Dr. Denise M. Akob
Associate Editor, ***BG***

Dear Dr. Akob,

Please find the revised version of our manuscript "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," which had been submitted for publication in ***BG***. The manuscript has been revised according to the referees' comments. The details of the changes made were shown in the follows. Also, the manuscript has been revised by a professional Academic Writing agency to correct the English language.

Thank you for your attention.

Sincerely,

Cheng-Wei Fan
.

Responses to Referee #1

Anonymous Referee #1
Received and published: 10 August 2017

Huang et al. studied the relationship between the plant derived long chain *n*-alkane contribution and the composition of the microbial community, specifically alkB degrading bacteria in three habitats of a subtropical rainforest. Their results show that the composition of the microbial community and the relative abundance of the alkB degrading bacteria are controlled by the *n*-alkane input of higher plants with higher amounts of alkB degrading bacteria in habitats with higher amount of litterfall, i.e. higher *n*-alkane contribution of plants.
I think the results are very interesting and a valuable contribution to the BG community. However, some improvements need to be done before the final publication of the manuscript.

General comments:
-the language needs further improvement. I am not a native speaker, but I would encourage the authors to consider that an expert may revise this manuscript, I think it would benefit a lot.

[Reply]
The manuscript has been revised by a professional academic writing agency to correct the English language.

-The "*n*" in *n*-alkanes should be italic
-The plural of *n*-alkane is *n*-alkanes
[Reply]
The changes have been made accordingly throughout the manuscript.

Specific comments

-page 1, line 10: its *n*-Alkanes, the '*n*' should be italic, adapt it in the whole manuscript

[Reply]

The changes have been made accordingly throughout the manuscript.

-p1, L12: I don't think levels is the right word, change it to 'concentrations' or 'amounts' or something more suitable

[Reply]

The word 'concentrations' was used as suggested on P1, L12.

-p1, L15: I would advise you to stay in present tense when writing about your results, they still show or demonstrate or . . .

[Reply]

We agree with the reviewer. The results were stated in present tense as suggested on P1, L15 and P1, L25.

-p1, L30: please add long chain *n*-alkanes with odd/even predominance

[Reply]

We rephrased the sentence on P1, L30.

-p2, L3: change 'inactive' to 'stable' or 'inert'

[Reply]

The word 'stable' was used as suggested on P2, L5.

-p2, L7: Maybe you can add what the end-product of the degradation is. Especially or researchers using *n*-alkanes as geochemical fossils it would be interesting to know which compounds were built from the *n*-alkanes.

[Reply]

We rephrased the sentence on P2, L8-L12.

-p2, L13 studies have shown. This occurs quite often in this manuscript, adapt it

[Reply]

The sentence was revised on P2, L19.

-p2, L18: Giebler et al. (2013)

[Reply]

The "(2013)" has been added (P2, L26).

-p2, L20 skip the citation here

[Reply]

The change has been made accordingly on P2, L28.

-p2, L20: alkane degrading bacteria

[Reply]

The phase "alkane degradation bacteria" has been revised to "alkane degrading bacteria" on P2, L28.

-p2, L20: might be
[Reply]
The sentence was revised to P2, L28-L30.

-p2, L23: the relationship… is more
[Reply]
“are” has been changed to “is” accordingly on P2, L33.

-p2, L24: it was shown
[Reply]
The sentence has been rephrased on P2, L33-L34.

-p2, L27: change ‘researches’ to ‘studies’
[Reply]
The sentence has been rephrased on P2, L34-P3, L2.

-p2, L32-p3L1: Please rephrase
[Reply]
The sentence has been rephrased on P3, L8-L11.

-p3, L2: change ‘applicable’ to ‘possible’ or something else
[Reply]
The sentence has been rephrased on P3, L16.

-p3, L10 change ‘employed’ to ‘used’
[Reply]
The word “employed” has been changed to “used” accordingly on P3, L24.

-p4, L11-14: So basically, your quantification and identification based on external standards? If so, then state it here
[Reply]
The sentence (p4, L11-14) was rephrased on P4, L24-25.

p4, L14: What was the recovery of the cholestane? Also, if you did not plan to correct your results for the recovery of the standards, why did you use them at all?
[Reply]
The recovery of the 5a-cholestane is 99.3± 6.9 %. An explanation of recovery of cholestane has been added to P8, L8-10.

-p7, L3: annual should be written in small letters
[Reply]
The word “Annual” has been changed in small letters on P7, L21.

-p7, L19/20: odd/even occurs two times, delete one of them
[Reply]
The redundant word ” odd/even” has been deleted (P8, L11).

-p7, L22: at the beginning of a sentence the 'n' in *n*-alkanes is written in small letters and the 'A' is written in upper case letters (*n*-Alkane)
[Reply]
The mistake has been corrected (P8, L11).

-p8, L1: which environmental parameters? Give some examples.
-p8, L2: reference?
[Reply]
An example with reference has been added on p8, L22-23.

-p8, L6: why?
[Reply]
An explanation of the variation of leaf *n*-alkane concentration within the same species has been added on P8, L23-28.

-p8, L16/17: skip the last sentence. There is no further statement that hadn't been said before.
[Reply]
The sentence was rephrased on P9, L6-7 to make it more clear.

-p11, L2-5: Delete the first two sentences
[Reply]
The first two sentences have been deleted.

-Figure 2: change 'traces' to 'chromatograms'. Also, why do you show the solvent peak?
[Reply]
The word 'traces' has been changed to 'chromatograms'
The solvent peak has been deleted.
In revised figure 2, the chromatograms of *Iles rotunda* from each habitat was showed (P8, L7).

-Figure 3: delete 'dynamic', change 'changes' to 'concentrations'
[Reply]
The words have been corrected as suggested.

Responses to Referee #2

Comments of Anonymous Referee #2
Received and published: 13 August 2017
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 Huang1,*, Bing-Mu Hsu1,*, Wei-Chun Chao2, and Cheng-Wei Fan1

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?

[Reply]

We appreciate the insightful criticizes. More detail information regarding *n*-alkane bacteria has been presented as supplementary results, including the phylogenetic trees of OTUs in *Proteobacteria* and *Actinobacteria* together with alkB contained bacteria. A summary table (Table S-1) with detail information of these OTUs is also presented.

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

[Reply]

The manuscript has been revised by a professional academic writing agency to correct the English language.

2. Specific Comments

Page 1

Line 20: bacteria not bacterial

[Reply]

The word ‘bacteria’ is used as suggested on P1, L20.

Line 29: please state how small a fraction.

[Reply]

The sentence has been rephrased on P1, L28.

Page 2

Line 8: Sentence not clear

[Reply]

The sentence has been rephrased on P2, L12-13.

Line 10: Probably dominated is not a clear statement.

[Reply]

The sentences have been rephrased on P2, L14-17.

Line 14: oil as in crude oil?
[Reply]
The phase 'crude oil' has been used on P2, L21.

Line 15: how upregulated? Values would be helpful to understand.
[Reply]
An explanation of upregulated added on P2, L21-24.

Line 21: what do you mean by seedbanks? Not clear how abundant they are
[Reply]
The word "seedbank" has been deleted and the sentence has been rephrased to make it clear on P2, L28-31.

Line 22: not clear what litterfall is and how to differentiate from other litter.
[Reply]
The sentence in –p2, L21-L23 has been rephrased on P2, L31-33.

Line 27: reference?
[Reply]
The reference (Schulz *et al.*, 2012) has been added on P3, L2.

Line 31: do you have diversity values for these forest plots?
[Reply]
The diversity values have been added to a supplementary table (Table S-2).

Line 34: how high? Is it statistically significant?
[Reply]
In the study, the litterfall in the ravine, windward and leeward area are 6.48±1.57 (t ha-1yr-1), 4.56±0.29 (t ha-1yr-1) and 5.16±0.64 (t ha-1yr-1), respectively. The litterfall in the ravine was 20% to 30% higher than that of leeward and windward habitats. The statistic results were not available.

Page3
Line1: why were those plots chosen from all the rainforest? Are they the most different?
[Reply]
This study aimed to see if differences in alkane concentration in natural habitats can alter the microbial community structure of alkane degraders. Therefore, the plots were selected to test this theory for their various alkane yields.

Line14: poor information on alkB
[Reply]
More information on alkB has been added on P2, L13-L15.

Line 19: why is there a difference in the number of stands in your plots?
[Reply]
The number of stands in the three habitats of Nanjenshan Reserve is mainly based on the availability in this area (P4, L2).

Line 21: What is the distance between stands? A map would be helpful to understand the experiment.
[Reply]
A map of the sampling sites was provided in an attached supplementary file (Figure S-1)as suggested. The horizontal distance between stands is about 50 m (P4, L2).

Line23: what is the effect of oven-drying the samples for 14 days? Is this the best approach? Reference?
[Reply]
We set the oven temperature at 40-degree, as this temperature may affect less on alkane composition during drying arrangement. Indeed, this temperature is good in preserving plant food for animals (Conklin–Brittain *et al.*, 2006). Later, we found that drying at 65-70 degree for 2 days can also serve the needs. However, for the integrity of the data set, we keep the 40-degree protocol in this study.

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.
[Reply]
The sample collection of litterfall was conducted from 2012 to 2013 to evaluate the litterfall yields and the alkane productivities from the 3 habitats. A total of 264, 12 x (6+4+12), stand samples were collected. The results of *n*-alkane analysis indicated that *n*-alkane productivity in the ravine was about two folds higher than those of windward and leeward habitats. It provides us with the rationale to study the microbial community structure. Therefore, the sample collection of litter layer from the 3 habitats for NGS study in bacteria was carried out during 2015-2016.

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)
[Reply]
We think these alkane degraders start to degrade the leaves after they fall on the ground (litter layer). We have some experience in SEM study trying to identify bacteria on litter-leaf. We find little if any bacteria can be found on the surface of freshly fallen leaves.

Line 24: how small are the leaf pieces? Are they macerated?
[Reply]
For the DNA extraction, several pieces of leaves from litterfall were randomly selected. They were cut into 2 mm x 2 mm chips and well blended. Around 0.1-0.2 g of leaf debris was weighed for DNA extraction as described in user manual -p15.
(http://www.mn-net.com/Portals/8/attachments/Redakteure_Bio/Protocols/Genomic%20DNA/UM_gDNASoil.pdf).

Page 5
Line 1: size of amplicon from your 16S rRNA PCR?
[Reply]
The size of the amplicon is about 460 bp.

Line5-11: Why are your primers so big? 40-50bp?
[Reply]
The full-length primer sequences include an adapter overhang nucleotide sequence and the V3-V4 primer pairs. The length of adapter overhang is about 33-34 bps and the primer for V3-V4 is 17 or 21 bps.

Line12: what are the expected sizes? A chart and PCR programs would be helpful to replicate the results.
[Reply]
The expected sizes are approximate ~460 bp. The detailed protocol including charts and programs were provided in the following pdf.
(https://support.illumina.com/content/dam/illumina-support/documents/documentation/chemistry_documentation/16s/16s-metagenomic-library-prep-guide-15044223-b.pdf).

Line19: is this information from metagenome DNA? How much DNA was extracted in each sample and included in the metagenomes? How many replicas?
[Reply]
The information described is from metagenome DNA. A flow chart was provided in Figure S-5 in the “Response to referee #2”.

Page 6:
Line5: 95% similarity at the nucleotide level or protein level? What is the size of the gene?
[Reply]
The similarity is at the nucleotide level. The size is about ~400 bp as showed in the supplementary table (Table S-1).

Line24: a bulk of the DNA extracted was used for this PCR? How many replicas?
[Reply]
For detail information, please see the Figure S-5 in the “Response to referee #2”.

Page7:
Line 8: diversity values for each site would be handy at this point.
[Reply]
The diversity values have been provided in the supplementary table (Table S-2).

Line10: effects of the environmental changes are not clear
[Reply]
The sentences have been rephrased on P7, L27 to P8, L1.

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.
[Reply]
Figure 1 is the result of litterfall, which includes litter-leaf, branches, flowers, fruits and plant debris, while table 1 is the result of litter-leaf.

Line28: Does this statement contradict 3.2?
[Reply]
To avoid the confusion, we have changed the title of 3.3 and the first sentence on P8, L16-L20.

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.
[Reply]
We have rephrased the sentences on P8, L23-L28.
We agree with that high concentration of *n*-alkane in some leaves might be very important at the bacterial level. However, we hypothesized that most of the bacteria on leaves start to grow after they contacted with soil (see above Page4 Line 15 reply).

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.
[Reply]
To make it consistent with figure 1, we revised figure 1 and figure 3 (P9, L2-L7).
The n value for the ravine, windward, and leeward habitats will be 12, 6, and 4, respectively. There are significant differences between litter-leaf, litter-layer and surface soli in all three habitats. Also, the differences between data from the ravine and the other two habitats are significant.

Line13: Conclusion from top or new litterfall?
[Reply]
The litterfall was collected once per month. The conclusion was made based on the results of annual summation.

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?
[Reply]
We rephrased the entire paragraph on P9, L8-L17.

Line 28: only at the phylum level.
[Reply]
Figure at the class level has been provided. Please see attached file in supplementary figure (Figure S-4).

Line 29: diversity values are still necessary
[Reply]
Diversity values have been provided. Please see attached file in supplementary table (Table S-2).

Page9
Line1: These diversity values would be good at the beginning of the papers results? Or as part of the introduction?
[Reply]
These values have been removed to the introduction as suggested (P3, L11-L14).

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?
[Reply]
We have provided the relative abundance of bacteria in three habitats at the class, order, and family levels in Figure S-4 (text on P9, L21).
The results indicated that the composition of the relative abundance of bacteria is similar between windward and leeward habitats. It's true that windward and leeward are close to each other (200 m). However, we would like to point out the height and number of plants varied drastically between windward and leeward habitats. These factors which affect the structure of plant vegetation don't have an impact on the structure of microbial communities.

Line8: how was this shown? By species?
[Reply]
The table of alkB lineage-like OTUs and the reference genes was provided in the supplementary table S-1.

Line 13: OTU's from metagenomes?
[Reply]
Yes, they are.

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?
[Reply]
We didn't bin the nucleotide reads. The amount and correlated bacteria and the picture of gene tree were described in figure S-2, figure S-3, and table S-1 as supplementary material.

Line22: OTU's are 16S rRNA?
[Reply]
Yes, they are.

Page9
Line29: the numbers look quite similar. Can you explain a bit more about the index?
[Reply]
Since we provided the detail biodiversity index in table S-2 and revised the figure 6, the paragraph in section 3.7 has been rephrased on P10, L22-L28.

Page10
Line1: what about abundance of OTU's?
[Reply]
Please see the detail information in the supplementary table S-1.

Page10
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.
[Reply]
An attached file (Figure S-4) was provided to illustrate the protocol. We rephrase the sentences (p7, L6-L19).

Page11
Line4: in the phylum level.
[Reply]
We think at least in family 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.
[Reply]
Please see detail in the supplementary figure S-2, figure S-3, figure S-4, and table S-1.

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.
[Reply]
We conducted semi-quantitative PCR to check the relative alkB gene levels in different habitats.

A schematic procedure has been provided to show the difference in sample preparation for NGS PCR and the semi-quantitative PCR.

Fig5 was taken from metagenome data?
[Reply]
Yes, it was.

Table1
nice table. the +, ++ language is not clear. It seems that more + would be more significant. What is a semi-quantitative score?
[Reply]
Please see above reply on Page7, Line28. We use the number of plus sign (+) to give a quick impression that species of high *n*-alkane concentration were found being abundant in the ravine habitat, resulting in its high *n*-alkane fluxes.

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!
[Reply]
We agree with the reviewer. We provided the GC-FID chromatograms of *Iles rotunda* in each habitat as suggested. The paragraph in 3-2 was rephrased as well to consist with figure 2 (P8, L6-L8)

Figure4
What about the genus or species level?
[Reply]
The data of relative abundance in order and family levels were provided as an attached pdf file (Figure S-4). There are too many numbers of genus or species to illustrate properly in a relative abundance figure. In this regard, we provide relative abundance in level and phylogenetic of *Actinobacteria* and *Proteobacteria*.

Figure6
What is the blue point and why was it taken out?
[Reply]
Figure 6 is the result calculated by the sequence. We add the circle to point out that location of *Actinobacteria*. To avoid confusion, the circle has been taken away. A revised figure 6 was attached.

Figure7B
Is this graph in percentage? Why are there values over 200?
[Reply]
Yes, it is in percentage. The average of band density levels in windward was given as 100%. It means that the relative copy numbers of alkB in ravine area were two folds higher than those of windward habitat.

Figure8
Interesting figure, leaves a few questions on difference between windward and leeward.
[Reply]
We agree with the reviewer.

3. Technical errors
Interestingly is used frequently throughout the paper.
[Reply]
Most of the words 'interestingly' have been either deleted or rephrased.

n-alkane
Page 2
Line 16: alkanes
[Reply]
The word 'alkanes' and '*n*-alkane' have been checked and rephrased throughout the manuscript as suggested.

Page 7
Line 15: all numerals are 3.1, 3.2 or 3-1 and 3-2? Make sure the format is the same.
[Reply]
We apology for the mistake. The format will be the same in the next version of the manuscript.

Page 10:
Line23: may be instead of suggesting
[Reply]
The sentence has been rephrased on P11, L17-L18.

Conklin–Brittain, N. L., Knott, C. D. & Wrangham, R. W. (2006). *Energy intake by wild chimpanzees and orangutans: Methodological considerations and a preliminary comparison.* . Cambridge.
Giebler, J., Wick, L. Y., Chatzinotas, A. & Harms, H. (2013). Alkane-degrading bacteria at the soil-litter interface: comparing isolates with T-RFLP-based community profiles. *FEMS Microbiol Ecol* **86**, 45-58.
Gómez-Coca, R. B., Pérez-Camino, M. d. C. & Moreda, W. (2016). Saturated hydrocarbon content in olive fruits and crude olive pomace oils. *Food Additives & Contaminants: Part A* **33**, 391-402.
Kostka, J. E., Prakash, O., Overholt, W. A., Green, S. J., Freyer, G., Canion, A., Delgardio, J., Norton, N., Hazen, T. C. & Huettel, M. (2011). Hydrocarbon-degrading bacteria and the bacterial community response in gulf of Mexico beach sands impacted by the deepwater horizon oil spill. *Appl Environ Microbiol* **77**, 7962-74.

Nie, Y., Chi, C. Q., Fang, H., Liang, J. L., Lu, S. L., Lai, G. L., Tang, Y. Q. & Wu, X. L. (2014). Diverse alkane hydroxylase genes in microorganisms and environments. *Scientific Reports* **4**, 4968.
Rojo, F. (2010). *Handbook of Hydrocarbon and Lipid Microbiology*. Springer-Verlag Berlin Heidelberg.
Schulz, S., Giebler, J., Chatzinotas, A., Wick, L. Y., Fetzer, I., Welzl, G., Harms, H. & Schloter, M. (2012). Plant litter and soil type drive abundance, activity and community structure of alkB harbouring microbes in different soil compartments. *ISME J* **6**, 1763-74.

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

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Abstract

Abstract. *n*-Alkane and alkane-degrading bacteria have long been used as crucial important biological indicators in paleoecology, petroleum pollution, and oil and gas prospecting. However, their the relationship between *n*-alkane and alkane-degrading bacteria in natural forests is still poorly understoodrelationships in natural forests are still poorly understood. In this study, long chain *n*-alkane (C_{14}-C_{35}) concentrationslevels of plants in litterfall, litter layer, and topsoil and the diversity and abundance of *n*-alkane-degrading bacterial community communities in litter layers were investigated in 3 three habitats across a lowland subtropical rainforest in southern Taiwan, : i.e. the ravine habitat, the windward habitat and leeward habitats in Nanjenshan. Our results demonstrated that the litterfall yield and productivity production and flux of long--chain *n*-alkane in ravine area were highest in the ravine habitats. among all habitats. However, long--chain *n*-alkane concentrations in all habitats were decreased drastically to a similar low level from the litterfall to the bulk soilformed a steep gradient to a similar level from the litterfall to the bulk soil in all habitats, suggesting a higher degrading rate of long--chain *n*-alkane degradation in ravine habitat. The oOperational taxonomic unit (OTU) analysis using from next--generation sequencing data revealed that the relative abundance of microbial communities in the windward and leeward habitats were similar to each other and different from that in the ravine habitat. Metagenomic data mining using the by NCBI database revealed that alkB--gene--associated bacterial (95 % DNA sequence similarity to alkB-containing bacteria95% similarity to alkB contained bacteria in DNA sequence) were most abundanthighest in the ravine area compared to other habitats. Empirical testing of litter -layer samples semiquantitative polymerase chain reaction for determiningby semi-quantitative PCR in alkB gene levels confirmed that the ravine habitat had higher alkB gene levels than the windward and leeward habitats. Heat map analysis revealed parallels in pattern colora parallel in the color pattern between the plant vegetation and microbial species -compositions of the habitats, suggesting a causal relationship between the plant *n*-alkane production and microbial community diversitythe diversity of microbial communities. This finding indicates indicated that the diversity and relative abundance of microbial communities in the litter layer were are affected by the *n*-alkane plant composition in the litterfall derived by plant vegetation.

Key words: n-alkane, Alkane degradation bacteria, alkB gene, Nanjenshan, Next generation sequencing, Microbial community

1 Introduction

Alkanes are saturated hydrocarbons that account accounted for a small fraction of the total organic carbon, with a concentration range in natural habitats of not more than a couple of thousand parts per million by weight (Gomez-Coca et al., 2016; Rojo, 2010).in natural habitats. In non-contaminated habitats, long-chain *n*-alkanes with an odd/even predominance are produced by plants or algae to act as chemoattractants or agents that protectare produced by plants or algae as chemo-attractants or as protecting agents against microbial invasion or water loss (Feakins et al., 2016; Koch et

al., 2009). Alkanes are exceptionally ~~especially~~ stable molecules that can survive in soils and sediment~~s~~, which ~~made~~ make them useful as biomarkers in paleoecology (Sachse et al., 2012). Because ~~Since~~ alkanes are the major component~~s~~ in petroleum, natural ~~nature~~ gas, and diesel fuel, the presence of environmental alkanes~~the existence of alkanes in environmental was~~ also serve~~d~~s as an index of oil contamination (Afzal et al., 2013). Although alkanes are very stable~~inactive~~, most ~~of them~~ can be degraded by several microorganisms. A~~s a~~lkane-degrading bacteria are commonly up-regulated when the concentration~~by the increases~~ of alkanes is increased; ~~,~~ it has thus been suggested that these bacteria be used ~~they have been suggested for using~~ as a biological indicator for oil and gas prospecting (Xu et al., 2013; Rasheed et al., 2012).

Extensive knowledge on the degradation ~~degrading~~ of *n*-alkane by microorganisms has been acquired in the past decades.~~accumulated during the past decades.~~ The first step of ~~n~~ enzymatic reaction is oxidization of n-alkane ~~alkane oxidization is the catabolizing~~ to the corresponding primary alcohol by alkane terminal hydroxylases. This step is crucial because activation of the alkane molecule requires an enzyme system that is not widespread (Rojo, 2010). The products can be further oxidized into a corresponding aldehyde and fatty acid, which are digested by most microorganisms. ~~Despite that some of the alkane degraders of algae and fungi have been discovered, bacteria are one of the most important microorganisms in long chain n-alkane degradations (Rojo, 2010; Rojo, 2009; Singh et al., 2012).~~ Although fungi and yeasts ~~bacteria~~ can degrade alkanes, most research has focused on the role of bacteria in the degradation of alkanes (Rojo, 2009, 2010; Singh et al., 2012). ~~under aerobic or anaerobic conditions, the decomposition of leaves in litter layer probably dominated by aerobic conditions (Wentzel et al., 2007).~~ To date, ~~The~~ alkane monooxygenase gene (alkB) hydroxylases ~~encoded~~ are the most commonly found alkane hydroxylases in both Gram-negative and Gram-positive bacteria.~~membrane-bound homologous protein is an inducible integral membrane-bound alkane hydroxylase that play an important role in aerobic alkane degradation (Beilen et al., 2003; Nie et al., 2014).~~ ~~Studies have showed that the level of alkB bacteria are highly dynamic in response to the fluctuation of environmental alkanes.~~More than 60 genera of aerobic bacteria and 5 genera of anaerobic bacteria are known to degrade n-alkanes (Nie et al., 2014). Although alkanes can be metabolized anaerobically, the growth of anaerobic alkane degraders is significantly slower than that of aerobic ones, especially in the litter layer environment where the oxygen supply is adequate (Wentzel et al., 2007). The alkB-encoded membrane-bound homologous protein is an inducible integral membrane-bound alkane hydroxylase that plays a vital role in aerobic alkane degradation (Beilen et al., 2003). Evidence has revealed that the number of alkB bacteria dynamically changes in response to a fluctuation in environmental alkanes. In soil or water environments, when polluted with crude oil, the alkane-degrading bacteria were up-regulated (Afzal et al., 2013). For example, a 10-fold increase in the relative abundance of oil degraders in contaminated versus clean sand was estimated for a Gulf of Mexico beach (Kostka et al., 2011). ~~Besides, I~~in oil and gas reservoirs, it has been shown~~ed~~ that the abundance of alkB bacteria was substantially higher ~~increased substantially~~ in the surface soils with no significant detection of soil alkane (Xu et al., 2013). The dynamic relationship between ~~of~~ alkane and alkB bacteria in soil from an agriculture ~~agriculture~~ research farm and a laboratory maintained~~or in lab~~ under controlled conditions has been previously studied.~~carefully studied before.~~ Giebler et al. (2013) reported ~~has showed~~ that in a controlled environment, sizeable communities of alkane-degrading bacteria, especially *Proteobacteria* and *Actinobacteria*,

were detected at the soil–litter interface when it was enriched with artificial alkane supplements.~~sizeable alkane-degrading bacteria were detected from soil litter interface when enriched with artificial alkane supplements, especially in Proteobacteria and Actinobacteria (Giebler et al., 2013).~~ The researchers demonstrated that many of the alkane-degrading bacteria were not detected before the alkanes were supplemented, suggesting that some alkane degraders may have been present in very low copy numbers and below the limits of detection. It is reported that the ~~The~~ substrate was discovered to modulate the biomass of microorganisms based on its initial concentration (Schmidt, 1992).~~They suggested that some alkane degradation bacteria in soil might represent as a seed bank and could grow opportunistically. Since substrate can selectively grow certain microorganisms and control the biomass based on its initial concentration (Schmidt, 1992),~~ Thus, different alkane ~~compositions~~yields of litterfall in various natural habitats may upregulate the growth of various alkane degraders. ~~it is reasonable to assume that litterfall might up-regulate the levels of alkB-degrading bacteria.~~ The relationship between alkB bacteria abundance and alkane~~The relationship of abundance of alkB bacteria and alkane~~ substrate ~~are~~ is more complicated in natural habitats. ~~It has been showed that t~~The dynamic changes in~~of~~ alkB- -degrading bacteria populations were demonstrated to be driven by numerous factors, such as the original source of the ~~also driven by many factors such as different sources of~~ alkane and soil type ~~(Schulz et al., 2012).~~. Although ~~the~~ knowledge of the regulatory mechanism regarding ~~of~~ environmental plant litter on soil bacterial levels and of the dynamics of alkane-degrading bacterial ~~dynamic~~ in natural habitats has been beneficial ~~very useful~~ and important in many aspects, studies ~~researches~~ on their dynamic changes are currently lacking ~~largely lacking currently~~ and should be conducted (Schulz et al., 2012).~~established.~~

The Nanjenshan Reserve, a lowland subtropical rainforest in southern Taiwan, consists of several ~~forest~~ dynamics forest plots for which ~~where the data of~~ forest structure, vegetation pattern, climatic, and topography data ~~in permanent study sites~~ have been collected at well- -established permanent study sites.~~ Up-to-date, f~~Four dynamic forest ~~dynamics~~ plots and a transect zone in the habitat have been documented since 1989 (Chao et al., 2010; Chao et al., 2007; Chao et al., 2008; Chen and Huang, 1986; Hsieh et al., 2000; Tsui et al., 2004; Fan et al., 2005). ~~(Chao et al., 2010; Chao et al., 2007; Chao et al., 2008; Fan et al., 2005; Hsieh et al., 2000; Tsui et al., 2004).~~ Among them, three plots captured our attention, namely plot I (120º 50’ 51” E, 22º 04’ 54” N), plot II (120º 50’ 36” E, 22º 04’ 52” N), and the Lanjenchi plot (120º 51’ 38” E, 22º 03’ 23” N). A ravine habitat is located across plots I and II, whereas leeward and windward habitats are located on plot III. Surveys conducted over the past decade have demonstrated that the annual yield of litterfall in the ravine habitat was higher than in those in the windward and leeward habitats. Moreover, studies have shown that in the leeward habitat, plant height was higher and plant density was lower when compared with the windward habitat (8.41±1.73 m vs. 4.63±0.88 m; 7,505 trees ha-1 vs. 20,065 trees ha-1). The plant height and density in the ravine habitat were 9.45±1.35 m and 4257 trees ha−1 (Chin, 2008). The alkane distributions and concurrent alkane-degrading bacteria have yet been uncovered. The litterfall plays an essential role in sustaining the microorganism food chain. Because the litterfalls in the habitats were different, investigations into the effects of litterfall on various habitats and microbial communities in these habitats are possible.~~Surveys conducted from past decade in the area have shown that the annual amount of litterfall in ravine habitat where located across plot I (120º 50’ 51” E, 22º 04’ 54” N) and plot II (120º 50’ 36” E, 22º 04’ 52” N) was higher than in~~

~~windward and leeward habitats where both located in Lanjenchi plot (120º 51' 38" E, 22º 03' 23" N). The alkane distributions and concurrent alkane degrading bacteria have not been uncovered yet. Since the litterfall in those habitats was different, it was applicable to investigate the effects of litterfall on the corresponding level of microbial community in these habitats.~~

E~~Many e~~nvironmental microbial communities are highly complex and diverse and difficult ~~recalcitrant~~ to culture under laboratory conditions. With the recent advent of next-~~-~~generation sequencing (NGS) technology and computational methods, we can conduct genome studies of microbes ~~on~~ in these habitats, which have numerous ~~that were known to have many~~ variables such as annual litterfall productivity and plant vegetation (Degnan and Ochman, 2012). We investigated the productivity ~~aimed to investigate the flux~~ of long-~~-~~chain ~~n-~~*n*-alkane in the pristine natural habitats of the Nanjenshan Reserve~~,~~ and explore their relationship with microbial communities, particularly emphasizing the correlation between ~~n-~~*n*-alkane production and bacteria contained the alkB gene. During the present 1 year ~~In this one-year~~ follow-up study, gas chromatography with a flame ionization detector (GC-FID) was ~~GC-FID~~ used to determine ~~was employed for determination of n-~~*n*-alkane (C_{14}-C_{35}) concentration. ~~The b~~Bioinformatics analyses and metagenomic data mining were performed to identify ~~carried out to reveal the~~ microbial communities and read numbers~~the numbers of read~~ of bacterial lineages that contained ~~carried~~ alkB genes in the three rainforest habits ~~of rainforest~~. Semiquantitative polymerase chain reaction (PCR) ~~Semi-quantitative PCR~~ was performed to test the results of the bioinformatics analysis.

2 Materials and methods

2.1 Sample description

A map of the sampling sites is provided in the supplementary file (Figure S-1). Twenty-two custom-made aluminum stands with frame (0.71 m × 0.71 m) ~~that~~ covered by a nylon mesh (1 mm ~~mesh~~i.d.) were used to collect litter-fall ~~in this study~~. Twelve stands were located ~~at~~ in the ravine habitat, whereas ~~while~~ ten~~10~~ stands were located ~~built~~ in the~~at~~ Lanjenchi plot. Of those on the ~~Among~~ Lanjenchi plot, six ~~are~~ stands were placed in the windward habitat and~~,~~ ~~whereas 4 are~~four in leeward habitat. The horizontal distance between stands was approximately 50 m. The number of stands in the three habitats of the Nanjenshan Reserve was mainly selected based on the stands available in this area. Each nylon mesh containing ~~with~~ litter-fall was collected and replaced ~~with new one~~ monthly form early October 2012 to late September 2013. After their ~~the~~ collection, ~~of~~ the litterfall samples~~, they~~ were sorted ~~out~~ into litter-leaf, litter-branches, litter-flowers, litter-fruits, and miscellaneous fractions. The s~~S~~orted samples were weighted after being oven-dried at 40 °C for 14 days (Conklin-Brittain et al., 2006). The litter-leaves~~litter-leaf~~ were further separated ~~identified~~ into genus and species. Samples of the litter-~~-~~layer and underlying soils from the three habitats were collected in 2015 and 2016, respectively. The~~All~~ collected samples, including litter-leaf, litter-~~-~~layer, and soils, were subjected to ~~n-~~*n*-alkane analysis and ~~litter-layer~~litter layer samples ~~in~~from each habitat were further subjected to~~used for the~~ DNA extraction and consequential assays.

The annual yield (litterfall or litter-leaf) in each stand was used to calculate the productivity of each habitat. The annual litterfall and litter-leaf yields in each habitat were calculated by averaging the annual stand weights. Statistically significant differences between habitats were evaluated through Student's t-test using the t-test calculator (format SD). ~~The monthly yields of litterfall in each habitat was calculated by dividing the total weighs of litterfall collected monthly in mesh nets by the area of the frame. Calculation of the annual yields of litterfall in each habitat was done by averaging the monthly yields of litterfall calculated.~~

2.2 Extraction of *n*-alkanes

~~2.2 Soxhlet extraction of n-alkanes~~

All samples from the litter-leaf (42 plant species ~~plants~~), and litter layer, and all soils ~~are~~ were dried and well homogenized. Approximately 0.5 g of ~~powder~~ samples powder ~~were~~ was dissolved in dichloromethane ~~dicholromethane~~ (250 ml) and Soxhlet extracted for 16 hours. A surrogate standard of 5a-cholestane (Sigma) was added to each sample before extraction. The extracts were concentrated ~~to the volumes of~~into 5-10 mL volumes using ~~by~~ a rotary evaporator with a water bath and further concentrated to reduce ~~in~~ the volume to approximately 1 mL, after which they ~~and~~ were fractionated using ~~by~~ silica gel chromatography (Silica gel 60, 3%). The ~~n-~~*n*-alkane fraction was eluted with 4 mL of hexane and reduced ~~volume~~ to a 1 ~~ml~~ mL volume under a stream of nitrogen. An internal standard of squalene (Acros) was added to the concentrated 1 mL of hexane fractions prior to instrumental ~~Gas Chromatography (~~GC~~)~~ analysis. Quantification of the ~~n-~~*n*-alkanes was performed using ~~on~~ a PerkinElmer Clarus 500 gas chromatograph equipped with an autosampler, PerkinElmer Elite-5 CB fused silica capillary column (30 m in length, 0.32 mm i.d., film thickness 0.25 µm), and flame ionization detector (FID). The GC oven temperature was programmed to increase from 70 °C to 310 °C at a~~the~~ rate of 4 °C/min^{-1}. The ~~n-~~*n*-alkanes between C_{14} and C_{35} were identified and quantified using ~~by~~ external ~~calibration~~ standards that contained known concentrations of all n-alkanes of interest (Dr. Ehrenstorfer), 5a-cholestane, and squalene. ~~, and all of the n-alkanes (Dr. Ehrenstorfer) of interest in this study. The concentrations reported in this study were not corrected by the recoveries of the standards.~~

The ~~measured n-~~*n*-alkane concentrations ~~of the~~in 42 litter-leaf samples ~~species~~ were measured and then ~~further~~ used to estimate the ~~n-~~*n*-alkane concentrations from the litter-leaf and to assess litter-leaf productivity ~~and fluxes of litter-leaf~~ in the three habitats. The n-alkane concentration in the litter-leaf from each stand (C_{stand}, in µg g^{-1}) was summed by weighting the concentration in one species in the litter-leaf by that species' proportion of the total litter-leaf collected in the stand as following formula:

$$C_{stand} = \sum_{i=1}^{42} \frac{W_i}{W_t} \cdot C_i$$

where C_{stand} is n-alkane concentration in the litter-leaf from each stand (µg g^{-1}), W_i is the dry weight of litter-leaf of each species (g), W_t is the dry weight of total litter-leaf (g), and C_i is the n-alkane concentrations (µg g^{-1}) of 42 litter-leaf samples.

Calculation of the litter-leaf n-alkane concentration in each habitat was then performed by averaging the n-alkane litter-leaf concentrations in all the stands. The annual n-alkane productivity in each stand was calculated by multiplying the n-alkane litter-leaf concentration in each stand by the annual yield of that stand. The n-alkane litter-leaf productivities of the stands in a habitat were then averaged to estimate the annual n-alkane productivity in the three habitats, which was evaluated using Student's t-test.~~The average of n-alkane concentrations of litter leaf in a habitat were summed up the weighting n-alkane concentrations of each litter leaf species by their ratios of the litter leaf yield collected to the total litter leaf yield collected in a habitat. The monthly average fluxes were the products of the estimated average monthly n-alkane concentrations of litter leaf and the monthly yield of litter leaf in the habitat. Calculation of the annual n-alkane of litter leaf flux in each habitat was done by averaging the monthly average n-alkane fluxes calculated of litter leaf.~~

2.3 DNA extraction

~~**2.3 DNA extraction**~~

~~Leaves of litter layer were cut into pieces for extracting the DNA of bacteria.~~For DNA extraction in the litter layer, several pieces of leaves from the litter layer were randomly selected, cut into 2 mm × 2 mm chips, and well blended. The ~~procedure for~~ DNA extracting procedure ~~wa~~is~~s~~ described in detail in the user manual, *Genomic DNA from soil (Macherey-Nagel)*. Briefly, a bulk mixture ~~of leaf fraction~~ (approximately 0.1−0.2 g ~~approximately~~) was transferred ~~into~~ to a tube containing ~~the~~ ceramic beads. Lysis buffer (lysis buffer SL1, 700 µ~~l~~L) and enhancer SX (150 µL~~l~~) were added to each sample and ~~subjected to~~ vortexed for 5 min at room temperature. Precipitate contaminants were removed through centrifugation ~~by centrifuge~~ for 2 min at 11,000 g. DNA supernatant ~~Supernatant with DNA~~ was transferred to a new tube, ~~added with~~ 150 µL~~l~~ of SL3 was added, and the mixture was ~~subjected to~~ vortexed for 5 s~~ec~~ before~~prior to the~~ precipitation by centrifugation ~~centrifuge~~ for 1 min at 11,000 g. The supernatant inhibitors ~~inhibitors in supernatant~~ were removed by using an inhibitor removal column. The column that bound with DNA was washed four times and dried ~~out~~ before DNA elution. DNA elution was performed by centrifuging for 30 s~~ecs~~ at 11,000 ~~×~~ g after ~~the~~ incubation with Buffer SE (50 µ~~l~~L). A final DNA extraction (approximately 30–100 ng uL−1 in 100 µL of Tris buffer) was eluted.

2.4 Sequencing using an Illumina MiSeq platform

The genomic DNA (12.5 ng) obtained from each habitat was used in amplicon PCR experiments, which were performed in triplicate. ~~2.4 Sequencing via an Illumina MiSeq platform~~

We performed ~~carry out~~ PCR amplification of 16S rRNA gene sequences at V3-V4 regions using Illumina's MiSeq system to create paired-end sequencing data. The experimental protocol was modified from the Illumina manual. The target sequence was amplified using ~~by~~ PCR ~~using~~ with mixed forward and reverse primers. The sequences of forward primers included an adapter overhang nucleotide sequence and V3–V4 primer pairs, which were ~~are:~~ 5'-TCGTC GGCAG CGTCA GATGT GTATA AGAGA CAGCC TACGG GNGGC WGCAG, 5'-TCGTC GGCAG CGTCA GATGT GTATA AGAGA CAGAC CTACG GGNGG CWGCA G, 5'-TCGTC GGCAG CGTCA GATGT GTATA AGAGA CAGTD CCTAC GGGNG GCWGC AG, and 5'-TCGTC GGCAG CGTCA GATGT GTATA AGAGA CAGGD RCCTA CGGGN GGCWG CAG. The sequences of the reverse primers ~~are~~were: 5'-GTCTC GTGGG CTCGG AGATG TGTAT AAGAG ACAGG ACTAC HVGGG TATCT AATCC, 5'-GTCTC GTGGG CTCGG AGATG TGTAT AAGAG ACAGT GACTA CHVGG GTATC TAATC C, 5'-GTCTC GTGGG CTCGG AGATG TGTAT AAGAG ACAGA CGACT ACHVG GGTAT CTAAT CC, and 5'-GTCTC GTGGG CTCGG AGATG TGTAT AAGAG ACAGG TTGAC TACHV GGGTA TCTAA TCC. After separation using agarose gel electrophoresis~~by electrophoresis in agarose gel~~, PCR products with expected sizes were purified from the matrix. The Illumina Nextera XT index kit was used in ~~the~~ second-stage PCR for the addition of the index. Capillary electrophoresis followed by a fluorescence-based method ~~were~~ was employed to qualify and quantify ~~the~~ libraries, respectively ~~respectively~~. Nucleotides ~~will be~~were sequenced using the~~by~~ Miseq sequencer for 18 dark cycles and 350 read cycles in the forward read, and 18 dark cycles and 250 read cycles in the reverse read. The data of forward and reverse reads were aligned using the analysis platform from (Genomic Workbench v.8.5) CLC bio ~~CLC bio's analysis platform (Genomic Workbench v.8.5)~~ with Q20 as a threshold to generate output ~~fasta~~ FASTA files.

2.5 Metagenomics library construction and analysis~~2.5 Metagenomics library construction and analysis~~

~~Fasta~~ FASTA files were further processed using the ~~by~~ sequence analysis tool, USEARCH. We merged all sequence fills, ~~together,~~ removed duplicates, and clustered the sequences into operational taxonomic units (OTUs) at 97 % pairwise identity with a minimum cluster size of ~~2~~ two to construct an OTU- reference library. Then, a comparison between samples and the reference library at a ~~level of~~ 97 % sequence identity level was performed ~~made~~ to create ~~a~~ OTU~~s~~ tables, which contained the number~~s~~ of DNA sequence read~~s~~ of each OTU. A 16s UTAX reference database was employed as a blast (Basic Local Alignment Search Tool) library. Finally, these data were combined ~~together~~ to determine the relative abundances and subsequent visual patterns of a heat map. Principal coordinate analysis (PCoA) was used to identify ~~in this study for knowing~~ the relative distance between OTUs. To plot ~~For plotting the~~ PCoA figures, ~~a~~ the calc_distmx command together with a phylip_lower_triangular parameter was used to calculate the distance of the OTUs. The axes file contained~~s~~ ~~the~~ plotting coordinates ~~for graphing the results~~and was derived from mothur using the ~~with~~ PCoA command. The PCoA figures were plotted using R program~~by R~~.

2.6 Proportion of alkB-gene-containing bacteria in the habitats estimated using the NCBI database

~~2.6 Estimation of the portion of alkB genes contained bacteria in the habitats by NCBI database~~

Metagenomic data mining was performed to search for~~carried out to look for the~~ microbial communities that may have had ~~which might contain~~ alkB gene. Because ~~Since the~~ bacteria in the same genus may have ~~contain~~ similar gene sequences and functions, nucleotide blasting was performed ~~carried out~~ to search for ~~the~~ homogeneous sequences in the OTUs. Bacteria with ~~We defined the lineage-like bacteria of a known bacterium were the microbes which the~~ DNA sequences ~~were~~ 95 % or more similar to a known gene were defined as lineage-like bacteria. NCBI database was used to construct ~~make~~ an alkB gene reference library. ~~To investigate the populations of alkB gene family in different habitats, we~~ We ~~first~~ collected ~~the~~ representative 16s gene sequences of all known alkB ~~-~~gene-containing~~ed~~ bacteria in the phyla *Actinobacteria* and *Proteobacteria* from the current~~ly~~ NCBI database to investigate the alkB gene family populations in different habitats. The alkB gene sequences in bacterial 16s rRNA ~~of bacteria~~ were downloaded to a reference nucleotide file and combined with the OTU~~s~~ librari~~es~~ of *Actinobacteria* and *Proteobacteria*.

To search the alkB family gene-like lineage ~~from~~ in the current OTU~~s~~ database, sequence alignment and phylogenetic analysis of the alkB nucleotide sequence were conducted using the Molecular Evolutionary Genetics Analysis 7 (MAGA 7) program. ~~The~~ MAGA 7 us~~ed~~ing parsimony, neighbor-joining, and maximum likelihood analyses ~~was used~~ to create a 16S rRNA ~~gene~~ phylogenetic gene tree. The ~~nucleotide blast program of~~ NCBI nucleotide blast program was employed ~~carried out~~ to test the similarity of DNA sequences between OTUs and adjacent references. DNA sequences with a similarity of more than 95 % were manually selected to determine the alkB-lineage OTU DNA sequence numbers read ~~numbers of DNA sequence read of alkB lineage OTUs~~ in the library. The Shannon-Wiener diversity index was used ~~in order~~ to calculate the plant diversity, overall bacteria communities, ~~of~~ and alkB-lineage bacteria and ~~it~~ was determined using the ~~generated in~~ PRIMER-5 software (Plymouth Routines in Multivariate Ecological Research).

2.7 Semiquantitative PCR to determine alkB gene levels in litter layer samples

~~2.7 Semi-quantitative PCR for alkB gene level from litter layer samples~~

Because ~~Since~~ alkB genes ~~was~~ are highly abundant and diverse, a standard method for alkB gene quantification was not available. We employed semi-quantitative PCR for measuring environmental alkB in a method that was modified from previous studies because ~~since~~ the alkB-targeting primer covers ~~had coverage among the bacterial strains of~~ *Proteobacteria* and *Actinobacteria* (Kloos et al., 2006; Jurelevicius et al., 2013). ~~(Jurelevicius et al., 2013; Kloos et al., 2006). Litter layer~~Litter layer samples from ~~3~~ three habitats in Nanjenshan were subjected to semi-quantitative PCR to quantify ~~study for quantifying~~ the DNA levels of alkB genes in the natural habitats. Litter layer samples from different stands in each habitat were randomly selected for sample collection at three time points. After collection, litter layer samples were sent

immediately to the laboratory at room temperature. Litter layer patches were randomly selected and cut into approximately 2 mm × 2 mm chips. Samples (250 mg) were taken for DNA extraction. Following DNA elution, 100 μL of buffer solution and 2 μL of eluted DNA were taken for semiquantitative PCR. The DNA was mixed with an ~~Following DNA extraction of litter layer samples, DNA eluate (2μl) were mixed with~~ alkB gene primer set (forward primer: 5'- AAY ACN GCN CAY GAR CTN GGN CAY AA -3', reverse primer: 5'- GCR TGR TGR TCN GAR TGN CGY TG -3'-, 1 μL~~l~~, 0.4 μM), 5 μL~~l~~ Fast-Run Taq Master Mix with Dye, and ~~16μl~~ 16 μL ddH2O with the final reaction volume of ~~25μl~~25 μL. Thermal cycling conditions for PCR were ~~included~~ an initial temperature of 95 ℃ for 5 min, ~~and~~ followed by 30, 27, or 24 cycles of 95 ℃ for 30 s~~ec~~, 55 ℃ for 30 s~~ec~~, 72 ℃ for 1 min and finial of 72 ℃ for 5 min. The expected amplicons of the PCR ~~are~~ were 548 bps. Following PCR, aliquots (5 μL~~l~~) of each amplicon were confirmed using ~~by~~ electrophoresis on ~~a~~ 1.5 % agarose gel followed by 0.5 μg/mL SYBR Safe DNA staining~~,~~ and imaging~~ed~~ using an ~~by~~ ultraviolet trans-illumination system. The band density average levels were compared with a windward habitat density setting of 100 %. Statistically significant differences were evaluated using Student's t-test.

3 Results and Discussion

3.1 Quantitative estimates of annual litterfall in the Nanjenshan Reserve ~~Quantitative estimates of Annual litterfall from the gathering in Nanjenshan Reserve~~

The ~~estimation of~~ annual average litterfall was estimated ~~calculated~~ from the litterfall ~~gathering~~ at the sampling sites. The annual yields of litterfall in the three~~from 3~~ habitats in Nanjenshan ~~was~~ are illustrated ~~showed~~ in figure 1. As shown~~ed on~~ in the records ~~of~~ from the past decade, the litterfall was higher in the ravine habitat than in the other habitats. Our results on the litterfall of Nanjenshan ~~have~~ indicated that the annual weight productivity ~~of weight~~ in the ravine habitat was approximately 30 % higher than in the leeward or windward habitat, which is consistent with previous studies. According to records from 1999 to 2007, seasonal litterfall in Nanjenshan ~~was~~ ranged from 2 ~~to~~ 7 (ton /ha^{-1}), the increases of litterfall were contributed ~~which was affected~~ by typhoons, precipitation, and the effects of monsoon seasons. In this study, the annual litterfall was discovered to be approximately 7-10 (ton ha^{-1})~~the litterfall was about 7 to 10 ton/ha~~, which is considered a high-volume when compared with the records.~~which was due to the effects of typhoon.~~ The primary reason for the large amount of litterfall during this study was the occurrence of typhoons. Because typhoons increase litterfall in the three habitats equally, the ranking in annual litterfall volume was not altered by the advent of typhoons. ~~Still, the change of weather did not affect the order of litterfall output in the habitats.~~ One of the reasons that the ravine habitat had more annual litterfall may be the topography of the ravine habitat, which is more suitable for plant growth than that of the other habitats.~~may lie on the topography of ravine habitat which was more suitable for the growth of plants.~~ T~~In fact, t~~he average height and diameter of cross section of plants in the ravine habitat were the largest of those in all the habitats~~highest~~. Because litter-leaf was ~~Since leaves were~~ the major part~~s~~ of

the litterfall in weight and in theand average n-*n*-alkane resourcesconcentration levels, we focused on the n-*n*-alkane levels in the litter-leaf of leaves in these habitats.

3.2 Example of *n*-alkane measurements in the Nanjenshan Reserve3-2 Typical example of n-alkanes measurement in Nanjenshan Reserve

The n-*n*-alkane concentration in litter-leaf leaves from 42 plants were measured using GC-FIDvarious kind of plants that harvested in this study were assayed. Figure 2 presents showed a representative GC chromatograms of the Iles rotunda leaves in each habitat. GC traces in leaves of *Bischofia javaniva*, *Cyclobalanopsis championii*, and *Lithocarpus amygdalifolius*, which were the most dominant tree species regarding the contribution of litterfall amount in ravine, windward and leeward habitats respectively. The recovery rates from the surrogate of 5a-cholestane ranged from 70 to 108 % and averaged at 99 ± 7 %, suggesting satisfactory extraction efficiency.of the experiment was determined to be 99.3 ± 6.9%. The n-alkane concentrations (ranging from 123 μg g^{-1} to 2,694 μg g^{-1}) calculated in this study were not corrected by the recovery rate. All samples exhibited an odd-even odd/even carbon-number predominance, with maximum at C_{31}, C_{29}, and C_{33}. . The distribution of aliphatic fraction showed the major n-alkanes components were from n-C_{27} to n-C_{25} with a distinctive n-alkane distribution pattern of leaves in each species. For example, the ratio of C_{29}/C_{33} was different in all three plants. *N*-*n*-alkane Alkanes of C_{14} to C_{26} were present in was only present in relatively lower amount. Further distribution analyses of the n-alkane litter-leaf components, such as the carbon preference index (CPI) and C_{29}/C_{33} ratio, for each species were needed to further identify the relationships between plant species and n-alkane distribution patterns. This paper study wasis the first to reveal the n-*n*-alkane levels of plant vegetations in Nanjenshan. More analysis was need to impose on the relationship between plant species and n-alkane distribution pattern.

3.3 Annual litter-leaf productivity and *n*-alkane productivity rankings

3-3 Annual Plant leaf productivity and their n-alkane productivity

A total of forty two42 plant species were identified in this study. Data from the of the list of plant species, revealed the total amount of leaves in the annual litterfallannual leaf in litterfall, and the resultant ranking order of leaf n-*n*-alkane concentration inof these plants were is presented given in table Table 1. The data of leaf n-*n*-alkane concentration and dry weight data were employed to make a gross estimate of the n-*n*-alkane productivity of the litter-leaf flux of litterfall in the Nanjenshan Reserve. The n-alkane concentration was discovered to change during leaf development and was affected by environmental parameters (Hoffmann et al., 2013; Jetter and Schäffer, 2001; Kahmen et al., 2011).It has been showed that the n-alkane concentration of leaves was dynamic during leaf development and was affected by environmental parameters (Kahmen et al., 2011; Jetter and ffer, 2001; Hoffmann et al., 2013). For example,

temperature and relative humidity affected the composition of n-alkanes in both *Acacia* and *Eucalyptus* in Australia (Hoffmann et al., 2013). In our pilot study, the n-alkane concentration variation among plant species was less than 15 % of the standard error, as determined through comparison of several samples from different stands and habitats for the same species. The ranges of leaf n-alkane concentrations between species were from 2,694 (μg g^{-1}) in Iles rotunda to 123 (μg g^{-1}) in *Alniphyllum pterospermum*. These data demonstrated that intraspecies variation in n-alkane concentration could be ignored compared with the interspecies variation. Therefore, using a representative plant n-alkane concentration was reasonable for estimating the overall litter-leaf n-alkane production in a habitat. When comparing the plant vegetation and leaf n-alkane concentration, the plants with leaves that had a high n-alkane concentration tended to grow in the ravine habitat; *Iles rotunda* and *Celastrus kusanoi*, for example.~~Since old leaves were affected less by environment variable, the internal variations within species might be slighter in leaves of litterfall. Besides, the leaves were gathered from habitats in the same area in a geographic scale, thus the differences of climate and geography were hardly to remarkably affect the n-alkane concentration of leaves within samples of the same species. In this study, the ranges of leaf n-alkane concentration between species were from 3000 (μg /g) to 200 (μg /g). We assume that the variable of n-alkane concentration within species can be ignored when compared with the variable between species. Interestingly, the leaves of plants which were of high leaf n-alkane concentration tended to grow at ravine habitat, such as *Ficus benjamina L* and *Iles rotunda*, to name a few.~~

3.4 Estimates of annual *n*-alkane productivity in the three habitats

~~### 3.4 Estimates of annual n-alkane productivity in three habitats~~

Figure 3 presents ~~showed~~ the ~~results of n-~~ *n*-alkane concentrations from the litter-leaf ~~litterfall~~ to the bulk soil ~~(fig 3A)~~ and the average litter-leaf ~~n-~~ *n*-alkane productivity ~~flux of litterfall~~ in the three habitats. ~~(fig 3B).~~ The average litter-leaf ~~n-~~ *n*-alkane concentration ~~of litterfall~~ was highest in the ravine ~~area when compared with other~~ habitats. MoreoverBesides, the ~~n-~~ *n*-alkane concentrations ~~of~~ in different layers formed a steep gradient from the litterfall ~~layer~~ to the bulk soil. The ~~n-~~ *n*-alkane flux ~~of~~ in the litterfall in the ravine habitat was approximately twice that in the ~~about twice as much as~~ other habitats ~~(fig 3B)~~. We concluded that the ravine habitat hadhas higher ~~n-~~ *n*-alkane input than the other habitats. This characteristic ~~character~~ offersed us an example of higher ~~n-~~ *n*-alkane input in the natural habitat to investigate the consequence of microbial community.

The balance between litterfall yield and decomposition affects the development of organic carbon in soil layers. This study and previous reports have indicated that the litterfall in ravine habitats is higher than that in other habitats. However, ~~Several effects such as decomposition, erosion and sediment transportation might play roles to govern the organic resource such as n-alkane (Kirkels et al., 2014; Quinton et al., 2006). Aa~~ study ~~in~~ of these habitats demonstrated ~~has shown~~ that the total

organic carbon ~~of~~ in the litter layer and bulk soil in the ravine habitat was equal to or less than that ~~lower than~~ in the windward and leeward habitats (~~Kuo~~Guo, 2010). Therefore, decomposition effects may be stronger in ravine habitats. Figure 3(a) reveals that the n-alkane concentration decreased significantly from litter-leaf to surface soil, which could be due to the effects of bacteria. It is plausible that other organic compounds were degraded in addition to the n-alkanes. ~~Those evidences did not favor the possibility of high mineralization of hydrocarbon in ravine habitats. Microorganisms were known to play many key roles in degrading the plant substratum.~~Several effects such as leaching, erosion, and sediment transportation could play roles in governing organic compound accumulation; therefore, we cannot rule out the diffusion effects of other physical factors (Kirkels et al., 2014; Quinton et al., 2006). We performed ~~carried out~~ NGS and bioinformatics studies to discover ~~unearth~~ the roles of microbial communities in *n*-alkane degradation~~on the degradation of n-alkane~~.

3.5 Bacterial composition in the Nanjenshan Reserve

~~**3.5 Bacterial composition in Nanjenshan Reserve**~~

NGS was performed ~~carried out~~ to reveal the microbial community in the ~~of~~ litter layer from the three~~3~~ habitats. Figure 4 show~~ed~~s the relative abundance ~~of~~ in ~~M~~metagenomic data of OTUs that were grouped ~~in~~ by phylum. More detailed information about the relative abundance in class, order, and family levels is presented in Figure S-42. Interestingly, a similarity was identified in microbial community structure between the windward and leeward habitats~~the microbial communities in windward and leeward habitats were similar to each other~~. Because ~~Since~~ the windward and leeward habitats are ~~were both~~ located on~~in~~ the same plot of a different orography, several parameters such as plant vegetation and soil properties were ~~also~~ similar between ~~both~~ the habitats. As mentioned previously, ~~T~~the most considerable ~~significant~~ differences between the windward and leeward habitats were the average height of plants and average number of trees per hectare~~. Study has showed that in the leeward habitat, the height of plants was higher and the plant density was lower, when compared with the windward habitat (8.41±1.73 meter vs. 4.63±0.88 meter; 7,505 tree/ha vs. 20,065 tree/ha). The plant height and density in the ravine habitat were 9.45±1.35 meter and 4257 tree/ ha (Chin, 2008). . Apparently,~~The physical parameters that affect~~ed~~ ~~the~~plant growth ~~of plants~~ in mountainous ~~mountain~~ area did no~~'~~t appear to affect the relative abundance of microbial communities. Conversely~~On the other hand~~, the ~~microbial~~ relative abundance of microbial communities in the ravine habitat was different from that in the windward and leeward habitats, with more members ~~in phyla~~ of the *Actinobacteria* phyla and fewer ~~less~~ members ~~in~~ of the *Proteobacteria* and *Acidobacteria* phyla. The more abundant microbial communities of the Actinobacteria phyla may have been because of higher n-alkane productivity in the ravine habitat.~~The up-regulation of might be due to the effects of increasing n-alkane input in the ravine habitat.~~ Although many phyla have been identified as carrying the ~~to carry~~ alkB gene, our data revealed that the only microbial communities carrying the ~~which related to~~ alkB gene in our study were~~were~~ *Proteobacteria*, and *Actinobacteria*.

3.6 Prediction of bacteria carrying alkB gene in Nanjenshan Reserve

The relative abundance of bacteria contained the aAlkB gene in phyla the *Actinobacteria* and *Proteobacteria* phyla from different habitats is illustrated were shown in figure 5. The phylogenetic trees of OTUs in the *Proteobacteria* and *Actinobacteria* phyla with alkB reference strains are shown in Figures S-3 and S-4. The nucleotide sequence similarities of OTUs and the adjacent alkB reference strains were manually blasted. Table S-1 reveals the nomenclature of OTUs in *Proteobacteria* and *Actinobacteria* with nucleotide sequences that had similarity of higher than 95 % when compared with adjacent alkB reference strains. The relative abundance of DNA sequence read in OTUs of alkB-lineage-like bacteria in phylum *Actinobacteria* were limited in leeward and windward habitats. Figure 5 illustrates that a significant number of reads in the OTUs whose sequences were similar to those of alkB-gene-containing bacteria was identified for the ravine habitat. On the other hand, a significant amount of sequence read in OTUs of that kind was found in the ravine habitat. Although the relative abundance of *Proteobacteria* in the ravine habitat was 20 % less than that in the other habitats (Figure 4), the number relative abundance of DNA sequence reads of alkB-lineage-like bacteria in the ravine habitat was greater were more than 1.5 times higher than the that in the windward and leeward areas (Figure 5)windward and leeward areas. To summarizesum up, there were twice as many the DNA sequence reads of alkB-lineage-like bacteria in the ravine habitat were twice higher than in the other habitats. Despite the relative abundance of DNA sequence reads in many OTUs of the alkB-lineage-like bacteria, Proteobacteria, and Actinobacteria phyla were limited in the leeward and windward habitats (Figure 5); these two habitats had small amounts of alkB-lineage-like bacteria (approximately 0.1 % relative abundance) in most OTUs (Table S-1). Our data provide evidence that there is a universal presence of alkane degraders in natural habitats.

3.7 PCoA of bacteria carrying alkB genes The principal coordinate analysis of bacteria that carried alkB genes

PCoA was used to visualize the similarities of DNA sequences in *Proteobacteria* and *Actinobacteria* from the three 3 habitats. The biodiversity index in OTUs of alkB-lineage-like bacteria and other sample types is shown in Table S-2. Figure 6 displays showed the distribution of a total of 240 OTUs in phyla the *Proteobacteria* and *Actinobacteria* phyla. The majority of OTUs (>90%) are present in two or three habitats. The phylum *Actinobacteria* was circled in pink except the one in blue while the OTUs not included in the red circle were belong to phylum *Proteobacteria*. At most OTUs (>90%), sequences can be found from 2 or 3 habitats. An initial capital letter in Figure 6 was used given to denote the OTUs with the most read numbers according to the largest source in read numbers of the habitats. The nomenclature of OTUs of the alkB-lineage-like bacteria OTUs and relative locations are presented in the figurewere showed on the figure. Thirty-three There were 34 OTUs that contained alkB-lineage-like bacteria in this study. Around 70 percent of the OTUs of them were

~~clustered together at location A, B and C.~~ The ~~corresponding~~ numbers of OTUs that contained alkB–lineage-like bacteria in the ravine, windward, and leeward habitats were 3~~1~~0, 3~~1~~0, and 2~~6~~5, respectively. The Shannon-Weiner index ~~in~~ of OTUs that contained alkB- lineage-like bacteria in the ravine, windward, and leeward habitats were 2.5~~5~~1, 2.39 and 2.46, respectively. Although the relative abundance of OTUs that contained alkB-lineage-like bacteria in the ravine habitat was more than twice that in the windward and leeward habitats (Figure 5), the Shannon–Weiner indices of the three habitats were similar, suggesting that the effective species numbers of alkB-lineage-like bacteria were almost identical. The results indicate the pre-existence of diverse alkB-gene-containing bacteria in natural habitats, with some bacteria so small in number that they are undetectable using normal PCR. They could, however, proliferate in the habitats with the appropriate substrate supplements. This finding is consistent with the results from a previous study using a laboratory controlled system and an agricultural research farm (Schulz et al., 2012; Giebler et al., 2013).~~Although some nomenclature of OTUs were given by a specific genus or species – such as, *alpha-Proteobacteria* in location A, *Stenotrophomonas* in location B, and *Mycobacterium* in location C to name a few, most of nomenclature of OTUs were pointed to uncultured bacterium clones. We concluded that the diversity of OTUs associated with alkB-lineage-like were greater in ravine habitat when compared with windward and leeward habitat.~~

3.8 ~~-~~Empirical testing of bacteria carrying alkB gene

In the bacterial metagenomics study, equal amounts of DNA were amplified to estimate the bacterial communities in each habitat. To compare the productivity of the alkB-gene-containing bacteria in different habitats, we conducted semiquantitative PCR to determine relative alkB gene concentrations.~~We carried out semi-quantity and heat map analysis of annual litterfall yield of individual species in each habitat to explore the relationship between microbial communities and plant vegetation patterns. The study of semi-quantitative PCR in alkB gene was carried out to verify the alkB gene numbers in those habitats.~~ Figure 7~~A~~(a) shows ~~showed~~ a triplicate study of DNA staining ~~in~~ of amplicons from the three habitats after various semi-quantitative PCR cycles. No bands were detected if fewer than 24 amplification cycles were performed~~the cycles of amplification were less than 24~~. Marginal PCR products could ~~can~~ be detected ~~with~~ by increasing the cycle number to 27. Figure ~~7B~~ 7(b) shows ~~showed~~ the statistical ~~statistic~~ results regarding ~~of~~ alkB gene levels in the three Nanjenshan habitats ~~of Nanjenshan, which~~and ~~confirmed~~ confirms that ~~the~~ bacterial alkB numbers were higher in the ravine habitat than in the other two habitats. ~~Alkane-degradation bacteria were known to exist ubiquitously in natural habitats.~~ We have thus provided evidence that litterfall and plant vegetation increase the abundance of alkane-degrading bacteria communities ~~the communities of alkane-degrading bacteria could be upregulated by litterfall and plant vegetation~~ in natural habitats.

3.9 –Exploring the relationship between ~~of~~ plant vegetation and microbial communities using ~~by~~ heat map analysis

Heat map analysis of plant species and read-number of OTUs versus habitats were performed carried out to investigate the relationship between the plant vegetation patterns and microbial communities in different habitats. The heat map presented in map of Ffigure 8 was based on the microbial community abundance (A) and plant productivity of litter-leaffall (B) from in different habitats, with the color intensity indicating the numbers of reads or weight of litter-leaffall (in logarithmic form log). In Ffigure 8(b)B, the color patterns of litter-leaffall yields levels of showed thatthe microbial abundance are was similar more similar to each other in the windward and leeward habitats, demonstrating indicating the similarity between the of composition of microbial communityies compositions in these two habitats. FurthermoreInterestingly, the litterfall data were also similar between the two habitatspresented in a parallel manner, which suggestsing an existence of a connection between plant vegetation species and microbial compositions. The pattern similarities similarity in the pattern in theof plant species and bacterial species can be easily explained by substrate-specific-induced growth responses substrate-induced growth response in the nature habitats. The data provided a supportive evidence that the diversity of bacteria in all OTUs were was affected by the plant vegetation, not only in quantity but also in species. In Figures 3, 5, and 7, an increase in n-alkane productivity is demonstrated to increase the number of alkB-gene-containing bacteria. In Figure 8, we showed that a substrate-specific relationship may exist between plant vegetation and microbial communities. Thus, a leaf in a natural habitat may upregulate some alkB-gene-containing bacteria while downregulating other types of alkB-gene-containing bacteria. A future study could include a litter-bag study that would enable better understanding of the relationship between individual factors within plant and bacterial species; for instance, one could study the interaction of a specific leaf species with a particular litter-bag study can be tested in future for leading us to a better understanding on the relationship between a specific leaf and the genus of n *n*-alkane -degradingdegradation bacteria or microbial community in general in different habitats.

4 Conclusions

We confirmed that the annual litterfall productivity of the ravine habitat was approximately 30 % higher than that of the windward and leeward habitats, whereas the long-chain n-alkane productivity in the ravine habitat was twice that in the other habitats in a lowland subtropical rainforest of Taiwan. The bacteria contained alkB gene was known to play crucial roles in degrading n-alkane, which have been applied in many sectors such as soil remediation and oil gas prospecting. In this study, the effects of n-alkane in litterfall input on the abundance of n-alkane degrading bacterial community in litter layer in natural habitats were investigated using GC/FID and NGS. We revealed that the Pplant vegetation not only affects the n *n*-alkane productivity input but also changes litter layer have profound impact to the microbial communitiesy of litter layer. In the ravine habitat, high productivity of litterfall leaf and n *n*-alkane productivity production resulted in an increase in the n-alkane-degrading bacteria in the litterfall.in plants have rendered the changes of microbial community and up-regulation of n-alkane degrading bacterial in litter layer. Increases in Although alkB -gene-containing associated bacteria were identified primarily in the in many phyla, they were primarily found in *Proteobacteria* and *Actinobacteria* phyla, with

NGS and semiquantitative PCR employed for determining alkB gene concentrations. ~~in the lowland subtropical rainforest in Taiwan.~~ Biodiversity analyses showed that the diversity of bacteria containing the alkB gene was similar among the different habitats, suggesting that natural habitats harbor diverse alkB-lineage-like bacteria with some species existing in very low copy numbers. Cross analyses regarding the quantity and quality of plant vegetation and structures of the microbial communities revealed that n-alkane-induced increases in alkB-gene-containing bacteria might have been substrate specific. To our knowledge, this is the first report on the regulation of n-alkane-degrading bacteria in litterfall in natural habitats. ~~Increasing of n-alkane input caused the rising of diversity and the shift of relative abundance in *Actinobacteria* and *Proteobacteria*, which have been confirmed by NGS and semi-quantitative PCR for alkB gene level. To our knowledge, this is the first report on the regulation of n-alkane-degrading bacteria by litterfall in rainforest.~~

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Ranking ~~order~~ of Leaf ~~n-~~ *n*-alkane concentration	Plant species	**Relative annual ~~Leaf~~Litter-leaf production ~~levels~~ (by dry weight)**		
		Ravine	Windward	Leeward
1	*Iles rotunda*	++++	++	+
2	*Ficus benjamin*a L.	+++++		
3	*Ficus benjamin*a L. f.	+++++	+	
4	*Diospyros eriantha*	++		++
5	*Reevesia formosana*	+++		
6	*Celastrus kusanoi*	+++++	+	
7	*Erycibe henryi*	+++		+
8	*Cyclobalanopsis longinux*	+	+	+++++
9	*Archidendron lucidum*		+++++	+++++
10	*Celtis sinensis* Pers.	+++		
11	*Vitis kelungensis* Moriyama	+	++++	++
12	*Ilex cochinchinensis*		+++	+++
13	*Rhaphiolepos indica*		+++++	+
14	*Castanopsis fabri Hance*		+	+++++
15	*Symplocos caudata* Wall.		++++	+
16	*Cryptocarya hainanensis*	++++	+	+
17	*Podocarpus macrophyllus*		+++++	+++
18	*Sapium discolor*	++	+	++++
19	*Illicium arborescens*	+	+++	+
20	*Syzygium formosanum*		+++++	
21	*Syzygium kusususense*	+	+++	+
22	*Machilus thunbergii*	+++++		
23	*Cyclobalanopsis pachyloma*	+	+++	+
24	*Lithocarpus formosanus*	+++++	+	+
25	*Bischofia javaniva*	++++	+	+
26	*Machilus zuihoensis*	++		+++++
27	*Ficus aurantiaca*	++++	+	
28	*Helicia formosana*	+++	++++	++++
29	*Cyclobalanopsis championii*		+++++	++
30	*Aglaia formosana*		+++	+++++
31	*Turpinia ternata*	+	++++	++++
32	*Aglaia elliptifolia*	++	++	+
33	*Castanopsis indica*	+++	+	+++
34	*Psychotria rubra*	++++	++++	+++
35	*Castanopsis carlesi*i			+++
36	*Schima superba*	+	+	++
37	*Antidesma hiiranense*	+	++	+
38	*Lithocarpus amygdalifolius*	+	++	+
39	Schizostachyum diffusum	+	+	++++
40	*Beilschmiedia fordii*	+	+	++++
41	*Beilschmiedia erythrophloia*	+	+	++
42	*Alniphyllum pterospermum*	++	++	+

Table 1: The ~~n~~ *n*-alkane levels of leaves, plant nomenclatures and their ranking in order of annual production in its habitat. The leaf ~~n~~ *n*-alkane concentrations in each species were presented in descending order. The number of plus sign characters (+) indicated the semi-quantitative score, which added information of the ranking in the order of annual litterfall yield of particular species in a habitat. Five plus sign (+++++) denoted the ranking number of 1to 5. That ranking of 6 to 10 denoted 4 plus sign (++++); that ranking of 11 to 15 denoted 3 plus sign (+++); that ranking of 16 to 20 denoted 2 plus sign (++); and that ranking after 21 denoted one plus sign (+). A blank space denoted none of leaf was harvest for entire year.

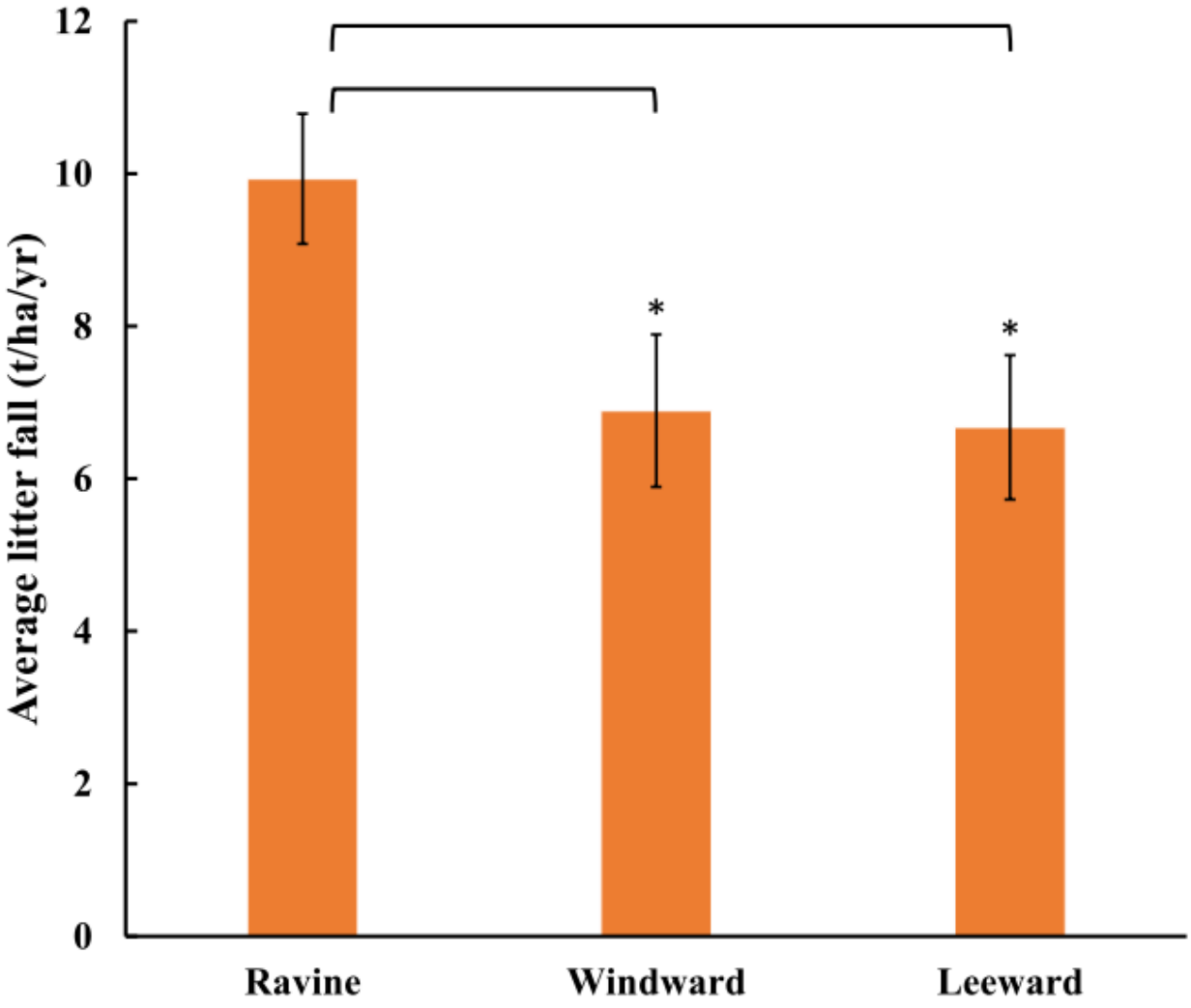
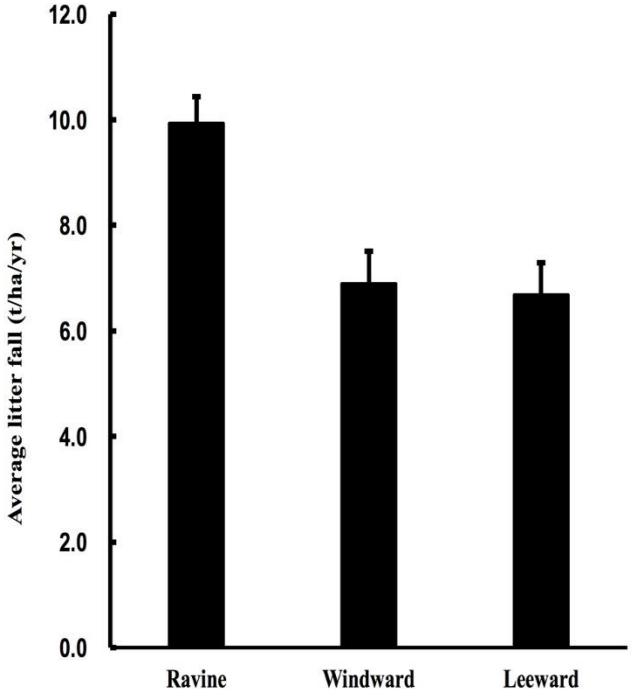


Figure 1. Annual litterfall in three habitats of Nanjenshan Reserve. Annual productivity of litterfall in ravine habitat were higher than windward and leeward habitats (* $p < 0.05$).

Figure



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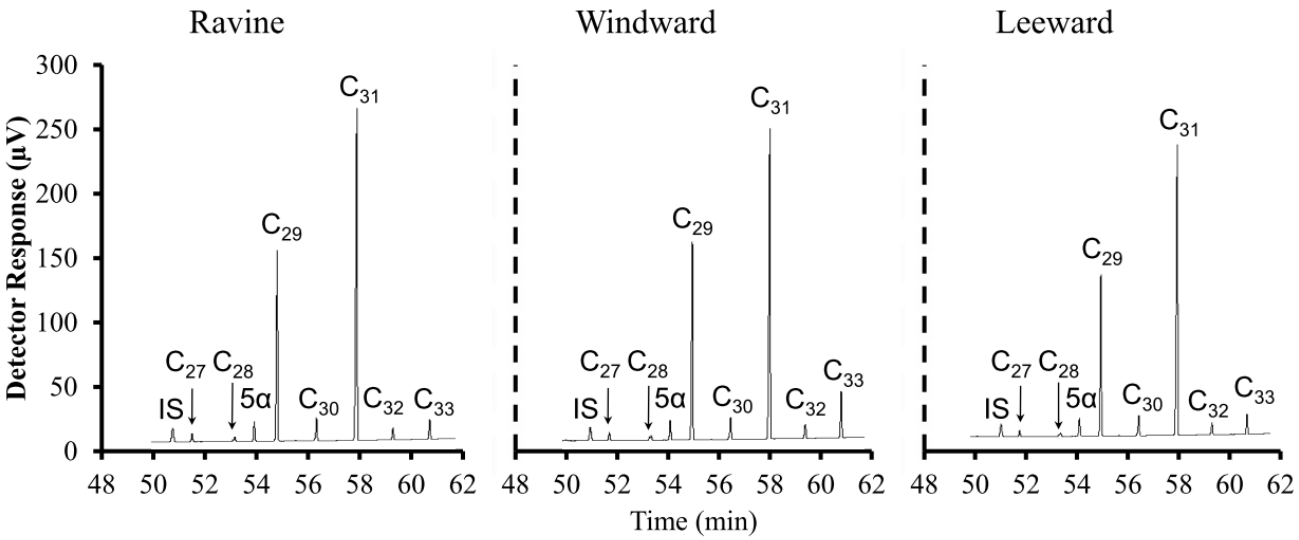

Figure 2. Representative GC-FID chromatograms of aliphatic hydrocarbons of *Iles rotunda* in each habitat.

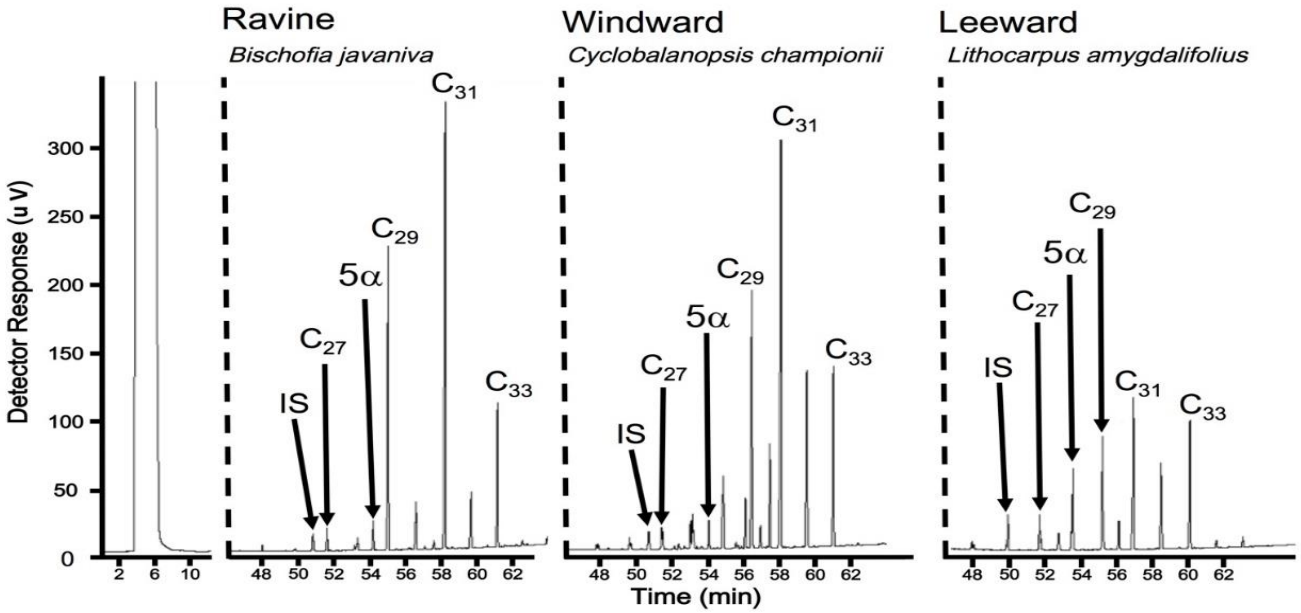

Figure 2. Representative GC-FID traces of aliphatic hydrocarbons of the dominated species in each habitat.

Figure 2

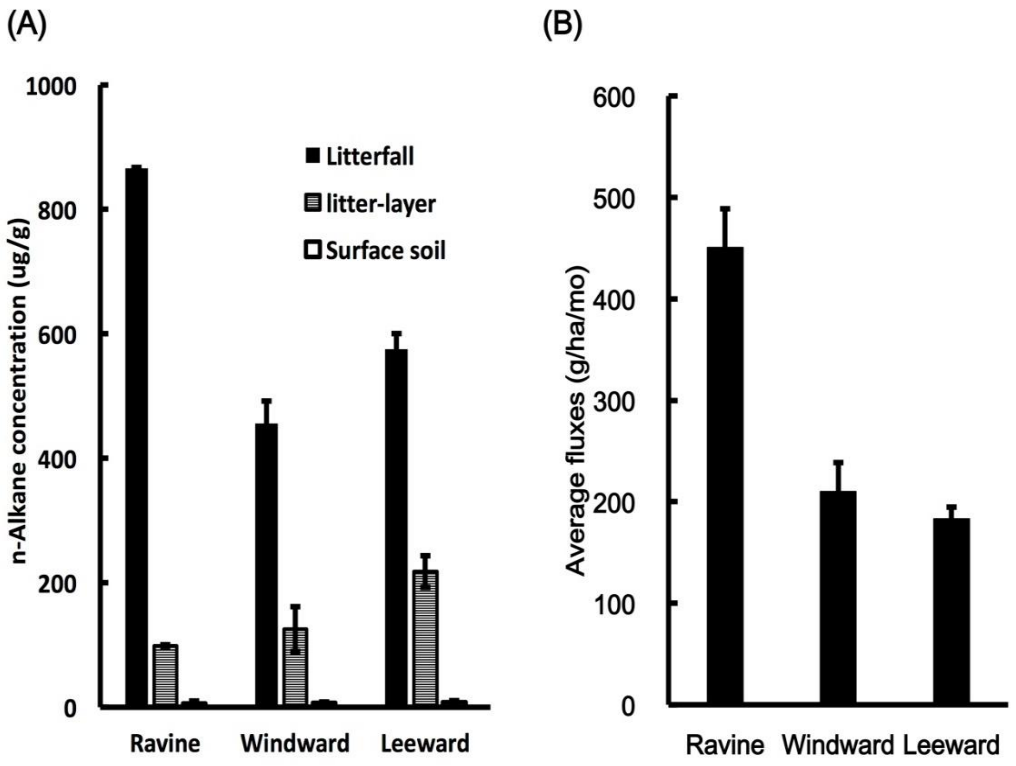

Figure 3. (A) Dynamic n-alkane level changes from litterfall, litter-layer to surface soil. (B) Estimated annual n-alkane flux generated by leaf of litterfall in 3 habitats.

Figure 3

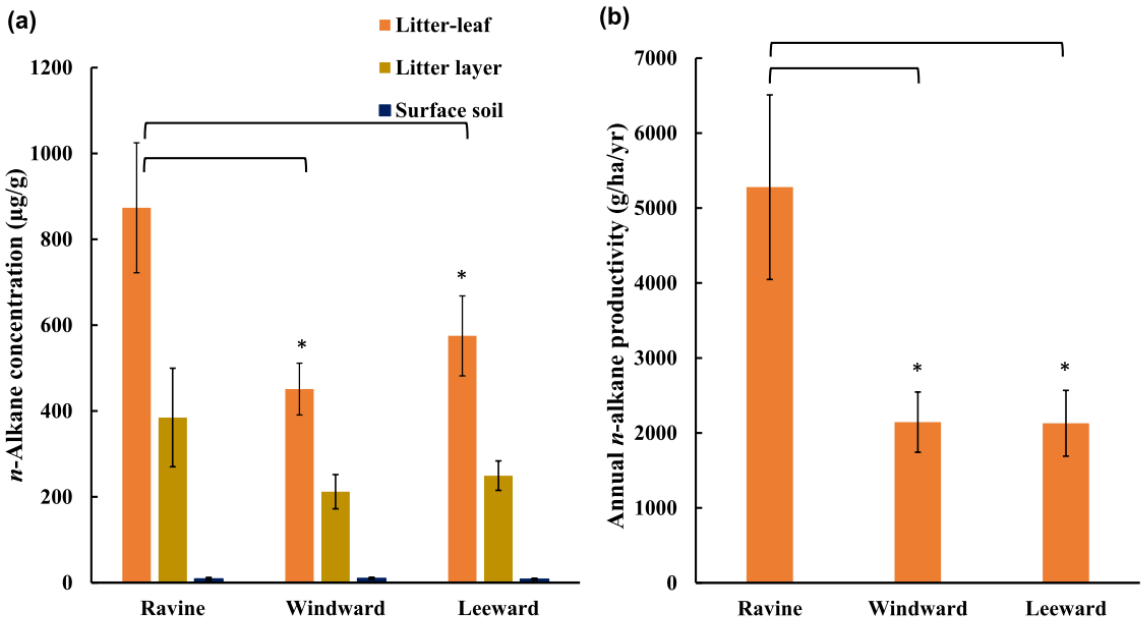

Figure 3. (a) *n*-Alkane concentration in litterleaf, litter-layer and surface soil. *n*-Alkane concentration in litterleaf was higher in the ravine habitat than in the other two habitats (* $p < 0.05$). (b) Estimated annual *n*-alkane productivity generated by litterleaf of litterfall in three habitats (* $P < 0.05$).

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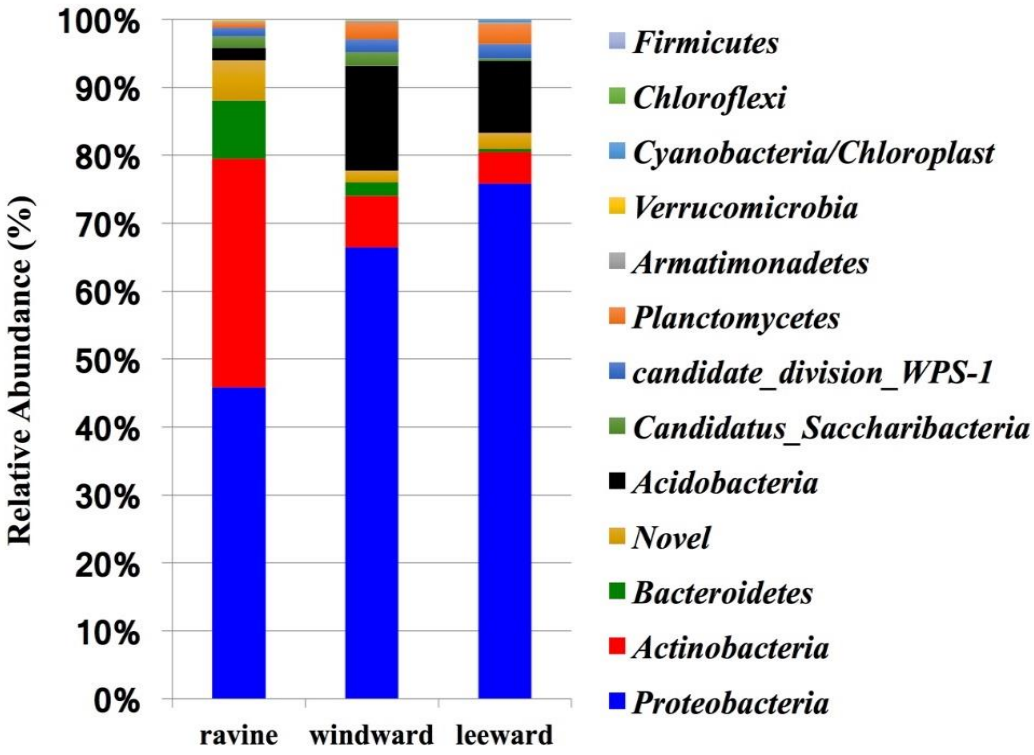
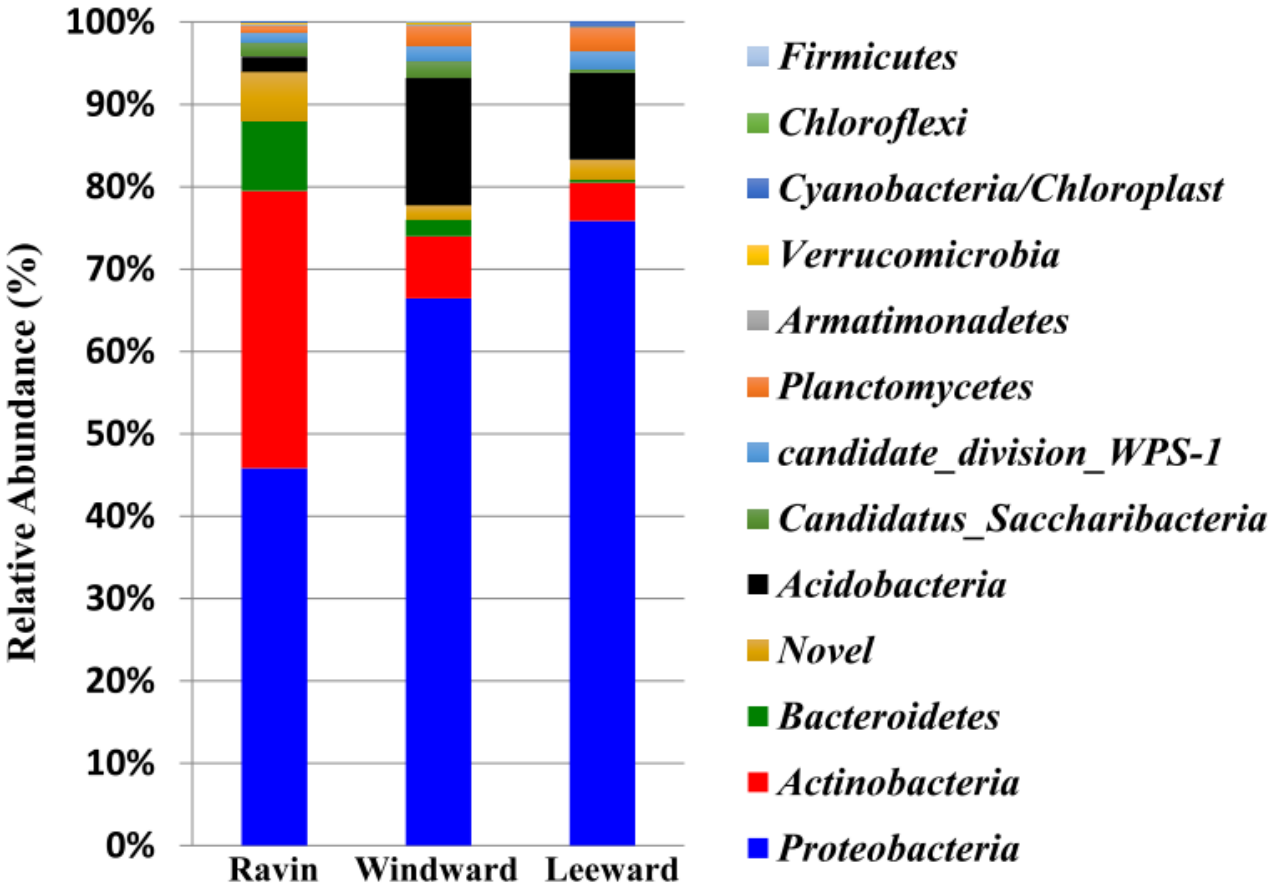


Figure 4. Microbial community structure in the three habitats of Nanjenshan Reserve. Bacterial lineages were indicated by phylum.

Figure 4



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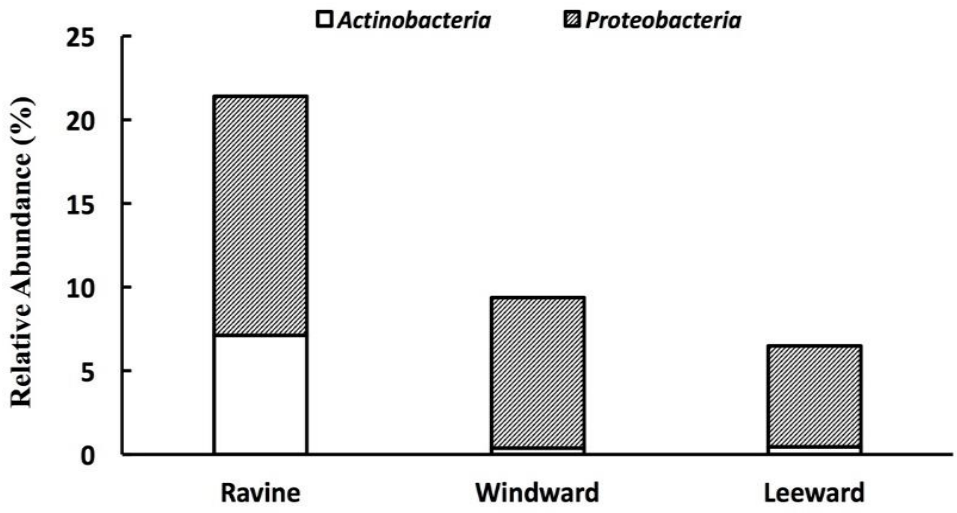

Figure 5 The relative abundance in microbial community of alkB gene-lineages. White block indicated the relative abundance of alkB gene in phylum *Actinobacteria* while the stripe block was in *Proteobacteria.*

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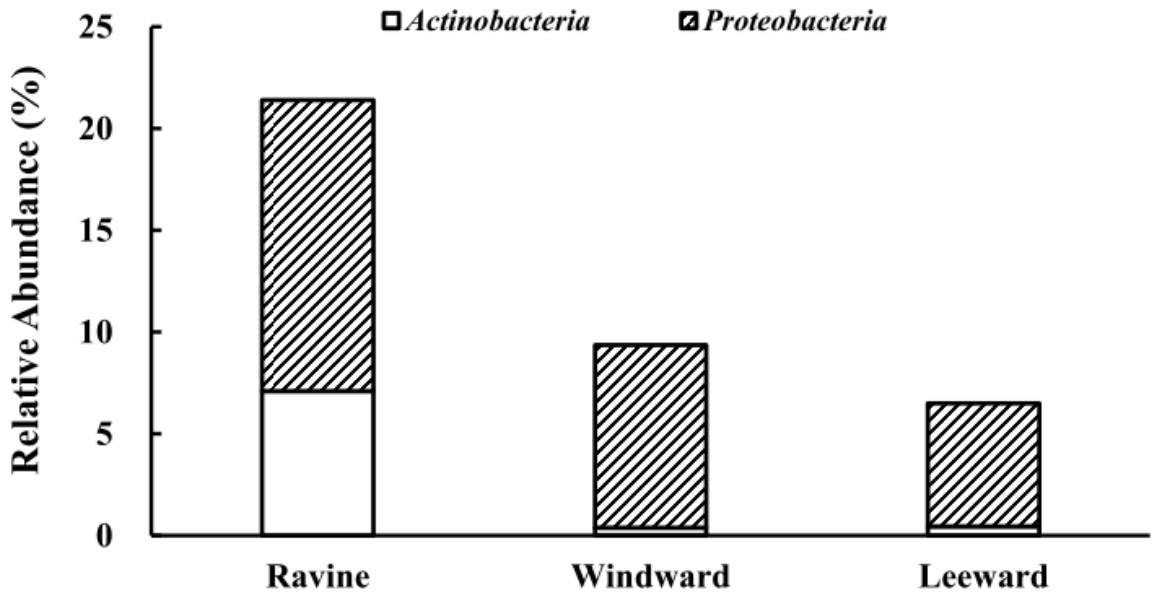

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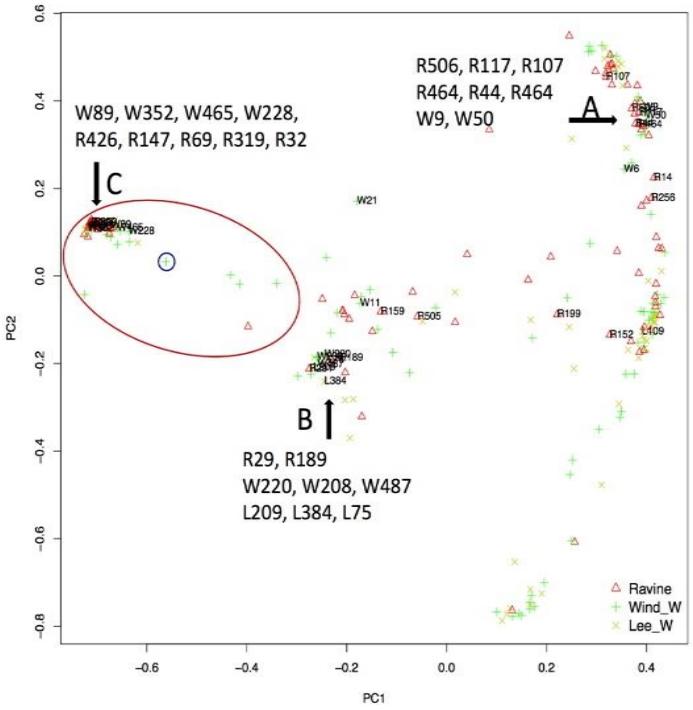


Figure 6. The PCoA plot of OTUs data in phylum *Actinobacteria* and *Proteobacteria*. The circle area in red was the phyla of *Actinobacteria* except the OTU with blue circle. The arrow bars indicated the OTUs of alkB gene lineage which clustered together.

Figure 6

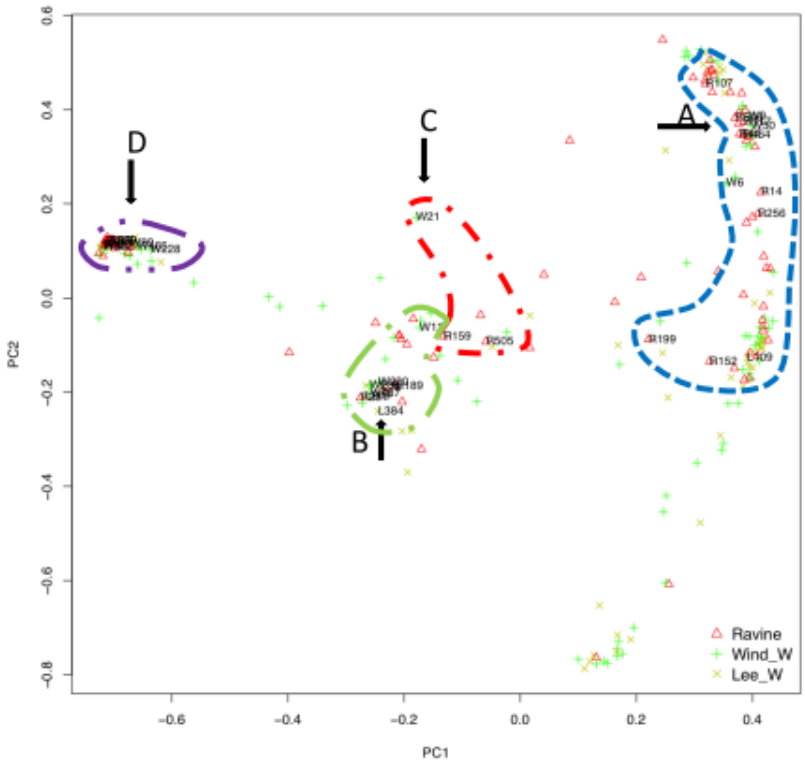


Figure 6. The PCoA plot of OTUs data in phylum *Actinobacteria* and *Proteobacteria* in three habitats. The circle areas in A, B, C and D are classes of a-*Proteobacteria*, b-*Proteobacteria*, g-*Proteobacteri*a and *Actinobacteria*, respectively.

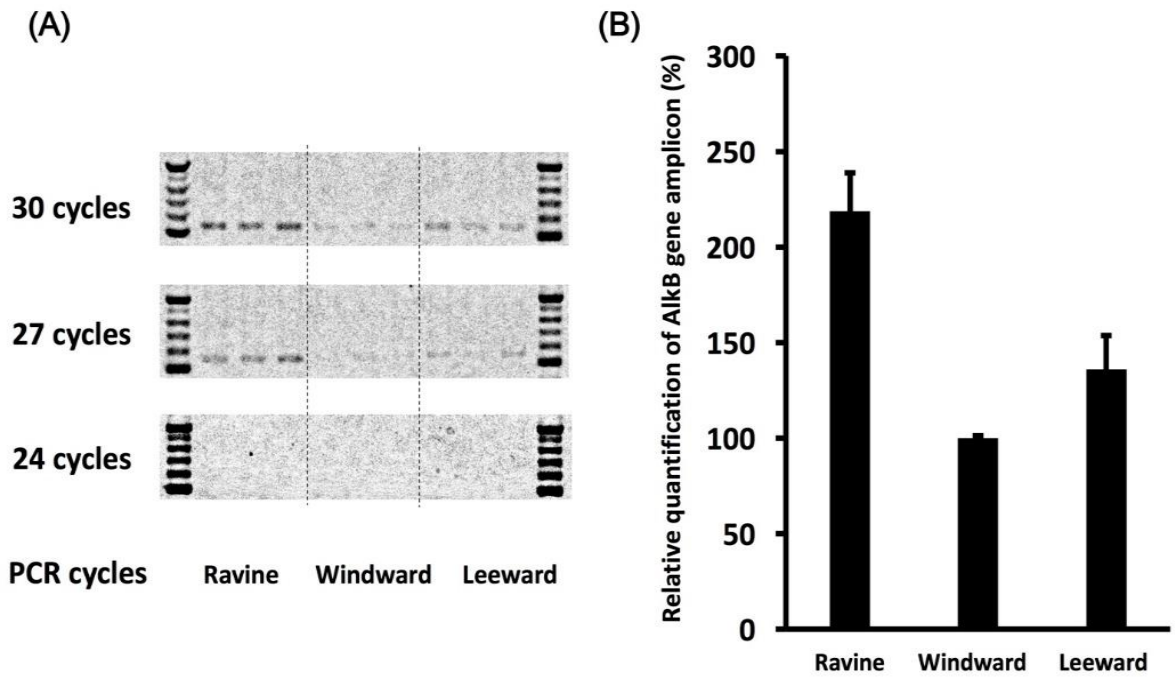


Figure 7. Semi-quantitative PCR of alkB gene results from samples of 3 habitats in Nanjenshan Reserve. (A) Agarose electrophoresis of alkB genes in different amplified cycles. Standard markers were loaded at both sides of samples. The samples from different habitats were separated by dash line. (B) The statistic results of semi-quantitative PCR after amplifying 30 cycle. The read numbers of alkB gene were significant higher in ravine habitat than windward and leeward habitats accordingly.

Figure 7

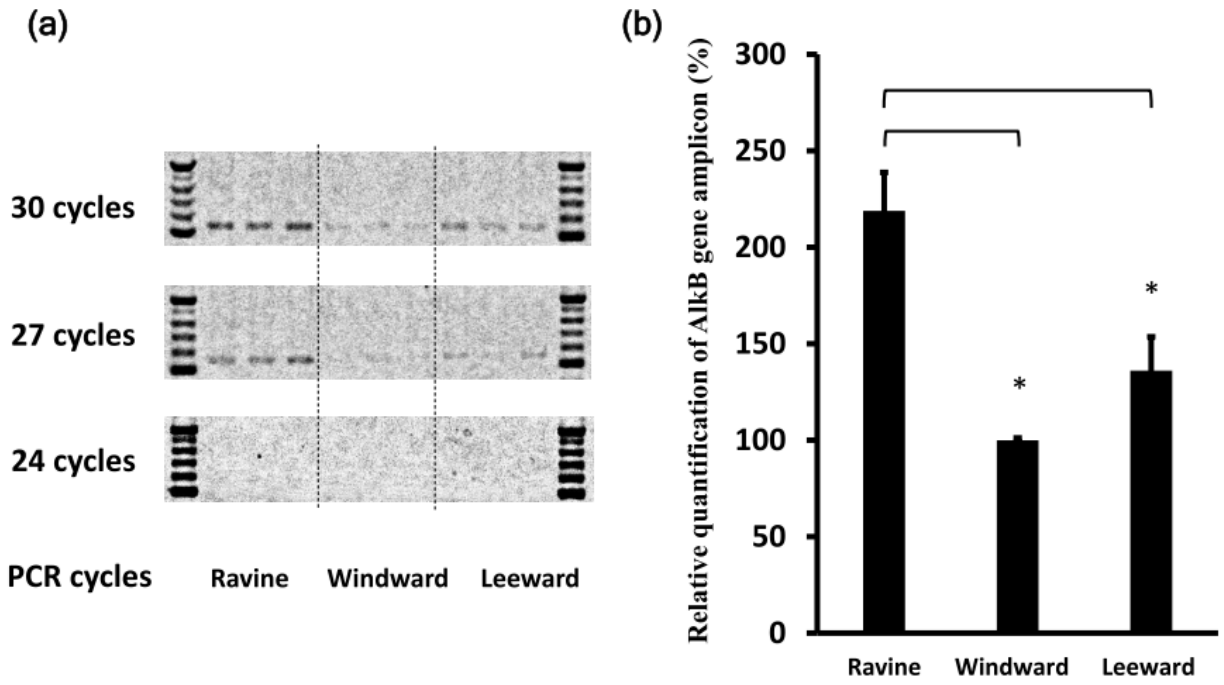


Figure 7. Semiquantitative PCR of alkB gene results from samples of three habitats in Nanjenshan Reserve. (a) Agarose electrophoresis of alkB genes in different amplified cycles. Standard markers were loaded at both sides of samples. The samples from different habitats were separated by dash line. (b) The statistic results of semiquantitative PCR after amplifying 30 cycle. The read numbers of alkB gene were significant higher in the ravine habitat than in the other two habitats (* $p < 0.05$).

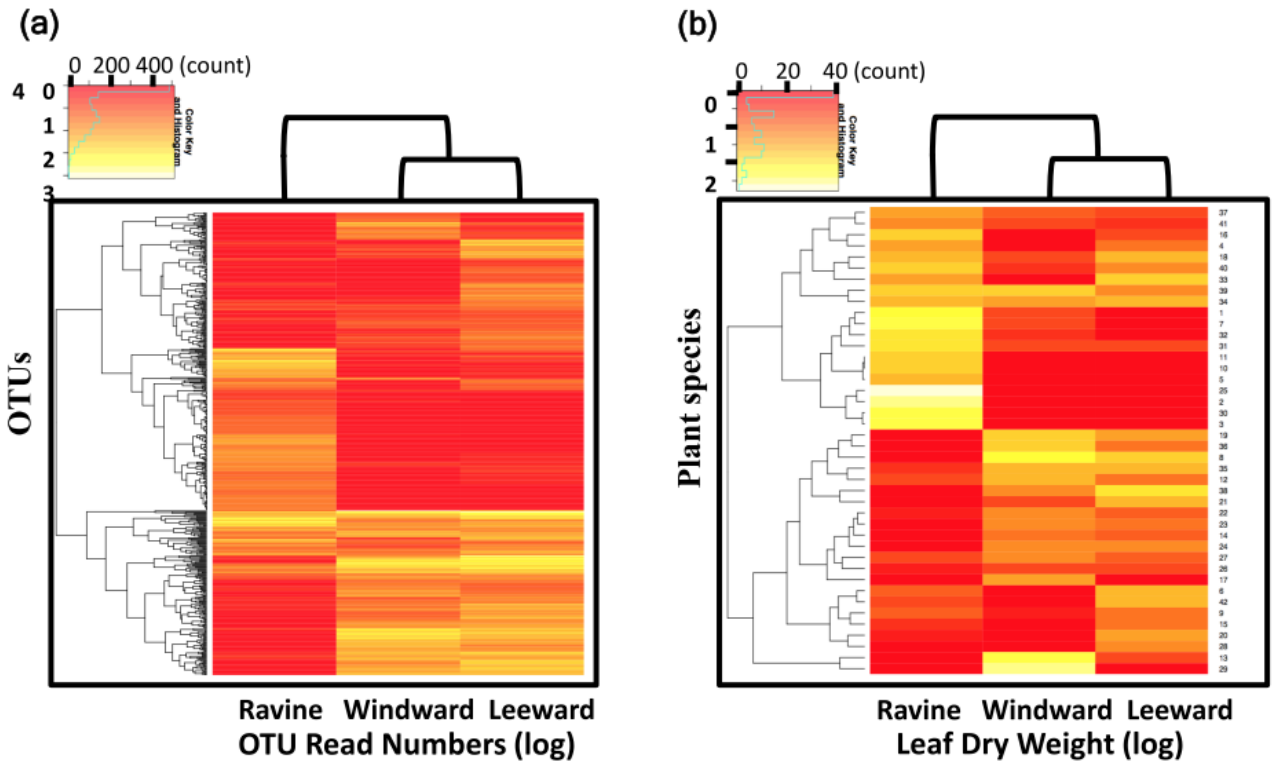
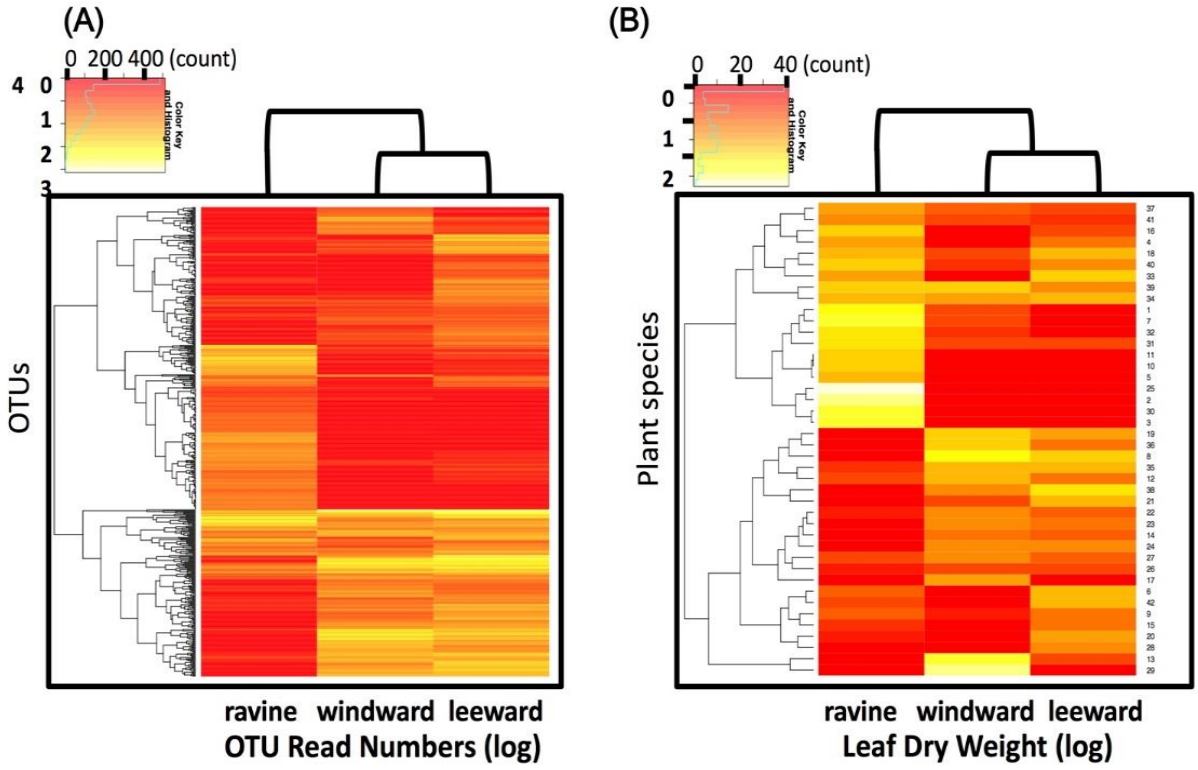


Figure 8. Heat map analysis of read-number of OTUs and annual litterfall productions of each plant species in three habitats. (a) Heat map of read-number of OTUs in three habitats. (b) Heat map of litterfall productions of plants in three habitats.



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