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## DEVELOPING SUSTAINABLE PRACTICES TO MITIGATE IMPACTS OF CLIMATE CHANGE ON NATURAL AND MANAGED TROPICAL PEATLANDS

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### SUMMARY

Southeast Asian peatlands are undergoing degradation through drainage and deforestation, accompanied by forest fires. This is occurring at unprecedented rates for agriculture and human settlement purposes. Exposure of carbon-rich peat to air has resulted in enhanced microbial-mediated peat oxidation emitting high levels of potent greenhouse gases (GHGs). Peat oxidation results in land subsidence thus increasing the risk of flooding that can affect the livelihoods of millions of people. The overall aim of this study was to provide an in-depth understanding of how land-use change and peat management practices affect the microbial ecology and physicochemical processes leading to peat oxidation and release of GHGs. We have used molecular marker-based approaches (microbial and metabolic profiles) that revealed the profiles were most influenced by variations in water table and land-use patterns, followed by age of drainage and peat thickness in that order. Plantations with mixed cropping had the least subsidence rates, where land subsidence is a proxy for peat loss by oxidation. We aimed to determine the functional potential of peat microbiome that is associated with peat oxidation from contiguous land-use types: degraded forest, degraded land and oil palm plantation sites. Metagenomic analysis revealed that genes belonging to aromatic compound biosynthesis and degradations were mostly associated with degraded forest and degraded land. *Actinobacteria* and *Firmicutes* were among the most abundant taxonomic groups, demonstrating their linkages in oxidation of tropical peatlands. The overall findings of this study will be useful in peatland management by providing a basis to focus early efforts on hydrological interventions and improving sustainability of oil palm plantations by adopting mixed cropping practices.

**Keywords:** *Greenhouse gas emissions, Mixed cropping practices, Metagenomics, Peat oxidation, Peat subsidence*

### INTRODUCTION

Peatlands are formed by the accumulation of partially decayed vegetation matter over millennial timescales in low-lying areas that are frequently waterlogged due to heavy rainfall or periodic inundation. Peatlands are a highly vulnerable natural resource that cover 50–70% of global wetlands (Gillespie, 2006) and sequester one-third of the world's soil carbon (Freeman *et al.*, 2012). In Southeast Asia, peatlands cover an area of nearly 25 Mha and store approximately 69 Gt of carbon, which is 77% of all the tropical peatland carbon pool (88.6 Gt), of which 65% (57.4 Gt of carbon) is in Indonesia itself, distributed within 23.4 million ha of peatland (Page *et al.*, 2011). Carbon density is relatively high in tropical peatlands compared to temperate or boreal peatlands; this is largely a consequence of deeper peat layers in the former, with peat thickness up to 20m recorded (Page *et al.*, 2002).

Southeast Asian peatlands are undergoing drainage and deforestation, including forest fires at unprecedented rates, making way for commercial plantations of oil palm and Acacia trees. These industrial-scale plantations on deforested, drained and burnt degraded peatland covered over 3.1 million hectares (approximately 20%) of the peatlands of Peninsular Malaysia, Sumatra and Borneo in 2010, an area expected to grow to 6.2 million hectares by 2020. Palm oil is the world's most important vegetable oil in terms of production quantity (USDA – FAS, 2010). In 2009 global palm-oil production amounted to 43.4 million tonnes, of which 47% was produced in Indonesia (USDA – FAS, 2010). The rapid expansion of oil-palm agriculture in Southeast Asia and other tropical regions is testament to an almost insatiable global demand for palm oil and the consequent financial lure to ramp up production. Oil palm has contributed greatly to regional, national, and local economies (Zen *et al.*, 2005). However,

this intensive agriculture has impacts on its sustainability mainly due to following reasons: (i) Peatlands under agriculture are undergoing oxidation due to microbial oxidation, leading to physical peat loss, also called subsidence (Mishra *et al.*, 2014); (ii) This peat loss would increase the flooding risk and the synthetic chemical load in the form of fertilizers would also be increased due to a loss of nutrients from the peat; (iii) Peat oxidation will also result in increased GHG emissions, which has resulted in Indonesia becoming one of the major global producers of GHGs.

In the tropics, we have a relatively poor understanding of the relationship of microbial diversity and factors influencing community structure for intact peatlands, as well as peatlands under land-use change. Given both the ecological and economic importance of these peatlands, it is useful to understand the differences among the various land-use patterns in degraded tropical peatland with respect to microbial ecology. Our approach is based on the ability of molecular profiling to capture shifts in community structure and metabolic profiling to reflect the functional outcome of metabolic activities of microbes, plant roots and their exudates, respectively. Using these two microbial profiling approaches, we report the effects of water table depth and oxygen availability, land-use patterns, age of drainage and peat thickness on bacterial diversity in degraded peatlands of Indonesia. Further to this, we have used next-generation sequencing approaches to determine the functional potential of the peat microbiome associated with peat oxidation.

## MATERIALS AND METHODS

### *Site description and sampling*

The study area is located in peatlands of the eastern part of Jambi province, Sumatra, Indonesia. The coordinates of sampling locations distributed across two broad areas, referred to as Site A and Site B, were 103°53'52.58" E, 1°43'12.47" S and 103°49'32.23" E, 1°40'58.24" S, respectively (described in Mishra *et al.*, 2014). In order to monitor the hydrological parameters, both rainfall and water table depths were measured periodically using rain gauges at strategic locations and dipwells along a transect. At each sampling location, a 1m<sup>3</sup> pit was dug. Three equidistant pits were used for sampling in each transect. We collected samples from above and below the water table.

### *Bacterial community structure (terminal restriction fragment length polymorphism (T-RFLP) analysis)*

Bulk peat gDNA was extracted using a ZR Soil Microbe DNA MidiPrep™ extraction kit (Zymo Research Corporation, Irvine, USA) based on the manufacturer's protocol, with minor modifications. TRFLP analysis was performed based on the protocol described in Mishra *et al.*, (2014).

### *Chemical analysis*

The bioavailable extract for microorganisms from peat was used for the analysis of anions, cations and dissolved organic carbon (DOC) based on the protocol described in Mishra *et al.*, (2014). Peat water samples were run through a Solid Phase Extraction cartridge using the protocol described in Mishra *et al.*, (2014).

### *Data analysis*

To analyze the variation in bacterial community structure as well as differences in metabolic functions due to the influence of the analyzed parameters (namely, water table, land-use patterns, age of drainage and peat thickness), multivariate statistical techniques (PRIMER 6, PRIMER-E, Ltd., Plymouth, United Kingdom) were used to calculate distance matrices using Bray–Curtis similarity indices and one-way ANOSIM (Analysis of Similarity) coefficients, following the steps mentioned in Mishra *et al.*, (2014). Similarly, to analyze the influences of geochemical traits on the bacterial community structure, Canonical Correspondence Analysis (CCA) was performed using Canoco (version 4.5 for Windows, PRI Wageningen, the Netherlands).

### *Metagenome 454 sequencing and data processing*

Whole-community metagenomic DNA was extracted as described above from above water table level of samples from three land-use types: (i) degraded forest; (ii) degraded land; (iii) oil palm plantations. The sequencing was performed on a Genome Sequencer (GS) XLR70+ FLX system (Roche Applied Science, Mannheim, Germany) using Titanium chemistry based on methods described earlier (Poinar *et al.*, 2006; Allentoft *et al.*, 2009). The paired read sequences were imported and the paired-end protocol of MEGAN5 was used (Huson *et al.*, 2011) to obtain taxonomic profiles of the three land-use patterns. Annotated taxonomic assignments were linked to functional potential using SEED and KEGG databases.

## RESULTS

### *Peat microbiome and functional potential in different land-use types do not differ qualitatively*

Metagenome sequence abundance based annotation indicated that the majority (77 – 81%) of the sequences belonged to bacteria (Table 1). Data for the microbiome from the degraded forest is only shown here. Within

microbial communities, *Proteobacteria* was the abundant phyla, followed by *Actinobacteria* and *Firmicutes* (Figure 1: Right panel). *Actinobacteria* was found to be higher (21% of total microbial communities) in abundance in the degraded forest compared to oil palm and degraded land (18% and 15%, respectively). The functional potential based on SEED annotations revealed a higher diverse set of functional categories as 5.756, 5.764 and 5.751, respectively, for degraded forest, oil palm plantations and degraded land (data not shown). Based on associations of different functional categories and geochemical characteristics with different land-use types, it was evident that the electron transfer reactions were mostly associated with oil palm plantations. The genes belonging to aromatic compound biosynthesis and degradations were mostly associated with degraded forest and degraded land. On the other hand, Archaeal-ribosomes/RNA metabolism categories were mostly associated with degraded land.

Table 1: General information for peat microbial metagenome Bp: means base pairs; % non-ACGT = Percentage of bp that are non- ACGT; Transl. reads = Translated reads (in silico); aa = amino acids

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

#### Relationship of peat oxidation with land-use pattern

Peat samples from mixed crop plantations, which have a more diverse plant cover than monoculture oil palm plantations, contain more DOC, have a more diverse metabolic profile, support a more diverse microbial community (both oxic and anoxic zone) and importantly experience a lower rate of peat subsidence; a proxy for oxidation-led peat loss (Figure 1: Left panel). However, the peat oxidation rates under these two land-use patterns followed a reverse trend, with higher rates in oil palm sites when compared to sites with mixed crop plantations (Mishra *et al.*, 2014).

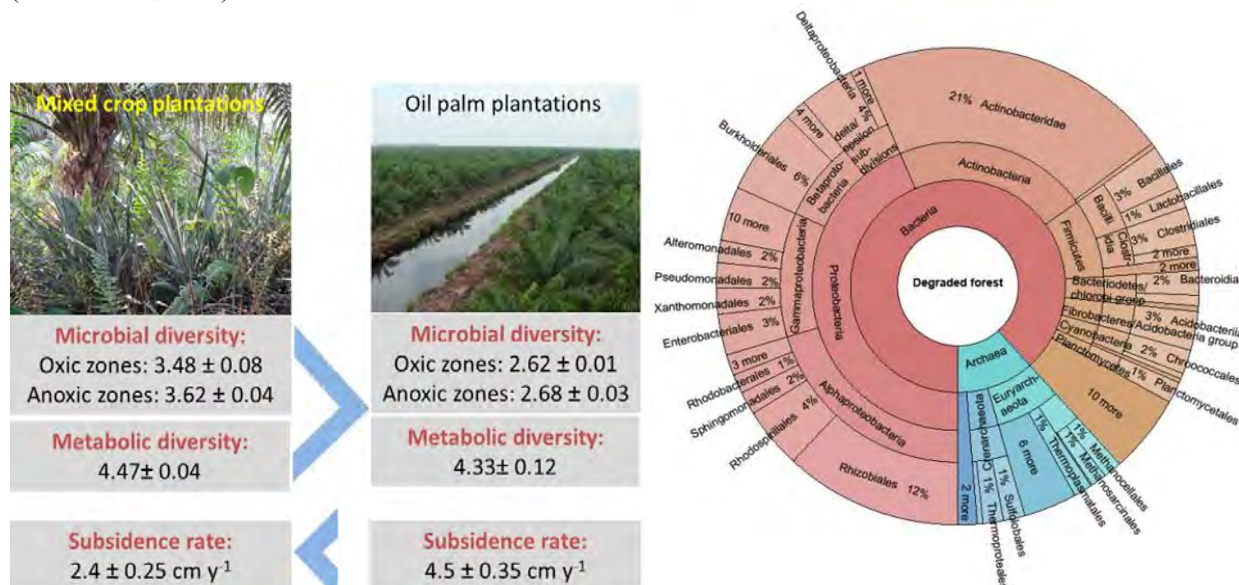


Figure 1: Left panel (Adapted from Mishra *et al.*, 2014) - Mixed crop plantations depicting higher microbial taxa and functional diversity compared to monoculture (oil palm), based on Shannon Diversity Index. Right panel- Representative figure of Peat microbiome from land-use type:

## DISCUSSION

Bacterial and metabolic markers that represent the complex nature of bacterial communities and metabolic processes of diverse biota, respectively, provided the resolving power to distinguish different habitats. This resolution ranged from the centimeter scale in depth measurements to the kilometer scale, where sites were distributed within the 48 km<sup>2</sup> of the study area. Our findings show that microbial profiles from peatland sites are most influenced by variations in water table and land-use patterns. The ability of microbial markers to distinguish the low- and high- water table sites shows their robustness in capturing differences in community structures despite the differences in the range of fluctuations in water level at these sites. Hence, microbial profiling presents a practical approach to monitor peat responses to both rapid short-term and long-term hydrological changes.

Oil palm plantation monocultures supported the least diverse bacterial communities and had the lowest levels of DOC. On the other hand, mixed crop plantations consisting of up to five plant species only, supported the most diverse bacterial communities and had the highest levels of DOC. In the tropical peatlands of Kalimantan, land conversion from secondary forest to paddy fields (monoculture plantations) led to a decrease in carbon content, together with a decrease in microbial abundance (Hadi *et al.*, 2001), which is consistent with our findings. Carbon levels increased when paddy–soybean rotation cropping was followed by a further decrease in microbial abundance. This possibly underlines the importance of adopting simultaneously mixed plantations rather than sequential crop rotations, as evident in our study. Low bacterial diversity in oil palm plantations, as seen in our study, can be sensitive to environmental pressures, thereby leading to a reduction in the productive period of plantations. These findings provide a good basis to adopt microbial ecology principles to encourage mixed crop planting in the existing plantations, in order to increase their microbial diversity, especially of beneficial microbes, which can lead to sustainable use of these plantations.

The deep sequencing-based approach reveals a more accurate prediction of microbial community abundance and their functional potential. In temperate peatlands, the carbon degradation is reported to be linked with the abundance of certain taxa, such as *Actinobacteria*, among others (Pankratov *et al.*, 2006; Mackelprang *et al.*, 2011; Tveit *et al.*, 2013; Tveit *et al.*, 2014). In the *Sphagnum* peat bogs of Russia, *Actinobacteria* has also been reported to play an important role in aerobic cellulose degradation (Pankratov *et al.*, 2006). A combined metagenomic and metatranscriptomic study on arctic permafrost peatlands (Tveit *et al.*, 2013 and 2014) revealed that *Actinobacteria* seemed to be particularly important, having a metabolic potential for carrying out several of the key steps in soil organic carbon degradation. In tropical peats, as reported in this study, similar abundance of these taxa was found. *Actinobacteria* and *Firmicutes* were the most abundant taxa after *Proteobacteria* in the metagenome data reported here, suggesting its potential role in the carbon degradation of tropical peat. This observation is consistent with the findings from marker-based approach (Mishra *et al.*, 2014), where *Actinobacteria* and *Firmicutes*, were among the predicated abundant species.

## CONCLUSION

We conclude that mixed crop plantations, which have a more diverse plant cover than monoculture oil palm plantations contain more DOC, have a high diverse metabolic profile, support a more diverse microbial community and, most importantly, experience a lower rate of peat subsidence; a proxy for oxidation-led peat loss. *Actinobacteria* and *Firmicutes* were among the most abundant taxonomic groups, demonstrating their linkages in oxidation of tropical peat. These findings provide a good basis to adopt microbial ecology principles to encourage mixed crop planting in the existing plantations, in order to increase their microbial diversity, which can lead to sustainable use of these plantations.

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