

Dear Dr. Chapman,

Thanks for the comments during the review process of the manuscript (MS No.: bg-2013-366).

We have addressed all the specific points systematically. These changes are described in the accompanied document.

We hope that these changes have improved the clarity of the manuscript. We have uploaded the following documents along with the accompanied responses:

1. Revised Fig. 3
2. Revised Fig. 5
3. Revised Suppl. Fig. S1

We look forward to your further comments and will be happy to make further amendments, as needed.

Sincerely,
Sanjay

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Author’s response to the comments rose by Reviewer 1 during the review process for the manuscript (MS No.: bg-2013-366) are as follows:

1. It would have been very informative to have had comparative sites within the indigenous peat swamp forest.

Author’s response: Thanks for pointing this out.

The intention in this study was to choose contiguous land-use types of varying degrees of land-use change so as to keep other confounding variables to be low. Site A and B constitute such contiguous land-use patterns, namely, ‘degraded forest’, ‘degraded land’, ‘oil palm plantations’, ‘mixed crop plantations’ and ‘settlements’.

As rightly pointed out, less degraded sites (intact peat swamp forest) from other non-contiguous areas will likely provide better separation of the effect of land-use change. Hence, we will include such sites in the follow-up studies.

2. The nMDS patterns are presented rather repeatedly and the number could be reduced.

Author’s response: Thanks for the suggestion.

We would therefore remove Fig. 5A, which is also pointed out in your comment no.9.

3. I was surprised that pH was not included among the “geochemical data”; pH has often been shown to have a major influence on microbial community structure. Possibly “dissolved inorganic carbon” could be a surrogate for pH.

Author’s response: pH was indeed measured from all the sites. The pH values of these peat sites were in the range of 3.2 to 3.5. These pH values do not show significant correlation with Shannon diversity indices for different land-use types and at both oxic and anoxic zones (see Table below).

Correlation statistics	Correlation of diversity at oxic zones with pH	Correlation of diversity at anoxic zones with pH
Linear correlation (Pearson)	0.963 (p=0.17)	0.921 (p=0.254)
Kendall’s tau	0.99 (p=0.11)	0.5 (p=0.9)
Spearman’s rs	0.99 (p=0.33)	0.33 (p=0.601)

So based on your comment, we suggest adding the following sentence at the end of line 5 on P14021:

“.....degraded land (Table 1). The pH values measured from all sites did not show any significant correlation with Shannon diversity indices from different land-use types at both oxic and anoxic zones.”

4. P14011 L16 “Bacterial and metabolic profiles: most diverse” – this was shown for the 16S rDNA data (Table 1) but not for the metabolic profiles.

Author’s response: Thanks for this comment.

We have added data for metabolic diversity in Table 1 (see modified Table 1 below). We will change the text in line 16 on P14011 as follows:

“.....oxygen availability. Mixed crop plantations had the most bacterial and metabolic profiles. Metabolic profiling.....”

Table 1: Shannon diversity indices for different land use patterns based on 16S rDNA and metabolic diversity.

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

5. P14012 LL2-4 This description really applies to coastal tropical peatlands. In other regions peatlands are often found in mountainous terrain, not inundated but sustained by high rainfall.

Author’s response: We will change the text on line 2-4 on P14012 as follows:

“Peatlands that are frequently waterlogged due to heavy rainfall or periodic inundation (Anderson, 1964).”

6. P14013 LL3-4 It is not clear what the difference is between “forest biomass” and “biomass”.

Author’s response: Thanks for this comment.

We will change the text on line 3-4 on P14013 as follows:

“Estimates, excluding forest biomass losses, fire losses and peat organic matter losses in the initial years after drainage.”

7. P14013 L14 I would say “In most ecosystems”.

Author’s response: Thanks for this suggestion. We will include this text as suggested.

8. P14013 L19ff The logic here needs changing, e.g. “While geochemical conditions affect microbial communities, they, in turn, affect their environment. Therefore, it is important to study microbial community composition”.

Author’s response: Thanks for this suggestion. We will include this text as suggested.

9. P14015 L17ff: Unfortunately these 5 classes do not map onto the 5 land-uses mentioned earlier, which is a bit confusing. It seems you have split the industrial plantation into two but missed out the pristine forest. It is a real pity that pristine forest was not included in the sampling; it would have enabled you to judge how far the microbiology had moved from the original land-use in all the various subsequent land-uses.

Author's response: The land cover described in Miettinen et al. (2012b) was re-grouped and subset of those land-use patterns falls in Site A and Site B, which were chosen for this study. Yes, industrial plantation was sub classified as mixed crop and oil palm plantations.

The intention in this study was to choose contiguous land-use types of varying degrees of land-use change so as to keep other confounding variables to be low. Site A and B constitute such contiguous land-use patterns, namely, 'degraded forest', 'degraded land', 'oil palm plantations', 'mixed crop plantations' and 'settlements'.

As rightly pointed out, less degraded sites (intact peat swamp forest) from other non-contiguous areas will likely provide better separation of the effect of land-use change. Hence, we will include such sites in the follow-up studies.

We will change the text on line 19-24 on P14015 to the following:

"The land-cover classes used in Miettinen et al. (2012b) were regrouped and reclassified for this study. The land-cover comprising of (1) slightly and moderately degraded peat swamp forest (PSF) (1656 km² or 49% of total mapped area) was classified as 'intact PSF', (2) heavily degraded PSF and secondary forest (543 km² or 16%) was classified as 'degraded forest', (3) shrubs, fern/grass and clearance area (712 km² or 21%) was classified as 'degraded land', (4) industrial plantation (279 km² or 8%) was classified as 'oil palm plantation' or 'mixed crop plantation', depending on land-cover in that area and (5) small holder mosaic and built-up areas (188 km² or 6%) was classified as 'Settlements'. For this study, the contiguous land-use types were chosen that were present in Site A and Site B. Intact PSF was not included in this study because of being non-contiguous."

10. P14016 L5 Muriate of Potash is Potassium chloride, so something is wrong here. Also I am surprised that having applied NPK, a further K source is added. In the oil palm plantations, rock phosphate is the common source of P. These details should be checked.

Author's response: Thanks for this comment.

We will change the text as follows:

"The main categories of fertilizer are nitrogen-phosphorus-potassium (NPK 16:16:16) and urea, which are applied three times a year, and potassium chloride (KCl) that is applied once a year."

11. P14021 L1As far as I can see in Figure 2B, the degraded land symbols (downward pointing triangles) are all within the oxic zone grouping.

Author's response: Thanks for this point. We understand it's a bit confusing. What we meant was: degraded land was not distinguished accurately between oxic and anoxic zones.

We will change the text on line 24 on P14020 - line 1 on P14021 to the following:

“The remaining samples clustered roughly into oxic and anoxic zones, although samples from degraded land from anoxic zones were mixed within oxic zones.”

12. P14021 L3ff There are no indicators of variability on the indices in Table 1 so it is difficult to judge whether these differences are significant or not.

Author's response: We do have variability on the Shannon diversity indices. They all fall within ± 0.17 and ± 0.08 in the oxic and anoxic zones, respectively.

We have added the following text to the footnote of Table 1:

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

13. P14023 L13 It is perhaps surprising to see a large influence of nitrate in the anoxic zones. Normally we might expect nitrate to disappear rapidly in zones already depleted of oxygen. The actual levels of nitrate are not given but any comment? Perhaps, as Fig. 2 indicates, the “anoxic” zones are not truly anoxic.

Author's response: The nitrate concentration in anoxic zones is 20-30 folds lower than the values detected in oxic zones.

We will add text as follows on line 14 in P14023 L14:

“.....zones. Nitrate levels were lower in the anoxic zones compared to oxic zones by 20-30 folds, which is likely to drive differences in their community structures. Bacterial communities.....”

14. P14024 L5 It needs to be recognised here and elsewhere in the discussion (L23) that the metabolic profiling does not look exclusively at bacterial metabolites. Some can equally come from fungi and other microbes. In fact, do we know the relative importance of bacteria and fungi in these soils?

Author's response: Thanks for pointing this out.

We agree to your suggestion and will add text as follows on lines mentioned below:

Line 4-6 on P14024: “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.”

Line 18-23 on P14024: “Compared with microbial profiles, those of metabolites were additionally able to differentiate land-use types from locations that are separated by nearly 8 km distance. In addition, metabolic profiling, which relies on markers derived from functions of diverse biotic communities and not just bacteria, provide a finer classification of peatland sites.”

15. P14025 L11 Tropical peatlands are wetlands themselves so you need to be more specific about the ones referred to here.

Author's response: Yu and Ehrenfeld (2010) have studied pinelands. We understand that peatlands are rather wetlands, however, the point that we wish to emphasize here is that the microbial profiling are able to capture the temporal fluctuations of water table. We will rephrase the text as follows from line 11-13 on P14025:

“Such changes in microbial community structure along a hydrological gradient have been reported in other natural ecosystems, such as in forested, temperate pine wetlands (Yu and Ehrenfeld, 2010). However, temporal fluctuations in water table in high and low water table sites leading to rapid drying and wetting, as seen here, were not present in the pine wetland study.”

16. P14027 L10ff To be fair, you only looked at the oil palm plantations and settlement sites; *Stenotrophomonas* sp. may well have been found elsewhere. One conclusion could be that the monoculture of oil palm is good in that it sponsors this organism, but I don't think you are trying to say that. Perhaps you need to refer back to Table 1.

Author's response: Thanks for pointing this out.

As we have not done this analysis for all land-use types, we take your suggestion. We will delete the sentence (line 10-12) on P14027:

~~“In oil palm plantations and settlement sites in our study, a plant growth promoting microorganism, *Stenotrophomonas* sp. was one of the most abundant species found”~~

17. P14039 Fig. 2. It is not clear what “n” is – the number of pairs or the total number? Also, it is not fully explained why so many of the BWT points are found within the AWT grouping (in addition to those in the low water subgroup).

Author's response: Thanks for this point.

To clarify what ‘n’ refers to, we will add the text below to the legend of Fig. 2a:

“ ‘n’= total number of independent measurements.”

We will replace the text from line 1-3 on P14021, “However....the time of sampling.” with the text below:

“BWT points found within AWT group are of two types. One of these BWT group is in a cluster that is highly influenced by low water table (as shown in the Figure 2b). This explains why this subgroup has both AWT and BWT points all coming from low water table sites. Of the remaining 8 BWT points within the AWT group, 3 belonging to oil palm plantation sites are right at the periphery of the BWT specific group. Another one (marked by arrow) is an outlier which was due to flooding. This leaves 4 BWT points belonging to degraded land, which we believe have high water table fluctuations as the water table is not controlled by hydrological interventions. While forested areas are also not managed, they are unlikely to have large fluctuations due to closed canopy. Thus, the degraded forest BWT point falls in the BWT specific group.”

18. P14040 Fig 3(B) The legend states “from above (oxic zones) and below (anoxic zones)” but in the figure itself it only mentions the former. There a discrepancy here; is something missing? Where are the anoxic points?

Author’s response: Thanks for pointing this out.

The figure is supposed to show only the oxic zones. We will therefore remove the word “below (anoxic zones)” from the legend.

19. P14042 Fig. 5 I am not convinced that 5A needs to be given. It is essentially a repeat of what has already been given in Fig. 2B except it shows only the anoxic zone values and gives a mirror image (which is immaterial). What is slightly curious is that the relative position of the arrowed point has changed somewhat; I am not sure how that is explained. I would recommend only presenting 5B (with the flooded site arrowed and possibly indicating those oil palm sites that are not really anoxic also). P14023 LL20-22 then needs to be deleted. It is less than convincing that the TRF patterns are separated on the basis of “habitat” while the metabolic profiles are separated on the basis of land-use. The separation of the flooded site is only one case.

Author’s response: As suggested, we will remove Fig. 5a. and present only Fig. 5b with modifications suggested. The legend for the revised **Fig. 5** will be as follows:

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

Fig. 5a (which is now removed as per your suggestion) was a subset of Fig. 2b and the number of points in the former was less. The stress value indicative of the fit between similarity indices and inter-object distance was less for nMDS plot in Fig. 5a than Fig. 2b. Therefore, there is possibility of shift in positioning of arrowed point. However, trend still remains the same.

The point that we would like emphasize here is that the bacterial profiles even though could marginally distinguish based on land-use patterns, it could significantly separate based on habitat (flooded and non-flooded). On the other hand, metabolic profiling could clearly distinguish based on land-use patterns as well as geographic locations. We will now rephrase the line 18-24 on P14023 as follows:

“Metabolic profiling of bacterial communities (Fig. 2b and Fig. 5). Bacterial communities from anoxic zones were marginally separated based on land-use patterns (Fig. 2b). The communities were separated based on habitat, as revealed by significant separation of the flooded site (indicated by arrow) from the non-flooded sites of mixed crop plantations (Fig. 2b). When comparing the functional data from metabolic profiling (Fig. 5), distinct clusters of different land-use types were formed. Metabolic profiling....”

20. Figure S1 There seems to be a discrepancy between the points (13) enclosed as Low water table dominated group here and those (8) labelled as Low water table in Fig 3B (all Medium peat depth). Figure 2B similarly has only 8 values in the oxic low water table group. Which is correct?

Author's response: Fig. S1 shows difference based on age of drainage. However, the 8 data points on the periphery belong to “low water table depths’ and “old age of drainage” We have replaced the text from “low water table dominated group” inside the Fig. S1 to: “Low water table sites (8 data-points on outer periphery)”

21. Figure S2 I am a bit confused here. You give values for LWT anoxic zones in B). However, from the methods, and reflected in Table 3, for the LWT sites you sampled above the water table at 20-30cm and 50 cm, both above the water table at 80 cm and hence both oxic. It is not clear which sites and depths were sampled. Also it would be useful to emphasize in the legend that these results refer to the settlements (HWT) and oil palm (LWT) sites only.

Author's response: Figure S2 represents predicted taxa group averaged over all sites based on water table conditions. For oxic zones of LWT sites, we have taken average of predicted taxa from both depths (20-30 cm and 50 cm). For anoxic zones of LWT sites, samples were taken from below water table. For HWT sites, all sites with high water table, DHFN, DHAN, DHPO, DHTO, DHPN, SHXN, MHXN and MHPN (details of this nomenclature given in legend of Fig. 1) were taken into consideration for calculation of predicted taxa separately from their respective oxic and anoxic zones.

As suggested, we will change the legend for Fig. S2 as follows:

“**Figure S2:** Relative abundance of bacterial population from all sites with high water table (HWT) and low water table (LWT), based on phylogenetic assignment using FRAGSORT from A) above water table (oxic) zones and B) below water table (anoxic) zones. For oxic zones of LWT sites, average of predicted taxa from depths, 20-30 cm and 50 cm from water table, were taken.”

22. Table S1 (Also in Fig. 4) We have significance values given for “all” canonical axes. However, is this correct? As I understand it significance values are assigned to each canonical axes and the significance of canonical axis 2 would be less than that of canonical axis 1.

Author’s response: We will modify Table S2 as follows indicating the significance values for first and all canonical axis:

Table S1: Relationship between the bacterial community structure and geochemical parameters as revealed by canonical correspondence analyses and Monte Carlo permutation tests performed separately for 16S rDNA gene-based T-RFLP data sets and geochemical data.

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

Technical corrections:

23. P14012 L6 Replace “world’s” with “the world’s”.
24. P14012 L8 Replace “tropical” with “the tropical”.
25. P14012 L11 Replace “deeper” with “a deeper”.
26. P14012 L17 Replace “decrease” with “decreases”. Also delete “in many cases” (in every case where the water table has been reduced, some peat will be newly exposed to oxidation).

Author’s response: Thanks for the comments 23-26. We will change the text as suggested.

27. P14013 L22ff Delete “Hence,” since the choice of molecular profiling, as such, does not follow from the preceding argument. I’m not sure that the following list of references is all that helpful; there are whole journals devoted to molecular profiling.

Author’s response: Thanks. We will delete ‘hence’ as suggested. Also we will reduce the reference list and the text will appear as follows:

“Molecular profiling approaches have been widely used to describe microbial diversity in soil, rhizosphere, extreme environments, freshwater and mangrove ecosystems (Bai et al., 2012; Chan et al., 2013; He et al., 2012; Nold et al., 2000; Xiong et al., 2010).”

28. P14014 L17 Replace “microbial” with “molecular”.
29. P14014 L20 Replace “molecular” with “microbial”.

Author’s response: Thanks for the comments 28-29. We will change the text as suggested.

30. P14014 L24 Sometimes you use “settlements” and sometimes “settlement”; it would be good to be consistent.

Author’s response: We would prefer to stick to “Settlements” and hence make it to “settlements” in the entire manuscript.

31. P14014 L26 Replace “influences” with “the influence of”.
32. P14014 L28 Replace “in” with “in the”. Replace “of the” with “of”.
33. P14015 L11 Replace “from” with “from the”.
34. P14016 L10 Replace “transect” with “a transect”. Replace “location” with “locations”.
35. P14016 L14 Replace “OX-N” with “an OX-N”.
36. P14016 L23 Replace “ZR” with “a ZR”.
37. P14017 L2 Replace “at” with “at the”.
38. P14017 L3 Replace “and” with “and the”.
39. P14017 L5 Replace “of” with “at”.
40. P14017 L6 Replace “at” with “for”.
41. P14017 L9 Replace “instructins” with “instructions”.

42. P14017 L21 Replace “gram” with “grams”.
43. P14018 L2 Replace “ion” with “an ion”.
44. P14018 L10 Replace “with” with “with an”.
45. P14019 L10 Replace “using” with “using a”.

Author’s response: Thanks for the comments 31-45. We will change the text as suggested.

46. P14019 L23 Clarify that this was for Settlements only.

Author’s response: Thanks.

We will replace the text on P14019 L23 as

“The samples were taken from both oxic and anoxic zones from Settlements site.”

47. P14020 L6 Replace “MEGA 5” with “MEGA5”.
48. P14020 L9 Replace “Vector” with “the vector”.
49. P14020 L11 Replace “301” with “302”?
50. P14020 L13 Replace “Genbank” with “GenBank”.
51. P14023 L15 Replace “a major” with “some”. Not convincing from the length of the sodium and chloride arrows that “a major influence” is warranted.
52. P14024 L16 Replace “scale” with “scales”.
53. P14025 L24 Replace “depth” with “depths”.
54. P14025 L28 Replace “sequester” with “dissipate”. Nitrogen is lost from the system, not gained.

Author’s response: For the comments 47-54, we will change the text as suggested in the manuscript.

55. P14026 L9 Delete “agricultural soil”

Author’s response: Sure. We will delete that text.

56. P14026 L11 Replace “utility” with “role”.
57. P14026 L17 Replace “peat” with “the peat”.
58. P14026 L21 Replace “loss” with “loss from tropical peatlands”.
59. P14027 L5 Replace “burnt” with “burning”.

Author’s response: For the comments 56-59, we will change the text as suggested.

60. P14029 L19 In the text you use 2a and 2b while the figures have 2A and 2B (Same for Figs 3 and 4).

Author’s response: We would prefer to use small alphabets. We will change it in the Figures.

61. P14040 Fig. 3. The format should follow the other figures, i.e. B) should be larger and on the right of A). Some colour in B) might be helpful.

Author's response: We have revised Fig. 3 as per your suggestion.

62. P14041 Fig. 4. The colour of the oil palm plantations appears to have changed; can it be the same as that in Fig. 2? Where it states “Environmental and geochemical data”, strictly these are all geochemical data; there aren't any environmental data.

Author's response: The software used to generate the the Fig. 2 and Fig. 4 are different and hence this colour change. The current represented colour is most similar in the available options that can match between the two softwares. Hence, the current colour of oil palm plantations is closest match between the two Figures. However, as per your suggestion, we will change the replace the text “Environmental and geochemical data” to “geochemical data” in the legend.

63. P14042 Fig. 5. The meaning of the arrow should be indicated here in the legend.

Author's response: As per your suggestion, we will delete the Fig. 5a and hence, legend for Fig.5 will be as follows. We have addressed this in our reply for comment no. 19.

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

64. Figure S1 Replace “New age of drainage (<10 years)” with “Drainage <10 years”. Replace “Old age of drainage (>10 years)” with “Drainage >10 years”. The original expressions are a little strange. See also Table 3 and legend to Fig 1.

Author's response: We have changed this in Fig. S1, Table 3 and also in legend to Fig.1.