

## Referee comment for Biogeosciences

### Research article:

Plant *n*-alkane production from litterfall altered the diversity and community structure of alkane degrading bacteria in litter layer in lowland subtropical rainforest in Taiwan

Tung-Yi Huang<sup>1\*</sup>, Bing-Mu Hsu<sup>1\*</sup>, Wei-Chun Chao<sup>2</sup>, and Cheng-Wei Fan<sup>1</sup>

### 1. General comments

It was a pleasure to read the manuscript and to be able to contribute comments to its review process. The manuscript does represent a substantial contribution to scientific progress with valuable data from three different and unique environments from a rainforest in Taiwan. The paper does establish a relationship between vegetation/litterfall and *n*-alkane degrading bacteria.

Some results tend to be very general and need more details, especially when referencing *n*-alkane degrading bacteria. Very general results in some figures, no details and not everything shown from what is stated in the conclusions. More details need to be presented, as supplementary results?

Finally, I would strongly suggest a native English speaker to help enhance the language of the written English in the paper.

### 2. Specific Comments

#### Page 1

Line 20: bacteria not bacterial

Line 29: please state how small a fraction.

#### Page 2

Line 8: Sentence not clear

Line 10: Probably denominated is not a clear statement.

Line 14: oil as in crude oil?

Line 15: how upregulated? Values would be helpful to understand.

Line 21: what do you mean by seedbanks? Not clear how abundant they are

Line 22: not clear what litterfall is and how to differentiate from other litter.

Line 27: reference?

Line 31: do you have diversity values for these forest plots?

Line 34: how high? Is it statistically significant?

#### Page3

Line1: why were those plots chosen from all the rainforest? Are they the most different?

Line14: poor information on *alkB*

Line 19: why is there a difference in the number of stands in your plots?

Line 21: What is the distance between stands? A map would be helpful to understand the experiment.

Line23: what is the effect of oven-drying the samples for 14 days? Is this the best approach? Reference?

Line24: why is there a difference of 3 years between sampling? Were this samples dried as well? How many samples were actually used during the analysis? It seems like there is a great amount of samples from what has been written.

#### **Page4**

Line 15: Litter leaves are fairly fresh fallen from the trees (less than a month). Are these *n*-alkane degradative bacteria on the leaves before they fall on the ground? (This is a question for the analysis)

Line 24: how small are the leaf pieces? Are they macerated?

#### **Page 5**

Line 1: size of amplicon from your 16S rRNA PCR?

Line5-11: Why are your primers so big? 40-50bp?

Line12: what are the expected sizes? A chart and PCR programs would be helpful to replicate the results.

Line19: is this information from metagenome DNA? How much DNA was extracted in each sample and included in the metagenomes? How many replicas?

#### **Page 6:**

Line5: 95% similarity at the nucleotide level or protein level? What is the size of the gene?

Line24: a bulk of the DNA extracted was used for this PCR? How many replicas?

#### **Page7:**

Line 8: diversity values for each site would be handy at this point.

Line10: effects of the environmental changes are not clear

Line 18: I looked at table 1 first before reading this and it doesn't correlate. Fig 1 is leaf production, is this different from litterfall from leaves? Not clear.

Line28: Does this statement contradict 3.2?

#### **Page8**

Line6: within species in the same sample? It looks like there is a 10-fold change and this might be very important at the bacterial level.

Line12: Figure 3 is a great figure! How many replicas? Can a statistical method be applied? Is it significant? I still have the question about new litterfall vs. old litterfall.

Line13: Conclusion from top or new litterfall?

Line20: how does this relate? New hypothesis is not clear according to the use of the reference. How was the total organic carbon established in the reference?

Line 28: only at the phylum level.

Line 29: diversity values are still necessary

#### **Page9**

Line1: These diversity values would be good at the beginning of the papers results? Or as part of the introduction?

Line3: remember to mention that it is at the phylum level in windward and leeward in the top litterlayer? Were windward and leeward too close? What is the distance between them? May this explain why ravine is different?

Line8: how was this shown? By species?

Line 13: OTU's from metagenomes?

Line 18: why? Do the *alkB* numbers correlate to your abundance of the organisms that have these genes? How diverse where your genes or do they all correspond to a specific organism? Did you bin your reads and identify the organisms that had the *alkB* genes?

Viewing the sequences would be interesting, or the most representative and a gene tree?

Line22: OTU's are 16S rRNA?

Line29: the numbers look quite similar. Can you explain a bit more about the index?

### **Page10**

Line1: what about abundance of OTU's?

Line13: poor information on DNA amounts included in each sample and amount of DNA in each plot, site. Could the results be caused because of the amount of litterfall? Not clear the difference between windward and leeward in the analysis yet. A bit more information would be appreciated.

### **Page11**

Line4: in the phylum level.

Line8: more details on the actual genus of Proteobacteria and Actinobacteria that changed would be important. Correlation to references of these organisms using *n*-alkanes.

Line11: most results are with relative abundance, what was the actual real abundance of these organisms? These differences might be an effect of the type of graph used?

DNA amounts from Fig4 are important.

Fig5 was taken from metagenome data?

### **Table1**

nice table. the +, ++ language is not clear. It seems that more + would be more significant. What is a semi-quantitative score?

### **Figure2**

One plant in each place? Why not the same plant in each place? The explanation in the text and what Table1 show do not correlate!

### **Figure4**

What about the genus or species level?

### **Figure6**

What is the blue point and why was it taken out?

### **Figure7B**

Is this graph in percentage? Why are there values over 200?

### **Figure8**

Interesting figure, leaves a few questions on difference between windward and leeward.

### 3. Technical errors

Interestingly is used frequently throughout the paper.

*n*-alkane

#### Page 2

Line 16: alkanes

#### Page 7

Line 15: all numerals are 3.1, 3.2 or 3-1 and 3-2? Make sure the format is the same.

#### Page 10:

Line 23: may be instead of suggesting