not regenerate if damaged (Daf, 2009). The shallow root system can grow up to 1- 2 m long and about 0.85 m deep (DHA, 2008). The pineapple intercropping system in our study was densely planted and none of the $P_{OP<1m}$ and D, measurements were more than 1 m distant from a mature pineapple plant. It is possible that all the measuring points had some influence of pineapple root respiration. The oil palm plants in the pineapple intercropping were less than a year old and contribution of their root respiration was presumably minimal. Although the $CO_2$ emissions from $P_{OP<1m}$ and D did not significantly differ between each other.

### 5.4.2 Effect of plant proximity on $CH_4$ emissions

High ground water level is essential to create anoxic conditions, required for anaerobic decomposition that produces $CH_4$ (Page and Hooijer, 2016). This was clearly reflected by distinctly high $CH_4$ emissions in pineapple intercropping site, which had water table above surface during the wet season measurements. All the other sites were

drained with relatively dry surface peat layers, and water tables below surface. Nonetheless, $CH_4$ emissions were very low and well under 1 mg $m^{-2}$ $hr^{-1}$ at all the sampling types in the studied sites including the pineapple intercropping site.

Another distinct trend was higher $CH_4$ emissions near the plants with adventitious root systems such as yam ($P_{YA<30cm}$) and pineapple ($P_{PA<30cm}$), even when the moisture level did not spatially vary between different sampling types within their respective sites (Tables 5.1& 5.2). Ground vegetation composition could significantly influence methane emissions from soil through root exudates, along with other environmental conditions such as moisture, temperature, pH and organic content (Bhullar *et al.*, 2014, Micallef *et al.*, 2009). It is plausible that root exudation and rhizosphere communities from yam and pineapple root systems support methanogenic communities contributing to the $CH_4$ emissions. Conversely, regions near mature live ($P_{OP<2.5m}$) and dead oil palm trees ($P_{OPD<1m}$) oxidised $CH_4$, this might be due to the mature trees rhizosphere communities containing methanotrophs, which also stayed after the death of the mature palm plants. However, the regions near young oil palm plants ($P_{OP<1m)}$ and $P_{OP<2m}$) emitted $CH_4$. Rhizosphere communities are known to change with different development stage of the plants (Chaparro *et al.*, 2014, Micallef *et al.*, 2009). It is possible that oil palm rhizosphere communities changed from greater abundance of methanogens to greater abundance of methanotrophs with increasing age. This also explains significantly higher $CO_2$ emissions observed at $P_{OP<2.5m.}$ Another factor that possibly influenced higher methane oxidation and increased $CO_2$ emissions near the mature oil palm in the first generation mono-cropping, was the observed significantly lower moisture level at $P_{OP<2.5m}$ than at D. as $CO_2$ emissions in tropical peatlands are known to increase with decreasing moisture (Couwenberg *et al.*, 2010, Hergoualc'h *et al.*, 2017, Jauhiainen *et al.*, 2005, Sangok *et al.*, 2017, Wakhid *et al.*, 2017).

## 5.5 Conclusion

The lack of variations in $CO_2$ emissions in relation to proximity to plants in most of the young cropping systems also potentially show that the autotrophic contribution to $CO_2$ emissions on these sites was very low. Autotrophic $CO_2$ emissions from peat swamp forest in dry conditions were known to contribute to >45% of the total $CO_2$ emissions (Hergoualc'h *et al.*, 2017, Murdiyarso *et al.*, 2017), while the plantations in peatlands were consistently recorded to have higher heterotrophic contributions to total $CO_2$ emissions (Dariah *et al.*, 2014, Hergoualc'h *et al.*, 2017, Matysek *et al.*, 2017, Murdiyarso *et al.*, 2010). Thus without appropriate above ground vegetation or hydrology for peat formation, ancient carbon stored on these peatlands are gradually lost into the atmosphere with high heterotrophic respiration under agricultural systems on peat. Though $CH_4$ emissions exhibited significant variations depending on the sampling proximity to different plants, the emissions were very low and their contribution to carbon loss and climate change is limited at these sites.

# Chapter 6

## 6. General discussion and conclusions

### 6.1 Soil microbial communities under different land-use types and their responses to environmental change

Soil microbial community structures in tropical peatlands were found to be highly sensitive to environmental changes and responded accordingly to changes in depth, land-use and season. As pristine tropical peat swamp forests are almost extinct in Peninsular Malaysia, secondary forests are increasingly used as the benchmark to study peat forests. Our results have shown that secondary peat forests have a distinct microbial phenotypic community structure to that of primary peat swamp forests. The evidence is this change in microbial community structure is essentially due to the drastically different hydrological conditions in secondary forest to that of primary forest. Similarly, the changes in community structure between seasons were also possibly explained by increased flooding in the wet season. A characteristic increase in the relative abundance of non-specific fatty acids was observed in typically wet

conditions such as in primary forest or in deeper peat or in wet season. Straight chain monounsaturated fatty acids were known to increase with flooding (Bossio and Scow, 1998), and all the unsaturated fatty acids were non-specific biomarkers in this study. Similarly the ratio between Gram-positive and Gram-negative decreased in wet conditions, with resultant increase in $CH_4$ emissions. This also explains the decrease in the ratio with increasing depth, as the moisture increases. This inverse response to moisture by Gram-positive and Gram-negative, gives new insight into the observed dominance of Gram-negative group in neotropical peatlands studied by Troxler *et al.* (2012) and dominance of Gram-positive group in most SEA peatlands in this study. Microbial community structures were dominated by bacteria under all land-uses, depth and seasons, by a great margin in SEA tropical peatlands. Relative abundance of different bacterial groups responded to changes in temperature, pH and organic matter content, while fungal relative abundance only responded to the changes in temperature. In most cases fungal relative abundance did not vary significantly between different treatments, implying significant influences of bacterial community composition in overall microbial community structure response to disturbances. In contrary to general observations that ecosystems with complex high organic input and acidic conditions generally favour fungi (Bardgett, 2005, Rousk *et al.*, 2009), this kingdom appears to take a 'back-seat' in microbial community structure at all studied land-use types in highly acidic and organic SEA tropical peatlands. Bacterial communities in general are unable to degrade complex soil organic matter and are often dependent on decomposition products from fungi (Moore-Kucera and Dick, 2008). A bacterial dominance in spite of this dependence on fungi in complex systems might be due to the availability of dissolved organic carbon and easily degradable carbon from leaf litter DOC in SEA peatlands (Yule, 2010), and other labile carbon

inputs such as fertilizers in agricultural systems. This bacterial dominance in tropical peatlands, irrespective of the land-use likely indicates higher potential for C loss in these ecosystems, as higher F:B ratio were known to stabilize C input and storage with higher C use efficiency (Malik *et al.*, 2016). So, drainage of these peatlands would enable increased bacterial activity, potentially effecting quicker loss of C than fungal-dominated systems (Malik *et al.*, 2016).

## 6.2 $CO_2$ emissions at different land-use types and environmental controls

Total $CO_2$ emissions were lowest at the primary forest site, this is plausibly due to the anoxic water-logged conditions inhibiting aerobic microbial activity all around the year. This was reflected in a dramatic increase in $CO_2$ emissions in the secondary forest, where the water level was below the surface. Hergoualc'h *et al.* (2017) found that heterotrophic contribution from a peat swamp forest with water table 25 cm below surface, similar to the range observed in the secondary forest in North Selangor, contributed to 45% of the total $CO_2$ emissions. Murdiyarso *et al.* (2017) found the heterotrophic contribution total $CO_2$ emissions from a secondary forest to be ca. 50%. Therefore it is plausible that almost all of the $CO_2$ emissions from anoxic peat in primary forest were derived from root respiration, and aerobic conditions in secondary peat forest added similar level of heterotrophic decomposition, resulting in $CO_2$ emissions that were double those observed in the primary peat swamp forest. Unlike oil palm plantations, the secondary forest systems have larger and denser root systems that in combination with aerobic decomposition result in higher $CO_2$ emissions than those generally observed in agricultural plantations.

Oil palm plantations in peatlands with similar dry aerobic surface zones have previously been reported to have lower autotrophic contributions (Dariah et al., 2014; Hergoualc'h et al., 2017; Matysek et al., 2017). However, in our study only mature oil

palm plants contributed significantly to increase in $CO_2$ emissions. The autotrophic emissions from younger oil palm plants aged 1-5 years did not significantly contribute to the increase in total $CO_2$ emissions, contradicting previous observations by Hergoualc'h *et al.* (2017). Among the intercropped plants, yam rhizosphere contributed to 23.4% increase in $CO_2$ emissions. This suggests that almost all of the $CO_2$ emissions reported in Chapter 3, from the 2nd generation oil palm mono-cropping system were derived from heterotrophic respiration and contributes to C loss. This is clearly reflected in organic matter and C content, which was half that observed in other land-use types including the 2nd generation intercropping sites.

Distinct seasonal variations were observed in 2nd generation plantations including the intercropping sites, with higher $CO_2$ emissions during the wet season, suggesting moisture limitation to microbial activity, which is generally observed in drier mineral soil systems (Werner *et al.*, 2006) . These seasonal variations were more severe in the mono-cropping system that had emissions cut down by half during the dry season from wet season, due to extremely dry conditions. Similar trends of seasonal variation due to moisture limitation were previously observed in some agricultural plantations on peat (Arai *et al.*, 2014b, Hadi *et al.*, 2005) and more consistently in moisture limited mineral soils (Abushammala *et al.*, 2016, Salimon *et al.*, 2004). In addition to severe peat drainage, the moisture limitation in the 2nd generation cropping systems may also be due to open canopy and increased evapotranspiration as drier open canopy systems in tropics are known to emit up to double the amount of $CO_2$ in wet season than in dry season (Arai *et al.*, 2014b, Chen *et al.*, 2002, Salimon *et al.*, 2004). However, the 2nd generation intercropping systems maintained negative correlations to moisture that were observed in forested peatlands. This was not the case for the 2nd generation mono-cropping site, which exhibited positive correlation with moisture, which is generally

observed in drier mineral soil systems (Huang and Hall, 2017, Werner *et al.*, 2006). The results evidently demonstrate that prolonged mono-cropping on peatlands cause severe damage to peat functions. However, intercropping of other crops with oil palm were shown to maintain most of the peat organic content and caused relatively lesser damage to other peat properties.

### 6.3 $CH_4$ emissions at different land-use types and environmental controls

Methane emissions were highest in the primary forest, due to waterlogged conditions throughout the seasons, however the $CH_4$ emissions were substantially lower than what is observed in northern peatlands or in neotropical peatlands. Nonethless, the secondary forest in North Selangor oxidised more $CH_4$ than other agricultural sites. This might be due to more active methanotrophic community at that site. Indeed, Jackson *et al.* (2009) did not find any methanogenic bacterial communities at the site, however they found other bacterial phyla containing methanotrophs. Pineapple intercropping systems during the wet season emitted uniquely higher $CH_4$ emissions, due to water logged conditions. However, $CH_4$ emissions were well under 1 mg $m^{-2}$ $hr^{-1}$, and other agricultural sites had $CH_4$ emissions near zero. Another notable characteristic was higher $CH_4$ emissions observed near intercropped plants with adventitious root systems such as yam and pineapple. This likely suggests that the rhizosphere of these crops supported methanogenic communities. This might also be due to labile substrate inputs through root exudation from these crops, as root exudation is known to accelerate $CH_4$ emissions through substrate quality (Girkin *et al.* 2018, Bhullar *et al.*,2014). However, $CH_4$ emissions at all these sites were very low in terms of global warming potential, in comparison to the scale of $CO_2$ emissions from these sites. $CH_4$ emissions were positively correlated with moisture, pH and temperature. Thus in peatland restoration processes, in addition to restoring the water

table level, other pristine peatland conditions such as high acidity and high canopy cover that help cooler microclimate, should be restored. Otherwise an increase in water table alone might potentially result in higher $CH_4$ emissions than what is observed in pristine peatlands.

## 6.4 Soil microbial communities and GHG emissions

Our study has demonstrated that Gram-positive relative abundance is positively related with $CO_2$ emissions suggesting that Gram-positive bacteria are the major group involved in extracellular microbial activity in SEA tropical peatlands. This was further supported by Gram-positive dominance in surface layers of all the studied land use types, except for land-use types with a water table above the surface such as the primary forest and burnt site that was turned into flooded shrub land. This complements the findings of Jackson *et al.* (2009) that extracellular microbial activity decreased with depth, while Gram-negative dominance increased suppressing Gram-positive group with increasing depth.

Gram-negative relative abundance was positively correlated to $CH_4$ emissions, relating to higher $CH_4$ emissions in the primary forest. This suggests the likely role played by microbial community structure on the observed differences between the SEA peatlands and neotropical peatlands on $CH_4$ emissions. Neotropical peatlands were known to have higher $CH_4$ emissions than SEA peatlands (Sjögersten *et al.*, 2014) and were also found to be overwhelmingly dominated by Gram-negative bacteria (Girkin, 2018, Troxler *et al.*, 2012). This is also reflected by higher $CH_4$ emissions in intercropping sites, that had the lowest G+:G- ratio of all the studied agricultural sites. However PLFA analysis does not detect methanogenic archaeal communities, as archaea contain ether-linked phospholipids, as opposed to ester linked fatty acids, in their cell walls (Gattinger *et al.*, 2003). So this positive correlation

between Gram-negative relative abundance and $CH_4$ emission might be due to an overall shift in the microbial community structure that accompanies the occurrence of methanogenic archaeal communities, or due to similar environmental conditions such as increased moisture favouring both Gram-negative and archaeal communities. Our results suggest that Gram-positive microbes are an important group in relation to aerobic microbial activity, while Gram-negative dominance in community structure was generally associated with lower $CO_2$ emissions and higher $CH_4$ emissions.

## 6.5 Conclusions

This study has demonstrated that peat microbial community structure in tropical peatlands, were dominated by bacteria, responds to depth, land-use change and seasons. The conversion of primary forest to secondary forest favours Gram-positive bacteria over Gram-negative bacteria, increased $CO_2$ emissions and decreased $CH_4$ emissions. However the secondary forest had minimal change to peat pH, organic matter, C and N content, but conversion to agricultural systems impacted all those properties, which was more pronounced in 2$^{nd}$ generation oil palm mono-cropping system that had lowest C content and highly variable heterotrophic C loss. Nevertheless 2$^{nd}$ generation intercropping systems maintained similar organic content as 1$^{st}$ generation mono-cropping systems and caused relatively less damage to peat properties. These results are significant because they shed light on previously unexplored tropical peatland microbial communities, their response to disturbances and their relationship with GHG emissions. The results showing detailed environmental and microbial impact of different oil palm cropping system is of particular importance, as it helps to make an informed decision on cropping practices for oil palm plantations that cover a vast area of tropical peatlands.

## 6.6 Implications for future work

Chapter 2 has explored the previously unknown tropical peat microbial community structure across the depth profile. The results have clearly shown that microbial community structure is distinct in different land-uses associated with oil palm expansion and also that the community structure varied consistently with depth. However PLFA analysis does not distinctly detect archaea, known to be a functionally important group in peatlands. Future studies could use phospholipid ether lipids (PLEL) analysis, or molecular techniques based upon nucleic acid analysis to study these functionally important organisms. Study of archaeal communities in complement with PLFA analysis would provide new insights into the observed changes in general microbial community structure, where involvement of archaeal communities is highly likely, such as the distinct shift noted at ca. 1 metre at all the studied land-use types (Chapter 2), and the observed relationship between Gram-negative PLFA relative abundance and $CH_4$ emissions (Chapter 4).

Chapter 3 has shown that prolonged oil palm mono-cropping was detrimental to peat properties, while intercropping ameliorated such detrimental effects. However the intercropping systems studied contained young oil palm plants, and intercropping may not be possible when oil palm plants mature, the canopy closes, and light becomes restricted in the understorey. These systems should be assessed again when they are converted from intercropping to oil palm mono-cropping to understand whether intercropping at initial growing stages of oil palm is beneficial to the peat properties and environment in the longer term. There should be further research on sustainability of intercropping systems in relation to the age and lifecycle of oil palm.

Chapters 3 and 4 have shown a strong effect of seasonality on surface peat microbial community structure in all the land-use types studied, even though the temperature

and moisture difference between the seasons were not significant for some sites such as forest sites and first-generation oil palm plantations. This seasonal difference in microbial community structure should be further explored by controlled experimentation containing a tropical peat control, suddenly flooded and drained (to replicate wet season rainfall) and constantly flooded, never drained (to replicate primary forest condition) tropical peat soils. This would further provide further insights on whether Gram-negative dominance in wetter peat in primary forest (Chapter 4) and in deeper layers of other land-use types (Chapter 2) is a truly consistent phenomenon.

Chapter 4 also demonstrated a strong link between Gram-positive relative abundance and CO2 emissions. As much of the microbial activity occurs through the release of extracellular enzymes (Jackson et al., 2013), measurement of the difference between activity of extracellular enzymes in a Gram-positive dominated peat and Gram-negative dominated peat would give further quantitative information about the significance of a dominant bacterial group in tropical peatlands. Activity of different microbial groups could be tracked using stable isotope (13C) probing (Wegener et al., 2016) to further understand the functional roles played by the different microbial groups.

Chapter 4 demonstrated that almost all the macronutrients and micronutrients were significantly lower in the secondary forest than in the primary forest and possible link to leaching through drainage. These differences in nutrient concentrations were also significantly correlated to GHG emissions. Despite the functional importance of these nutrients for both microbial communities and GHG emissions, the impact of drainage on tropical peatlands nutrient content is virtually unknown. This should be

investigated with controlled experiments on nutrient leaching though drainage in tropical peatlands.

Chapter 5 demonstrated that proximity to a plant significantly influenced CO2 emissions only for mature oil palm and yam crops. However, further experimentation with the use of techniques such as trenching that completely separate rhizosphere influence from bulk soil is needed, to fully understand the autotrophic and heterotrophic contribution to CO2 emissions in different oil palm cropping systems. Chapter 3 had shown that microbial community structure was significantly different between the two 2nd generation intercropping systems studied that is also different from 2nd generation oil palm mono-cropping. However, influences of rhizosphere-related processes of different crops on this change in microbial community structure is not known. Future studies could usefully explore the differences in microbial community structure between the bulk soil and rhizosphere zone of different crops, to fully assess the impact of these different cropping systems in tropical peatlands.

# Appendix 1

**Table A1:** Table of GPS coordinates for the location of each collected core with respect to land-use type

| Site | Forest | Drained | Burnt | Young OP | Mature OP |
|---|---|---|---|---|---|
| **1** | N03°41'42.9'' E101°11'04.7'' | N03°38'51.3'' E101°20'32.6'' | N03°27'56.5'' E101°26'29.08'' | N03°39'49.8'' E101°20'38.1'' | N03°24'43.6'' E101°20'2.8'' |
| **2** | N03°29'02.4'' E101°15'07.3'' | N03°38'04.2'' E101°20'41.2 | N03°25'53.1'' E101°20'12.6'' | N03°39'15.8'' E101°20'28.3'' | N03°25'26.3'' E101°20'05.3'' |
| **3** | N03°41'49.7'' E101°11'08.5'' | N03°38'42.0'' E101°20'35.0'' | N03°28'10.6'' E101°26'29.7'' | N03°39'17.8'' E101°20'25.2'' | N03°25'16.7'' E101°19'48.6'' |
| **4** | N03°29'55.6'' E101°12'01.5'' | N03°39'30.7'' E101°19'19.9'' | N03°27'45.3'' E101°26'30.3'' | N03°39'43.6'' E101°19'42.5'' | N03°27'55.8 E101°27'09.8'' |
| **5** | N03°39'08.2'' E101°17'43.4'' | N03°39'34.2'' E101°19'24.7'' | N03°25'56.2'' E101°20'14.0 | N03°39'40.2'' E101°19'51.4'' | N03°24'49.9'' E101°20'10.0'' |

# Appendix 2

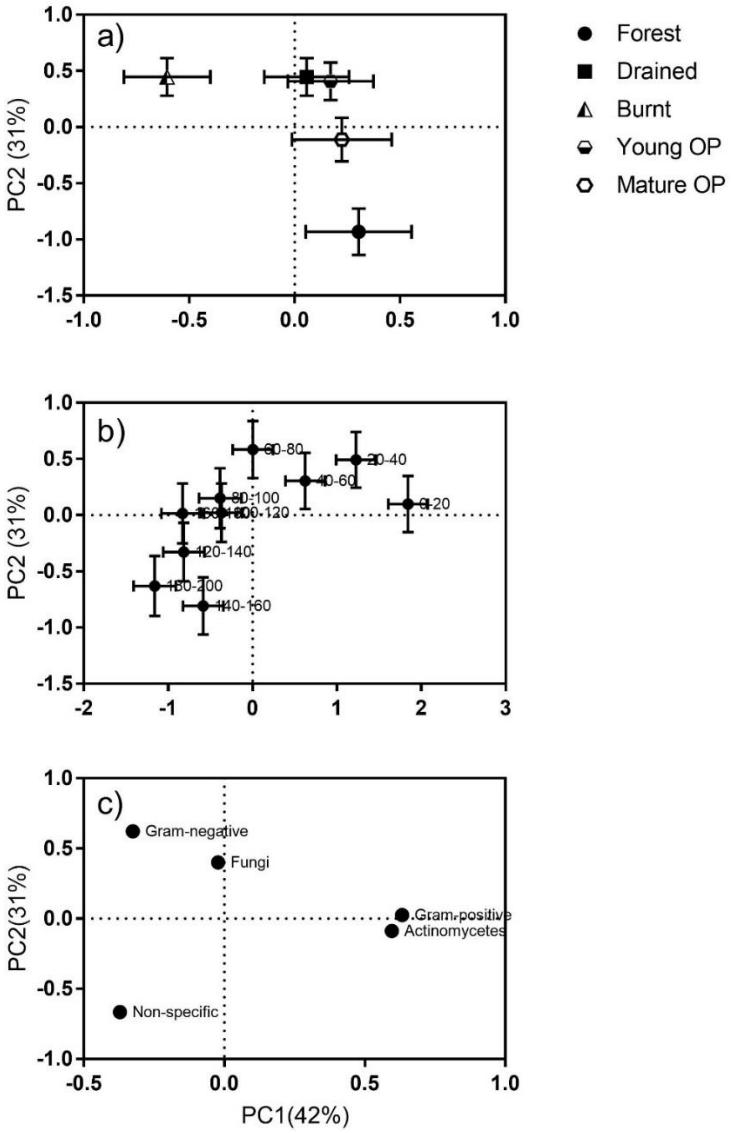



**Figure A2:** Effect of land-use type upon phenotypic structure of soil microbial communities determined by PLFA analysis, as shown by principal component (PC) analysis. (a) ordination of PC1 and 2 with respect to land-use type (OP = oil palm); (b) ordination of PC1 and 2 with respect to soil depth (point labels denote depth of sample in cm from surface; (c) associated loadings, point labels denote PLFA type. For (a, b) points denote means (n varies, see text), whiskers denote standard errors.

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