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INCREASED CO_2 EMISSIONS DUE TO REWETTING OF DEGRADED TROPICAL PEATLANDS UNDER OIL PALM PLANTATIONS

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SUMMARY

Peatlands occupy 44.1 Mha worldwide, of which nearly 15% have been drained and deforested mainly for commercial agriculture. This degradation has resulted in emissions of 1.3 Gt CO_2 yr^{-1}, which excludes the considerable source of emissions arising from peat fires. From the global area under degraded peatlands, nearly 3.1 Mha is present in Southeast Asia, mainly drained and deforested for commercial plantations of oil palm and *Acacia*. The current rate of carbon emissions from these peatlands under commercial plantations is estimated at 230-310 Mt CO_{2e} yr^{-1}. This degradation has led to physical loss of peat resulting in its subsidence, which is a proxy for CO_2 emissions. As drainage of these peatlands has been associated with significant increase in greenhouse gas (GHG) emissions, hydrological restoration by rewetting to bring back the water table levels near to the peat surfaces, is increasingly being considered as an option to reduce peat degradation and GHG emissions. In this study, we have aimed to understand the mechanistic basis of CO_2 emissions in the tropical peatlands, and the effect of rewetting on these processes. We have conducted laboratory-based microcosm studies and have shown that large CO_2 emissions results due to direct CO_2 emissions from different oxic and anoxic state of peat associated with water saturations, and not linked to methane oxidation to CO_2. We also report here metabolic pathways from directly measured metabolites involved in carbon mineralization that are associated with CO_2 emissions and their changes resulting from controlled rewetting. Our findings from the microcosm study corroborate with our field-based monitoring study of peat subsidence that undergo drying-rewetting and subsiding at a rate of 4.4 cm/yr. Finally, we provide field-based GHG emission from different oxic and anoxic state of peat associated with water saturation that corroborates with our microcosm study.

Keywords: *Greenhouse gas emissions, peat oxidation, microcosm study, metabolomics, peat subsidence*

INTRODUCTION

Peatlands are formed by the accumulation of partially decayed vegetation matter in low-lying areas under water-logged conditions. Peatlands occupy 44.1 Mha worldwide, of which nearly 15% have been drained and deforested mainly for commercial agriculture (Page *et al.,* 2011). This degradation is resulting in emissions of 1.3 Gt CO_2 yr^{-1}, which excludes the considerable source of emissions arising from peat fires (Joosten *et al.,* 2010). The current rate of carbon emissions from these peatlands under commercial plantations is estimated at 230-310 Mt CO_{2e} yr^{-1}.This degradation leads to physical loss of peat resulting in subsidence of its surface, which is a proxy for CO_2 emissions (Couwenberg and Hooijer, 2013; Hooijer *et al.,* 2012).

Hydrological restoration by rewetting is increasingly being considered as an option to reduce peat degradation and GHG emissions. The effectiveness of rewetting in reducing emissions has been evaluated mainly by two approaches: (i) direct hydrological interventions in the field and mesocosms and (ii) extrapolation of correlations of water table depth with GHG emissions. In a recent study from temperate peatlands, methane emissions were higher, where peat surface was rewetted till the top when compared to those, which were rewetted till 20 cm below peat surface. However, CO_2 levels were only marginally affected compared to CH_4 levels (Karki *et al.,* 2015). In case of tropical peatlands, hydrological restoration did not reduce emissions from the degraded sites upon rewetting (Jauhiainen *et al.,* 2008). In contrast to field- and mesocosm-based studies, extrapolations based on correlations of water table and GHG emissions have shown that rewetting is associated with reduction in CO_2 emissions. Sites that have relatively low water table have marginally high subsidence, when compared to sites that have relatively high water table (Couwenberg and Hooijer, 2013). Similarly, a trend of low CO_2 emissions has been reported from sites in tropical peatlands with high water table, when compared to low water table sites (Jauhiainen *et al.,* 2005, 2012). Based on these studies, it has been predicted that rewetting in SE Asian peatlands can lead to substantial reductions of net greenhouse gas emissions (Couwenberg *et al.,* 2010). Hence, based on the different reports on the outcomes of the effects of hydrological interventions on GHG emissions using experimental approaches and from modelling studies, it still remains to be established whether rewetting indeed can reduce GHG emissions.

Relationships between water table and GHG emissions can be explained, if the basis of microbial oxidation of peat biomass can be better understood. We have previously shown that fluctuations in water table in low water table sites is linked with changes in bacterial community structure through oxygen and nutrient availability on the one hand and selection of bacteria that can withstand drying–wetting cycles on the other hand (Mishra *et al.,* 2014). The mechanism of large amounts of CO_2 emission and how rewetting affects peat oxidation in the hot tropics leading to these emissions, needs to be better understood. In order to understand the mechanistic basis of CO_2 emissions in the tropics, and the effect of rewetting on these processes, we have conducted microcosm studies and corroborated our findings with the field studies.

MATERIALS AND METHODS

Study site description and sample collection

The study area is located in peatlands of the eastern part of Jambi province, Sumatra, Indonesia (Site map is described in Mishra *et al.,* 2014). The land-use type from where samples were collected was oil palm plantation, having mostly low water table (82±3 cm below peat surface). Peat samples were collected from three depths, (i) 20-30 cm below peat surface- referred as *oxic zone*; (ii) 20-30 cm above water table- referred as *partial oxic zone* and (iii) 20-30 cm below water table- referred as *anoxic zone* (based on dissolved oxygen data). Peat water samples were collected in sterile falcon tubes to conduct rewetting perturbation. These samples were then shipped to Lawrence Berkeley National Laboratory (LBNL), USA, where part of this study (microcosm set-up, measurements of CO_2 and CH_4 emissions using respirometer) was performed.

Microcosm set-up, sampling design and monitoring

In order to understand the microbial physiological responses leading to gas emissions (CO_2 and CH_4) before and after rewetting, a microcosm experimental set-up was designed and samples for microbial and metabolic changes were collected before and after rewetting. Three replicates of sample from respective zone (1. Oxic, 2. Partial oxic, 3. Anoxic) were bottled-up in serum bottles and were then installed in Micro-Oxymax respirometer (Columbus Instruments). A perturbation of rewetting was generated in the middle of the experimental period.

Peat microbial metabolomics and environmental traits

In order to understand the microbial metabolic profiling and predictive functional potential before and after rewetting, samples from each time points were run through in-house developed metabolomics workflow, described in (Benke *et al.,* 2015). In order to monitor the hydrological parameters and subsidence rates, same methodology was adopted as described in Couwenberg and Hooijer, (2013). Physicochemical analysis for anions and cations was also performed based on the methodology described in Mishra *et al.,* (2014).

RESULTS

Effect of oxygen availability and rewetting on microbial CO_2 emissions (Microcosm study)

CO_2 emissions were significantly higher than CH_4 emissions in all the measured zones at all different time–points measured (Figure1). CO_2 emissions were at baseline levels in the autoclaved samples (controls) compared to high levels of emissions in the non-autoclaved samples (Figure 1, Left panel). Thus, establishing that CO_2 emissions are due to microbial respirations only. CO_2 emissions were similar for the first 6 days from the start of microcosm study, indicating an adaptation time of approximately 144 hrs (6 days) for the microbial communities to adapt to the set up. The rates of CO_2 emitted were highest in the anoxic zones (increased 65% after 6 days), followed by –oxic and then partial –oxic zones (35% and 30% after 6 days, respectively), indicating anaerobic CO_2 production dominates such emissions from tropical peatlands. These rates were further increased in the anoxic zones. In the –oxic and partial –oxic zones, the rates at which CO_2 was released increased by 22% and 25%, respectively, upon rewetting. Methane emissions, on the other hand, did not have a clear trend for the emissions patterns from the –oxic, partial –oxic or the anoxic zones (Figure 1, Right panel).

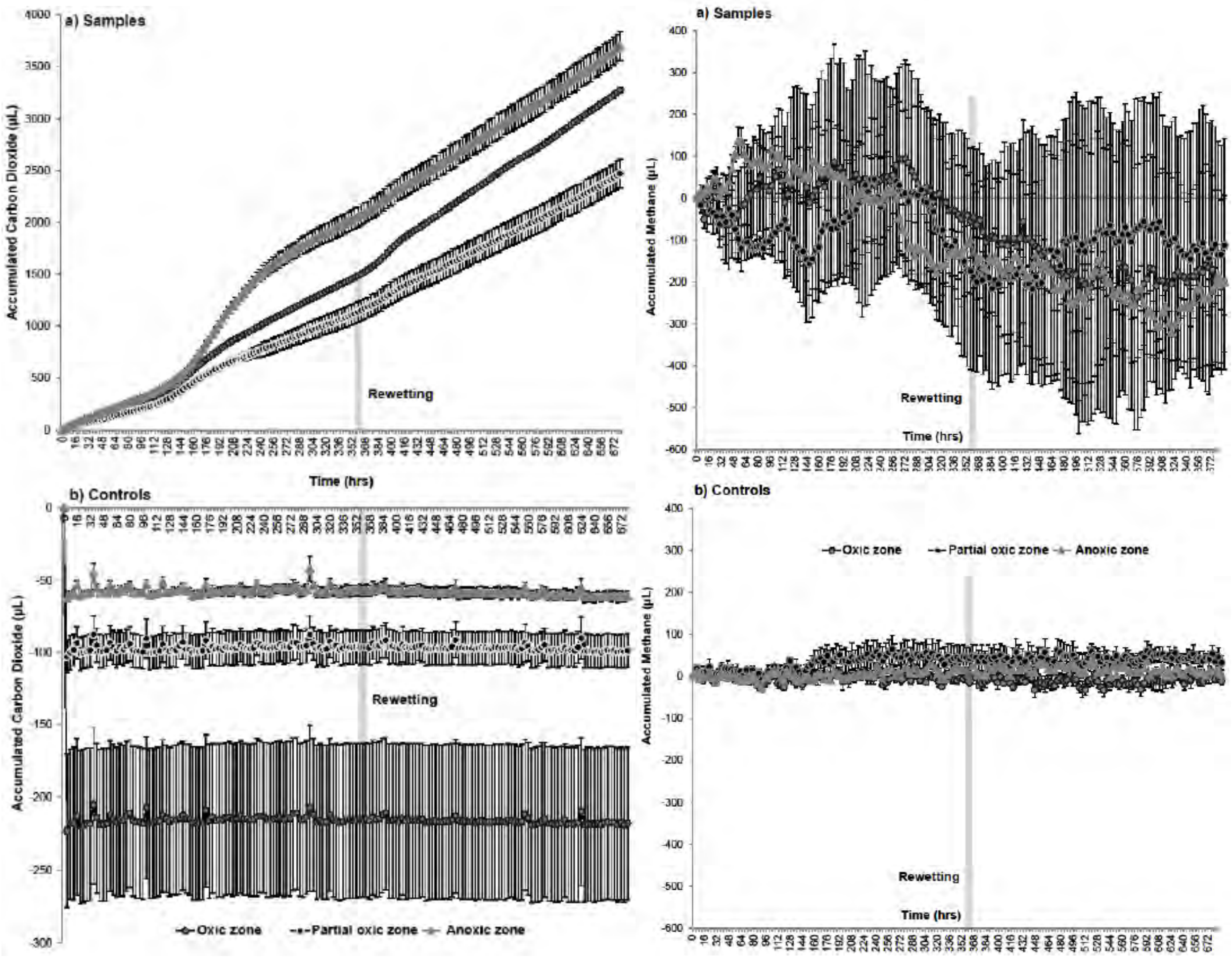


Figure 1: Left Panel- Accumulated carbon dioxide from -oxic, partial-oxic and anoxic zones of peatlands under oil palm plantations, over time before and after rewetting for samples (a) and controls (b). **Right Panel:** Accumulated Methane from -oxic, partial-oxic and anoxic zones of peatlands under oil palm plantations over time before and after rewetting for samples (a) and controls (b).

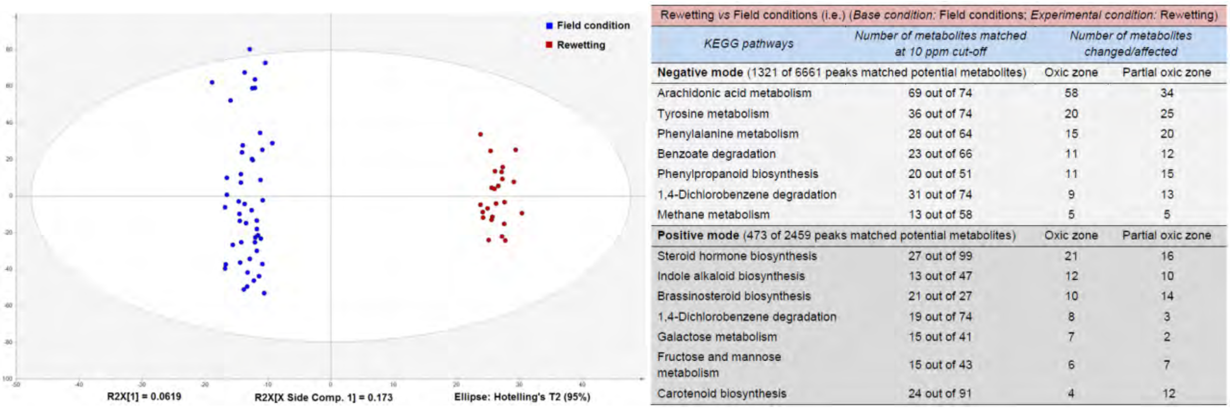
Effect of rewetting (rainfall inundation and hydrological management) and oxygen availability on subsidence rates (proxy for carbon emissions) and GHG emissions, respectively (Field studies)

Following similar trend as of CO_2 emissions in the microcosm study, its proxy trait *(i.e.)* subsidence from same sites showed that peat oxidation did not slowed down upon inundation due to rainfall. The average annual subsidence rate based on data monitored for 3.5 years was 4.4 cm/yr. During low water table times, peat oxidized at the subsidence rate of 5.7 cm/yr and did not stop during high water table times (continued subsidence rate: 5.4 cm/yr). Based on this field data, it is clearly evident that during rewetting due to rainfall inundation and hydrological management, peat oxidation do not stop. This is similar to findings reported in Couwenberg and Hooijer (2013).

Similarly, peat mean GHG concentrations were lower at depths that were close to the peat surface. On the other hand, GHG concentrations was generally highest from peat profiles at the depth of 100 cm and 150 cm depth, i.e. in water logged conditions (anaerobic conditions). This corroborates to our findings obtained in microcosm study, where anaerobic CO_2 dominates the gas emissions from degraded tropical peatlands under oil palm plantations.

Metabolic functions of the microbial communities

OPLS–DA analysis showed that the metabolic profiles between field conditions and rewetting were slightly different at variation of 17% (Figure 2, Left panel). Selected pathways, which got annotated in KEGG and had highest number of metabolites being shifted are shown in Figure 2 (Right panel). The changes during rewetting when compared to field conditions, shows that more than 3-fold of predicted metabolites (1321 of 6661 peaks – negative mode *vs* 473 of 2459 peaks – positive mode) was identified in negative mode when compared to positive (Figure 2, Right panel). Most of these pathways belonged to secondary metabolism.



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

Figure 2: Left Panel- OPLS-DA analysis of the microbial metabolic features excluding blanks to estimate the variations between metabolic changes during field conditions (blue) and after rewetting (red). Variation in X-and Y- axis are 17.3% and 6.2%, respectively. **Right Panel:** Functional metabolic potential of microbial communities between field and rewetting conditions based on annotation using KEGG pathways. The second columns show the number of metabolites present in our data that were matched in respective pathways showing potential metabolites. The third and fourth column depicts the number of metabolites that got affected due to rewetting.

DISCUSSION

Microorganisms have a variety of evolutionary adaptations and physiological acclimation mechanisms that allow them to survive and remain active in the face of environmental stress. The stress in our study could be rewetting. Microbes acclimatize to immediate stress by altering their allocation of resources from growth to survival pathways. These microbes may use the material to support growth and survival [1. Cryptic growth (Chapman and Gray 1986)], to enable attack on recalcitrant soil organic matter [2. Priming (Fontaine *et al.,* 2004; Battin *et al.,* 2008)], or to fuel processes such as 3. Denitrification (Sharma *et al.,* 2006) under stress conditions. During rewetting, microbes generally dispose osmolytes rapidly, either by respiring, polymerizing, or transporting them across the cell membrane (Wood *et al*., 2001). The consequence of disposing of osmolytes could lead to enhanced production of CO_2, DOC, and nutrients released on rewetting (Schimel *et al.,* 2007), as found in this study.

The top 5 metabolic pathways that are affected by rewetting, as identified from microcosm study (Figure 2, right panel), are also highly abundant in the field-based peat metagenome analysis (data not shown). The metabolic changes associated with aromatic aminoacid, xenobiotics and carbohydrate metabolism after rewetting were affected in our study. In the study about rewetting in organic soils, it is demonstrated that aromatic ring compound, amino-acids, glucose, and acetate metabolism decreased after rewetting (Tate, 1979) with aromatic ring compound metabolism to be highly affected upon rewetting. Upon rewetting, compounds, such as, arachidonic acid, linoleic acid and other lipid metabolism were found to be affected in the current microcosm study. The changes in metabolite concentration in phenylalanine metabolism upon rewetting are linked with adaption of tropical peat microbial communities to degrade recalcitrant lignocellulosic materials.

Drying-wetting mainly occur owing to low water table depths and high rainfall patterns, such as, in tropical peatlands. In our microcosm study which demonstrated that there was no effect on CO_2 emissions upon rewetting, shows linkage of fluctuations of water table with peat oxidation. In a study from farmed organic soils in temperate region, it is shown that CO_2 emission rates increased up to 5-fold following wetting (Prieme and Christensen, 2001). In a field study from Kalimantan, it is reported that there was CO_2 emission were not lowered pre- and post-hydrological restoration (Jauhiainen *et al.,* 2008). From our data both on laboratory-based microcosm and field-based study, shows that it is the direct anaerobic and aerobic CO_2 that dominates the large CO_2 production from this part of the world, rather than methane oxidation to CO_2. Hence, a combined approach of hydrological and microbial-based solution is needed to lower the peat oxidation in this region.

CONCLUSION

From this study, we conclude that elevated carbon dioxide emissions and negligible amount of methane emissions are attributed to low water table depths. At such low depth, there would be higher frequency of rewetting during rainfall events. We also show here that large amount of CO_2 emissions from this region is attributed to high amount CO2 production from both aerobic and anaerobic zones of peat, rather than CH_4 oxidation to CO_2. Lastly, it can be concluded that metabolic functions, such as, metabolism of aromatic compounds, amino acids, xenobiotics and carbohydrate metabolism that are distributed among diverse taxa are likely to govern the changes (non-reduced CO_2 emissions) upon rewetting.

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