We would like to appreciate referee #2 for the valid comments, which we believe has instructed us to improve the quality of this manuscript. In this document, all of the comments were responded one by one. Some material which is helpful to address the comments was also provided as attached files.

Responses to Referee #2

Comments of Anonymous Referee #2

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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 for the insightful criticizes. More detail information regarding *n*-alkane bacteria has been presented as supplementary results. The data include the phylogenetic trees of OTUs in Proteobacteria and Actinobacteria together with alkB contained bacteria. A summary table 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 will be revised by professional academic writing agency to meet the writing standard of native English speaker once the contents of the manuscript were settled. All the words which have been remarked by referees have been completely revised and replaced as suggested throughout the manuscript.

2. Specific Comments

Page 1

Line 20: bacteria not bacterial

[Reply]

The word ‘bacteria’ is used as suggested.

Line 29: please state how small a fraction.

[Reply]

The sentence in –p1, L29 has been rephrased as follows.

‘Alkanes are saturated hydrocarbons that accounted for a small fraction of total organic carbon no more than a couple of thousand ppm (parts per million by weight) range in natural habitats (Gómez-Coca *et al.*, 2016, Rojo, 2010).’

Page 2

Line 8: Sentence not clear

[Reply]

The sentence in p2, L8 is to address the critical role of bacteria in long chain n-alkane degradations. The sentence has been rephrased as follows.

‘Although fungi and yeasts can degrade alkanes, most research has focused intensely on the role of bacteria in the degradation of alkane (Rojo, 2010; Rojo, 2009; Singh et al., 2012).’

Line 10: Probably dominated is not a clear statement.

[Reply]

We have rephrased the sentence as follows.

‘Although alkanes can be metabolized anaerobically, the growth of anaerobic alkane degraders is significantly slower than that of aerobic ones especially in the environment of litter layer where oxygen supply is adequate (Wentzel et al., 2007). To date, more than 60 genera of aerobic bacteria and 5 genera of anaerobic bacteria can degrade *n*-alkanes (Nie *et al.*, 2014).’

Line 14: oil as in crude oil?

[Reply]

The phase ‘crude oil’ has been used to replace the word ‘oil’.

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

[Reply]

The value of alkane degraders is added and the sentence is rephrased as follows.

‘For example, an estimation of a 10-fold increase in the relative abundance of oil degraders in the contaminated versus clean sand have been described in Gulf of Mexico beach (Kostka *et al.*, 2011).’

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

[Reply]

The phrase “seedbank” has been rephrased to make the sentences clear.

‘Many of the alkane degraders were not detected before the supplements of alkanes, suggesting that some alkane degraders might have presented in very low copies under the detective limitation. They could be induced when the appropriate substrate was supplemented.’

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

[Reply]

The sentence in –p2, L21-L23 has been rephrased.

‘It has been revealed that substrate modulated the growth of microorganisms and controlled the biomass based on its initial concentration (Schmidt, 1992). Thus, different alkane abundance of litterfall in various habitats might up-regulate the growth of various alkane degraders.’

Line 27: reference?

[Reply]

The reference (Schulz *et al.*, 2012) has been added.

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

[Reply]

The diversity values have been added as a supplementary table (Table S-2).

Line 34: how high? Is it statistically significant?

[Reply]

In the study, the author concluded that 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. In other words, the litterfall in the ravine was 20% to 30% higher than that of leeward and windward habitats based on the calculation from research in eight years prior to 2008. The statistic results were not available.

Page3

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

[Reply]

The aim of this study was to see if differences in alkane concentration in natural habitats can alter the microbial community structure of alkane degraders. Therefore, the ideal plots to test this theory will be in the habitats of similar environmental parameters except for their alkane productions.

Line14: poor information on alkB

[Reply]

Some information on alkB has been added. The sentence in L14 has been rephrased. “AlkB proteins hydroxylate alkanes at the terminal position. AlkB are so-far the most commonly found alkane hydroxylases distributed in both Gram-negative and Gram-positive bacteria. A detail review about alkB has been addressed (Nie *et al.*, 2014).”

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.

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

[Reply]

A map of the sampling sites has been provided in an attached supplementary file (Figure S-1). The horizontal distance between stands is about 50 miters. There are 6 and 4 stands at windward and leeward habitat in the Nanjenshan plot, respectively.

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]

Three points need to be addressed here. First, although it has been demonstrated that the litterfall in ravine habitat is higher than in windward and leeward, the alkane flux in these habitats has not been discussed before. Second, none of the research work has previously done to analyze the differences of *n*-alkane compositions/abundance in the tree species at these plots. Third, after completing analysis of *n*-alkane, we realized that the *n*-alkane fluxes in ravine habitat were about two folds higher than those of windward and leeward habitats, which provide the rationale to study the differences in microbial community structure in these habitats. Therefore, the sample collection of litterfall was conducted from 2012 to 2013, while the sample collection for NGS study in bacteria was carried out during 2015-2016. A total of 264, 12 x (6+4+12), samples was used to calculate the *n*-alkane concentration.

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. 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 detail 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 in L19 is from metagenome DNA. A flow chart was provided as showed in supplementary Figure (Figure S-5).

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 attached supplementary figure (Figure S-5).

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 in –p7, L9-L11 has been rephrased as follows.

'In this study, the annual litterfall amount is about 7-10 ton/ha (fig 1), which is considered high-volume when compared with the past record. The primary reason for causing large amount of litterfall in the year was the effect of typhoons. Since the typhoons equally increase litterfall amount in these habitats, the ranking in annual litterfall volume is not changed by the typhoons. One of the reasons that ravine habitat has a larger amount of annual litterfall may lie on the topography of ravine habitat, which is more suitable for the growth of plants.'

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 one 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]

This section (-p7, L28) addresses the issue which is different from 3.2.

In this section, the leaf *n*-alkane concentration in the 42-plant species have been analyzed and ranked in descending order. The leaf *n*-alkane concentration and

corresponding annual litter-leaf production of the 42-plant species in the 3 habitats were showed in table 1. The reason we use plus sign (+) to denote the leaf production instead of real numbers was 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.
To avoid the confusion, we would like to change the title of 3.3 and the first sentence as follows.

'3-3 Annual plant litter-leaf production and the ranking order in leaf *n*-alkane concentration
A total of forty-two plant species were identified and their leaf *n*-alkane concentrations were analyzed in this study. The leaf *n*-alkane concentration and the corresponding annual litter-leaf production are listed in a descending ranking order of *n*-alkane concentration as showed in table 1. The annual net weights of leaves in each plant from the 3 habitats were expressed as the number of plus sign (+) to denote their relative leaf production.'

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]
There maybe is a misunderstanding here.
The sentence in –p8, L6 should be 'We assume that the variation of *n*-alkane concentration in **the same species** can be ignored when compared with the difference **between species**.'.
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.

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 revise figure 1 and figure 3. In the previous version of figure 1, we calculate each number by amount/month. In the revised version, we simplify the calculation by (total amount in weight)/year. Therefore, the n value for the ravine, windward, and leeward habitats will be 12, 6, and 4, respectively. In the previous version of figure 3, the numbers of litter-leaf come from the months that match with the collection of litter-layer and surface soil samples.

However, we think it might be better to do the same way as it has done in figure 1. Therefore, the sample sizes of litter-leaf in figure 3 are identical to that of figure 1. The sample sizes of litter-layer and surface soil are 4, and 11 from each habitat. 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]

In this paragraph, we addressed that relationship of litterfall and the total organic carbon in the land. Since the litterfall in ravine habitat is higher than the others, the organic carbon supposedly could be build up over time. However, a study in these habitats has shown that the total organic carbon of litter layer and bulk soil in the ravine habitat was equal or lower than in the windward and leeward habitats (Kuo, 2010). In other words, the relatively large amount of litterfall in ravine might be either decomposed by microbes or carried away by other effects. We rephrase the entire paragraph in –p8, L18-L23 as following.

‘The balance between litterfall production and the decomposition affects the development of the organic carbon in soil layer. This study together with previous reports indicated that the litterfall in ravine habitat is higher than the others. However, a study in these habitats has shown that the total organic carbon of litter layer and bulk soil in the ravine habitat was equal or lower than in the windward and leeward habitats (Kuo, 2010). Therefore, the decomposition effects might be greater in the ravine habitat. In the figure 3A, we showed that *n*-alkane concentrations decrease significantly from litter-leaf to surface soil, which could be because of the effects of bacteria. It is plausible to speculate that other organic compounds were degraded as *n*-alkanes were. Since several effects such as leaching, erosion and sediment transportation might play roles in governing the accumulation of the organic compounds, we do not rule out the diffusion effects of other physical factors (Kirkels et al., 2014; Quinton et al., 2006). We carried out NGS and bioinformatics

studies to unearth the roles of microbial communities on the degradation of *n*-alkane.

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 introduction as suggested.

In introduction p3, L1, the paragraph mentions about the difference of the 3 habitats, which is a good place to add these values. We rephrase the paragraphs as follows.

-p3, L1

“Among them, three plots aroused our attention, namely plot I (120º 50’ 51” E, 22º 04’ 54” N), plot II (120º 50’ 36” E, 22º 04’ 52” N) and Lanjenchi plot (120º 51’ 38” E, 22º 03’ 23” N). Ravine habitat located across plot I and plot II, while leeward and windward habitats located at plot III. Surveys conducted from past decade have shown that the annual amount of litterfall in ravine habitat was higher than in the windward and leeward habitats. Moreover, a 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).”

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 (Figure S-4). 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. However, we would like to point out the height and number of plants varied drastically between windward and leeward habitats. In other words, 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. Please see them in attached file for detail.

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 needs to be rephrased. After putting all indices together, we agree with the viewer that the numbers were similar. We change our interpretation of data. The paragraph was changed as follows.

'PCoA was used to visualize the similarities of DNA sequences in *Proteobacteria* and *Actinobacteria* from 3 habitats. Figure 6 showed the distribution of a total of 240

OTUs in phyla *Proteobacteria* and *Actinobacteria*. The nomenclature of OTUs of alkB lineage-like bacteria was showed on the table S-1. The biodiversity index in OTUs of alkB lineage-like bacteria and other sample types were showed in table S-2. Although the relative abundance in OTUs that contained alkB lineage-like bacteria in the ravine habitat was more than twice higher than those of windward and leeward habitats (figure 5), the Shannon-Weiner indices of three habitats were similar, suggesting the effective numbers of species were almost identical. The data provide the evidence that very low copies of the alkB gene contained were preexistent in natural habitats under the detective limitation by normal PCR procedure. They could be fostered when the appropriate substrate was supplemented. This finding is consistent with the results from the previous study in a laboratory controlled system and an agricultural research farm (Giebler *et al.*, 2013, Schulz *et al.*, 2012).'.

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 starting at the beginning of –p6, L24.
'Litter-layer samples from 3 habitats in Nanjenshan were subjected to semi-quantitative PCR study for quantifying the DNA levels of alkB genes in natural habitats. Litter-layer samples around different stands in each habitat were randomly selected for sample collection at 3 time points. After the collection, samples of litter-layer were sent to the laboratory in room temperature as soon as possible. The patches of litter-layer were randomly selected and cut into approximately ~2mm x ~2mm chips. Two hundred and fifty mg of samples were taken for DNA extraction. Following the elution of DNA in 100 µl buffer solution, 2µl of eluted DNA were taken for semi-quantitative PCR. The DNA was 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' ,1µl, 0.4µM), 5µl Fast-Run Taq Master Mix with Dye, and 16µl ddH2O with the final reaction volume of 25µl. …'

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]

In the study of microbial community, since an equal amount of DNA was used for PCR, it is no good to compare the abundance of these organisms (Figure S-5). That is why 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]

Like we have addressed in the previous reply on page 7 and Line 28, we use the number of plus sign (+) to denote their relative amount of leaf production. The reason we use plus sign (+) to denote the leaf production instead of real numbers was 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. The real number (x) of leaf production were used in log(x) to have heat map figure as showed in figure 8.

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.

'**3-2 Typical example of *n*-alkanes measurement in Nanjenshan Reserve**

The *n*-alkane concentration of leaves from 42-plant was assayed in this study. Figure 2 showed a representative GC chromatograms in leaves of *Iles rotunda* in each habitat.'

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 manuscript.

Page 10:
Line23: may be instead of suggesting
[Reply]
The word 'suggesting' is used as suggested.

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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

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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.

Phylum	OUT	Relative Abundance (%)			Reference of bacteria with alkB gene	Sequence ID	Identities
		ravine	windward	leeward			
Proteobacteria	litter14	4.5	1.0	0.2	*Agrobacterium fabrum*	NR_074266.1	377/382(99%)
	litter44	2.2	0.2	0.1	*Rhizobium etli*	U28916.1	376/381(99%)
	litter50	2.1	0.9	0.6	*Ensifer adhaerens*	CP007236.1	375/382(98%)
	litter117	1.6	<0.1	<0.1	*Mesorhizobium amorphae*	NR_024879.1	371/384(97%)
	litter6	0.7	1.6	1.2	*Bradyrhizobium diazoefficiens*	AB909430.1	380/382(99%)
	litter159	0.6	<0.1		*Stenotrophomonas maltophilia*	AM743169.1	390/406(96%)
	litter464	0.4	<0.1	<0.1	*Sinorhizobium meliloti*	CP003933.2	377/384(98%)
	litter189	0.4	<0.1		*Stenotrophomonas maltophilia*	AM743169.1	394/408(97%)
	litter291	0.3			*Delftia acidovorans*	CP000884.1	387/408(95%)
	litter199	0.3	0.1	<0.1	*Caulobacter crescentus*	AE005673.1	384/384(100%)
	litter256	0.3	<0.1	0.1	*Bradyrhizobium diazoefficiens*	AB909430.1	376/387(97%)
	litter506	0.2	<0.1		*Mesorhizobium ciceri*	CP002447.1	375/382(98%)
	litter152	0.1	0.1	<0.1	*Caulobacter crescentus*	AE005673.1	370/384(96%)
	litter109	0.1	<0.1	0.7	*Ochrobactrum anthropi*	CP000758.1	374/382(98%)
	litter9	0.1	1.6	0.4	*Ochrobactrum anthropi*	CP000758.1	369/382(97%)
	litter21	0.1	0.7	<0.1	*Enterobacter lignolyticus*	CP002272.1	398/407(98%)
	litter505	<0.1	<0.1	<0.1	*Pseudomonas putida*	LT799039.1	396/408(97%)
	litter487	<0.1	0.2	0.2	*Burkholderia pseudomallei*	CP009163.1	401/413(97%)
	litter75	<0.1	0.2	1.4	*Burkholderia phenoliruptrix*	CP003863.1	402/409(98%)
	litter11	<0.1	1.2	0.4	*Burkholderia phenoliruptrix*	CP003863.1	399/409(98%)
	litter384	<0.1		<0.1	*Herbaspirillum seropedicae*	CP002039.1	384/406(95%)
	litter209	<0.1	0.2	0.5	*Burkholderia phenoliruptrix*	CP003863.1	399/410(97%)
	litter208		<0.1		*Ralstonia solanacearum*	AL646052.1	394/406(97%)
	litter220		0.1	0.2	*Burkholderia pseudomallei*	CP009163.1	389/407(96%)
Actinobacteria	litter32	4.0	<0.1		*Amycolatopsis orientalis*	NR_042104.1	375/389(96%)
	litter69	1.6	<0.1		*Amycolatopsis orientalis*	NR 042104.1	373/391(95%)
	litter426	0.5	<0.1	<0.1	*Streptomyces virginiae*	NR 115622.1	382/386(99%)
	litter147	0.4	<0.1	<0.1	*Amycolatopsis orientalis*	NR 042104.1	366/387(95%)
	litter319	0.3			*Amycolatopsis vancoresmycina*	NR 025565.1	370/390(95%)
	litter89	0.1	0.2	0.3	*Mycobacterium smegmatis*	X52922.1	382/387(99%)
	litter228	0.1	0.1	0.1	*Mycobacterium kansasii*	FR822390.1	378/399(95%)
	litter352	<0.1	<0.1	<0.1	*Mycobacterium abscessus*	LC149865.1	370/388(95%)
	litter465		<0.1	<0.1	*Mycobacterium kansasii*	FR822390.1	391/403(97%)

Table S-1. The alkB lineage-like OTUs and the reference genes.

Sample type	Habitat	Biodiversity Index		
		Shannon-Weiner	effective numbers of species	Evenness
Plant vegetation	Ravine	3.26	26	0.69
	Windward	3.64	38	0.78
	Leeward	3.78	44	0.78
Bacteria	Ravine	4.43	84	0.76
	Windward	4.22	68	0.70
	Leeward	4.12	62	0.72
OTUs of AlkB-lineage	Ravine	2.51	12	0.74
	Windward	2.39	11	0.70
	Leeward	2.46	12	0.76

Table S-2. Biodiversity index of plant vegetation, bacteria, and AlkB-lineage like OTUs.

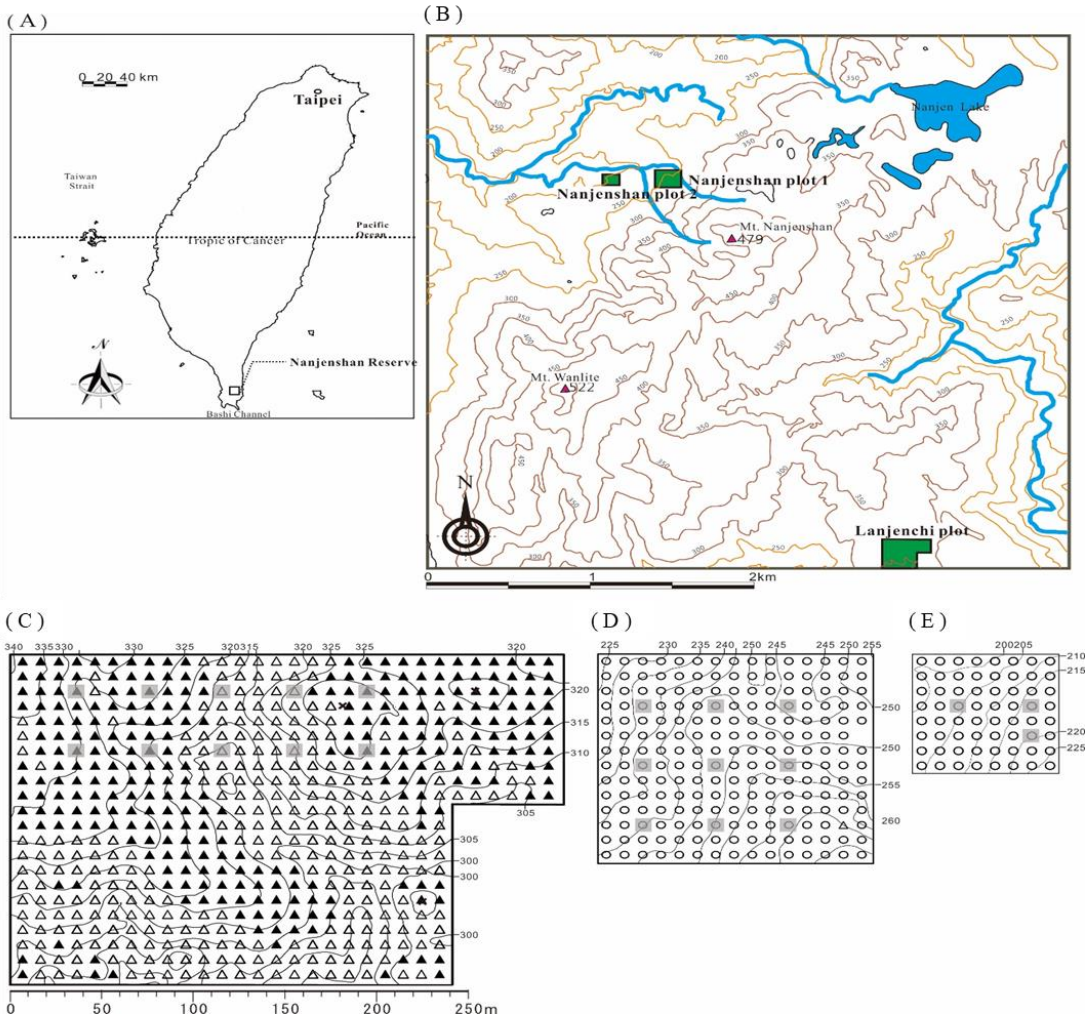


Figure S-1. Location of the sampling site (from Chao et al, 2010). (A) Map of Taiwan. (B) Map of the Nanjenshan Reserve. (C) Stands set of windward habitat (▲) and Leewind habitat (△) in Lanjenchi plot. (D) Stands set of ravine habitat (○) in Nanjenshan plot I. (E) Stands set of ravine habitat (○) in Nanjenshan plot II.

This figure was produced with permission of original author Wei-Chun Chao who is the co-author of this study.

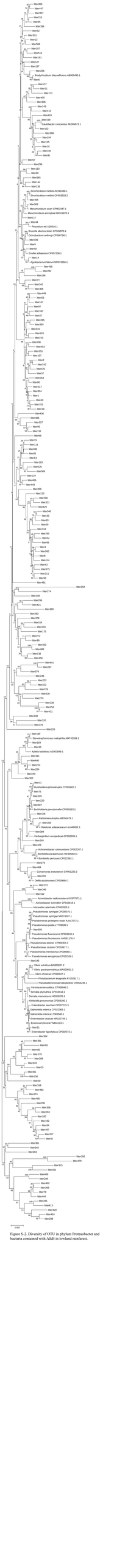


Figure S-2. Diversity of OTU in phylum Proteaobacter and bacteria contained with ARB in lowland rainforest.

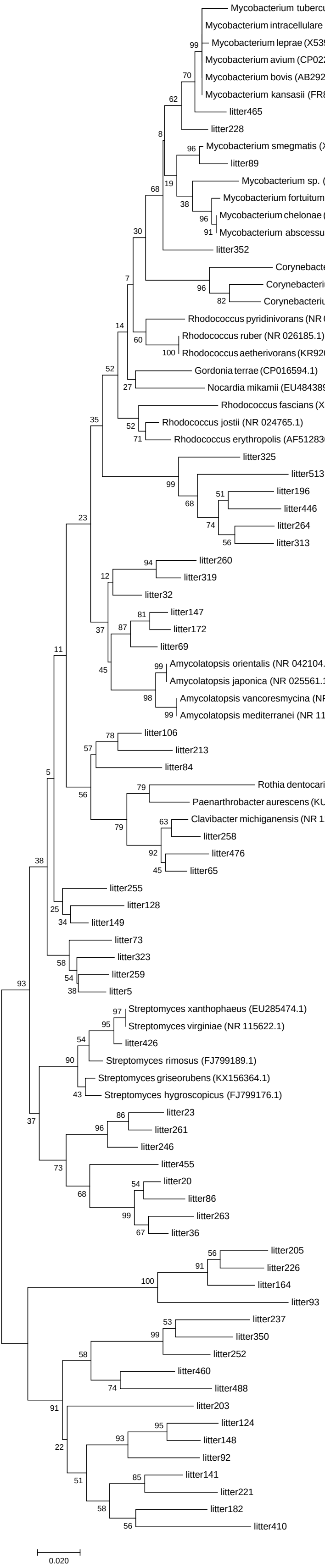


Figure S-3. Diversity of OTU in phylum Actinobacteria and bacteria contained with AlkB in lowland rainfarest.

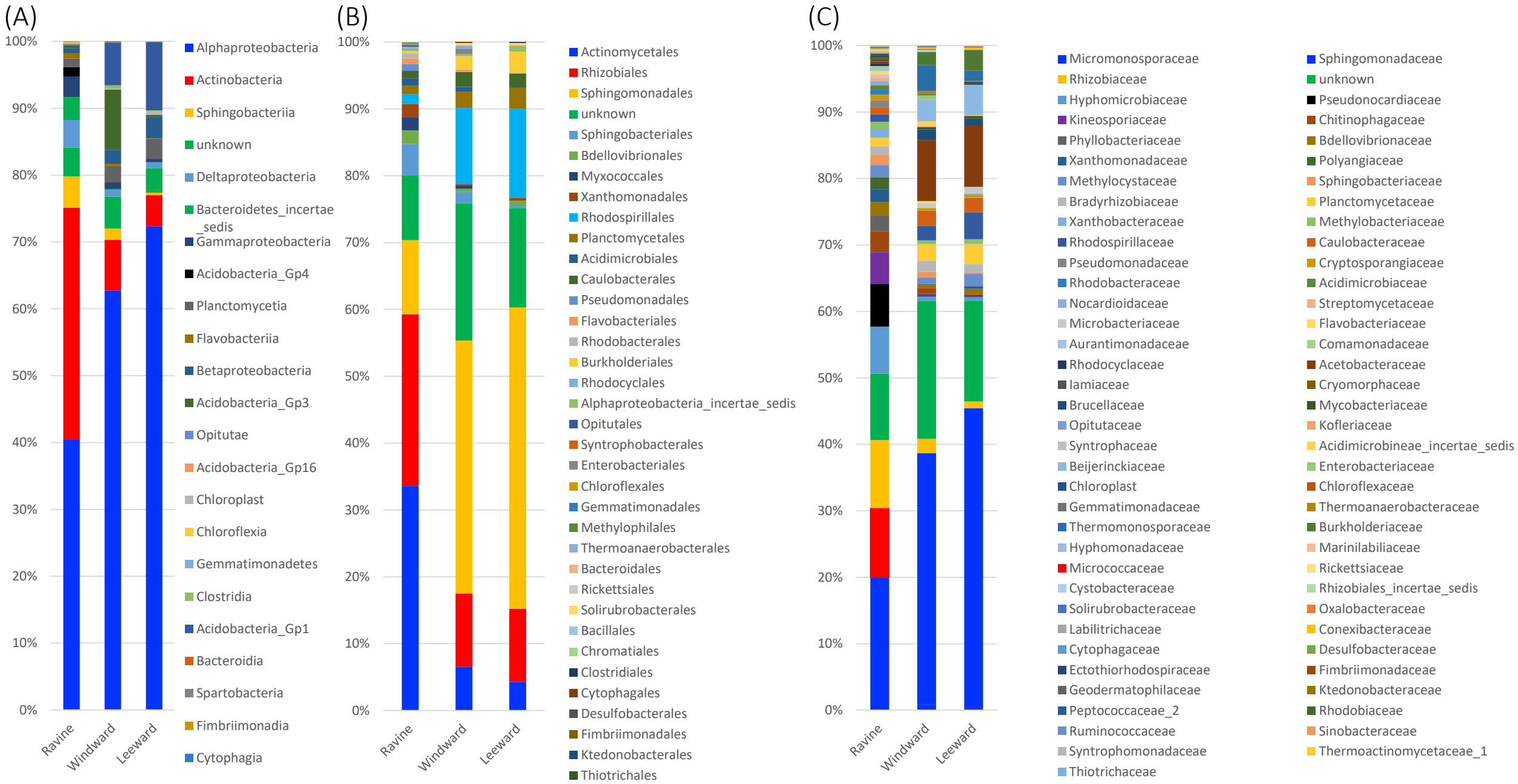
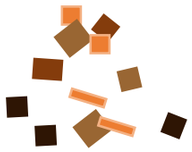


Figure S-4. Microbial community structure in the three habitats of Nanjenshan Reserve. Bacterial lineages were indicated in (A) class, (B) order, and (C) family.

(A)

Patches of litter-layer were randomly selected.
A total of three batches was carried out in this study.



The patches were cut into approximately ~2mm x ~2mm chips. Take 250 mg of samples for DNA extraction.

One hundred μL of DNA was Eluted in buffer.

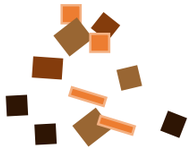
Take 2 μL of DNA for PCR .

Semi-quantitative PCR for AlkB gene

AlkB gene expression

(B)

Randomly select patches from litter-layer



Cut into approximately ~2mm x ~2mm chips. Take 0.1-0.2 g of samples for DNA extraction.

A final extraction of DNA (approximately 30 ~100 ng/ μL in 100 μL Tris buffer) was eluted.

Amplicon PCR experiment was performed in triplicate. In each experiment, 12.5 ng of DNA was used in each experiment.

The final product of **Amplicon PCR** was mixed for conducting **Index PCR**.

MiSeq Metagenomics sequencing

Figure S-5. (A) Flow chart for Semi-quantitative PCR. (B) PCR flow chart for NGS.