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Interactive comment on "Ammonium and nitrate additions differentially affect soil microbial biomass of different communities and enzyme activities in slash pine plantation in subtropical China" *by* Chuang Zhang et al.

Chuang Zhang et al.

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We are grateful for the reviewers comments and we agree to substantial changes to the manuscript in response to the valuable input from the two reviewers. Please find our response to reviewers comments on behalf of all co-authors. Our response to the reviewer's comments are detailed point by point below.

Yours sincerely

Chuang Zhang, Xin-Yu Zhang, Hong-Tao Zou, Liang Kou, Yang Yang, Xue-Fa Wen,

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Sheng-Gong Li, Hui-Min Wang, Xiao-Min Sun

Response to reviewers comments

RC1 Anonymous referee #1

1. The data analyses is not enough or suitable. 1) The authors mentioned that using two factors randomized block variance of analyses (ANOVA) to test the differences between the treatment and the sampling season. However, after reading the whole manuscript, I did not find any results from this methods. For example, if there is two factors, it must have the possible interaction effect between two factors. Actually, from Table 2, I have found several important interaction effects for DOC, Nitrate, Fugal et al. However, the authors did not discuss this at all.

Response: We added the results of interaction effects to the result section. “The soil pH and ammonium contents were either treatment- or time-independent. There were interaction effects between the treatments and the sampling time on the soil DOC and nitrate contents ($P<0.01$, Table 1).” (line 238-240) “Both the treatment and the time of sampling significantly influenced the soil microbial biomass of the different communities ($P<0.01$). Total PLFAs, bacteria, G−, and G+/G− were either treatment- or time-independent. There were also interaction effects between treatments on sampling time and fungi, actinomycetes, G+, AMF, SAP, and the fungi/bacteria ratio (Table 1).” (line 252-255) “There were significant influences from both treatment and sampling time on the measured absolute enzyme activities ($P<0.01$). Activities of BG, AP, and PPO were either treatment- or time-independent, and there were interaction effects between the treatments and sampling time on activities of aG, BX, CBH, NAG, and PER (Table 1).” (line 271-274) We also discussed the possible reasons resulted in the interaction effects at the discussion section. “The N treatments also varied significantly on a seasonal basis and there were interaction effects between N treatments and seasons on the contents of some PLFA biomarkers and enzyme activities (Table 2). Climate conditions, plant growth, the amount of litter returned, and plant-soil-microorganism systems

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varied across the three seasons. The temperature ranged from 13.5 to 27.6 °C, and precipitation ranged from 88.2 to 176.6 mm, across the three seasons (Fig. S1), and did not limit the growth of microorganisms. The positive relationships between PLFA biomarker contents and soil moisture contents indicate that soil moisture had a strong influence on soil microbial community biomass. There may be interaction effects between plant growth, the mass and quality of litter, plant-microbe competition, and soil nutrient dynamics. For example, compared with the control plots, the soil DOC contents were lower, and soil nitrate contents stayed the same in June (the growing season) in the ammonium treatment, but the soil DOC and nitrate contents were higher in the ammonium and nitrate treatments in March and October (the non-growing season, Fig. 2). This indicates that there was stronger competition between plants and microbes for available C and N in June than in March and October, and that there were interaction effects between plants and microbes on soil C and N availability. This might explain the interaction effects between N additions and seasons on the activities of C and N-acquisition enzymes. The effects of interactions between N additions and season on the AMF PLFA contents, along with available C and N dynamics, may result from plant growth as plant-AMF symbiotic systems may be influenced by fine root biomass." (line 354-371)

2) Another question is that I have some confused why the authors took three measurements for PLFA biomarkers, even we knew that the variance of PLFA measurements varied a lot. Is there any important reasons to choose these three different months and what the ecological meanings are? evenly sprayed onto the plot once per month. Did this mean that the frequency of spray the nitrogen 12 times per year?

Response: "We collected soil samples in March, June, and October of 2015, to represent spring, summer, and fall." (line 169-170) "The atmospheric conditions and plant-derived litters differed between the three seasons, and so indirectly affected the soil microbial biomass and enzyme activities of different communities. We collected soils from three seasons so that we could investigate the synthetic responses of soil micro-

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bial biomass and enzyme activities to ammonium and nitrate additions and to obtain improved information to support predictions of the effects of elevated N depositions on C, N, and P cycling." (line 172-177) The frequency of spray the nitrogen was 12 times per year. We showed at "The NH4Cl or NaNO3 were dissolved in 30 L of tap water and evenly sprayed onto the plots once a month, i.e. 12 times per year." (line 161-162)

Other minor comments:

1. In introduction and discussion section: the authors cited papers in different ways, please format it.

Response:Revised as recommended.

2. The authors should improve their English grammar for a lot. There are a lot of small mistakes in the whole manuscript.

Response: We have our revised version manuscript professionally edited by a native English speaker colleague, Dr Deborah Ballantine from the United International College, Beijing Normal University and Hong Kong Baptist University, Zhuhai, Guangdong Province.

RC1 Anonymous referee #2

General Comments:

1) In general, the data are much more complex across time than the authors present. It would be nice if the results are as clean as suggested in the topic sentence of each discussion paragraph, but it is simply not the case because many of the results are time-dependent. For this reason, the authors need to greatly expand the interpretation and discussion of the treatment x time interaction that is presented in Table 2. Further, to help the reader reason through the data, I think it would be beneficial to collapse the data to not include the 3 sampling times for those factors that do not exhibit a significant treatment x time interaction. For example, Fig 2(i) can be reduced to three bars for control, ammonium N, and nitrate N because there was not a significant interaction.

Response: We discussed the possible reasons resulted in the interaction effects at the discussion section. “The N treatments also varied significantly on a seasonal basis and there were interaction effects between N treatments and seasons on the contents of some PLFA biomarkers and enzyme activities (Table 2). Climate conditions, plant growth, the amount of litter returned, and plant-soil-microorganism systems varied across the three seasons. The temperature ranged from 13.5 to 27.6 °C, and precipitation ranged from 88.2 to 176.6 mm, across the three seasons (Fig. S1), and did not limit the growth of microorganisms. The positive relationships between PLFA biomarker contents and soil moisture contents indicate that soil moisture had a strong influence on soil microbial community biomass. There may be interaction effects between plant growth, the mass and quality of litter, plant-microbe competition, and soil nutrient dynamics. For example, compared with the control plots, the soil DOC contents were lower, and soil nitrate contents stayed the same in June (the growing season) in the ammonium treatment, but the soil DOC and nitrate contents were higher in the ammonium and nitrate treatments in March and October (the non-growing season, Fig. 2). This indicates that there was stronger competition between plants and microbes for available C and N in June than in March and October, and that there were interaction effects between plants and microbes on soil C and N availability. This might explain the interaction effects between N additions and seasons on the activities of C and N-acquisition enzymes. The effects of interactions between N additions and season on the AMF PLFA contents, along with available C and N dynamics, may result from plant growth as plant-AMF symbiotic systems may be influenced by fine root biomass.” (line 354-371) We also simplified the figure that the indexes were treatments-independent, and the figures were shown at Fig. 1and Fig. 3.

2) More can be done with soil enzyme data to forward the authors main hypotheses and ideas that are introduced in line 119. For example, enzyme data can be presented as ratios of C acquiring/N acquiring and/or C acquiring/P acquiring. Such analysis will provide a clearer avenue to draw conclusions about whether microbes can alter how resources are allocated to scavenge for nutrients under different conditions.

Response: “We compared the stoichiometry of C and P to N-acquisition enzyme activities by ln(aG+BG+CBH+BX) and lnaP to lnNAG, respectively (n=27).” (line 210-212) “When compared to control, the ratios of C to N-acquisition enzyme activities were about 0.2 higher, the ratios of N to P acquisition enzyme activities were about 0.1 lower, and there were no obvious differences in the ratios of C to P acquisition enzyme activities in the ammonium and nitrate treatments.” (line 277-280) “The ratios of C or P to N acquisition enzyme activities were higher in the ammonium and nitrate treatments than in the control plots, and the N-acquisition enzyme activities per unit of microbial biomass were lower in the ammonium and nitrate treatments than in the control (Fig. 5), indicating that microorganisms secreted enzymes in line with the economic theory. Measured absolute enzyme activities were positively correlated with soil pH and ammonium contents, and negatively correlated with nitrate contents (Fig. 6). The inhibitory effects of N on the soil absolute enzyme activities may be more closely related to abiotic factors, i.e. soil pH and nitrification, than biotic factors (Kivlin et al., 2016).” (line 339-346)

3) Is there any ecological rationale for the March/June/October time points? What is the climatic variation across these times? Also, I assume soil moisture was measured, and if it was, those data should be presented and included in all analyses (including the RDA). Soil moisture has been shown to be a major driver of microbial community composition.

Response: “We collected soil samples in March, June, and October of 2015, to represent spring, summer, and fall.” (line 169-170) “The atmospheric conditions and plant-derived litters differed between the three seasons, and so indirectly affected the soil microbial biomass and enzyme activities of different communities. We collected soils from three seasons so that we could investigate the synthetic responses of soil microbial biomass and enzyme activities to ammonium and nitrate additions and to obtain improved information to support predictions of the effects of elevated N depositions on C, N, and P cycling.” (line 172-177) “Soil water contents (SWC) were measured

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by the oven drying method (105 °C).” (line 184-185) “SWC were positively correlated with soil PLFA biomarker contents, but were not correlated with the absolute enzyme activities (Fig. 6).” (line 302-303) “The temperature ranged from 13.5 to 27.6 °C, and precipitation ranged from 88.2 to 176.6 mm, across the three seasons (Fig. S1), and did not limit the growth of microorganisms. The positive relationships between PLFA biomarker contents and soil moisture contents indicate that soil moisture had a strong influence on soil microbial community biomass.” (line 357-360) The data applied to RDA analysis included soil moisture contents, and the figure was shown at Fig.6. Average monthly atmospheric temperature and precipitation at the study site during 2015 were shown at Fig. S1.

4) The manuscript needs to be edited for grammar, flow, and word choice. The writing is poor and must be improved significantly in order to be publishable.

Response: We have our revised version manuscript professionally edited by a native English speaker colleague, Dr Deborah Ballantine from the United International College, Beijing Normal University and Hong Kong Baptist University, Zhuhai, Guangdong Province.

Minor comments: Line 169: Is there rationale for the dose of N applied? Any relation to predictions for future N deposition in the region?

Response: “Background atmospheric wet N deposition of about 33 kg N ha−1 yr−1 comprises 11 kg N ha−1 yr−1 as ammonium and 8 kg N ha−1 yr−1 as nitrate (Zhu et al., 2014). We established a control and test plots at the experimental sites. We equally added two types of N to the test plots, i.e. ammonium (Nammonium) as ammonium chloride (NH4Cl) and nitrate (Nnitrate) as sodium nitrate (NaNO3), at an annual rate of 40 kg N ha−1 yr−1. This rate was about double the background N wet deposition.” (line 154-159)

Line 229: How were the 3 sampling times considered for the RDA? Was the RDA ran on data from one of the three sampling times? Or from average data across the sampling

times? Given the treatment by time interaction, this point needs clarification.

Response: The response of soil biomass of different microbial communities and enzyme activities to N treatments was similar in the three sampling seasons, so all of data in the treatments and seasons (n=27) was applied to RDA analysis. And we added the n value to statistical analyses section. (line 231)

Line 315: Others have shown that N addition disproportionately effects soil fungi and may stimulate soil bacteria (for example, see doi 10.3389/fmicb.2016.00259 and 10.1128/AEM.01224-14). This dynamic may also help explain the increase in DOC observed with N addition.

Response: We have added that " Moreover, the higher soil DOC concentrations observed in the nitrate-addition treatments (Fig. 2) may be attributed to changes in the diversity of the composition of saprophytic bacteria (Freedman and Zak, 2014; Freedman et al., 2016)." (line 324-326)

Table 2: I think P-values can be removed from this table. Given that significant values are bolded, P-values are redundant and make the table busy for the reader.

Response: Revised as recommended, please refer to Table 1.

Please also note the supplement to this comment:
https://www.biogeosciences-discuss.net/bg-2017-179/bg-2017-179-AC1-supplement.pdf

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