

## ***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.***

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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.

Comments in response to Referee 1

The effect of atmospheric nitrogen deposition on forest ecosystems has become one important issue and popular research topic in recent decades across the world. The

C1

aim of this manuscript is to explore the differential effects of ammonium and nitrate on soil microbial communities and enzyme activities. The ideas are interested and worth to do. In general, I think the manuscript could be published after taking the following questions. Response: We would like to thank you for the helpful and constructive comments, which further improved the manuscript. We have carefully revised our manuscript to take account of your comments and suggestions. Please find below our responses (blue font) to comments (repeated in an italic font). The page and line numbers mentioned here refer to the latest revision of our manuscript simultaneously submitted with all figures as a single PDF file.

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<sub>-</sub>, and G<sub>+</sub>/G<sub>-</sub> were either treatment- or time-independent. There were also interaction effects between treatments on sampling time and fungi, actinomycetes, G<sub>+</sub>, 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

C2

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 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?

### C3

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) 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.

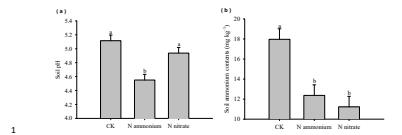
Please also note the supplement to this comment:

<https://www.biogeosciences-discuss.net/bg-2017-179/bg-2017-179-SC1-supplement.pdf>

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Interactive comment on Biogeosciences Discuss., <https://doi.org/10.5194/bg-2017-179>, 2017.

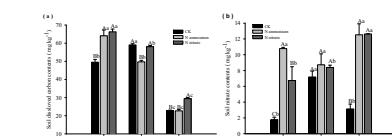
### C4



1  
2 Fig.1 The effects of ammonium and nitrate additions on soil pH and ammonium contents. Small letters  
3 represent significant differences between treatments ( $P < 0.05$ ), error bars represent means  $\pm$  standard  
4 errors ( $n=9$ ).

**Fig. 1.**

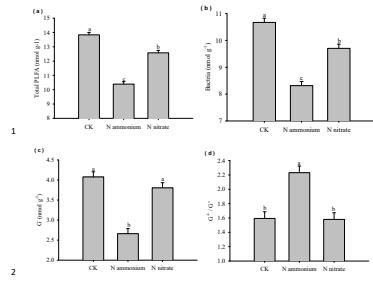
C5



1  
2 Fig.2 The effects of ammonium and nitrate additions on soil nitrate and DOC contents for each  
3 sampling event. Capital letters represent significant differences between the treatments ( $P < 0.05$ ), and  
4 small letters represent significant differences between the sampling events ( $P < 0.05$ ), error bars  
5 represent means  $\pm$  standard errors ( $n=3$ ).

**Fig. 2.**

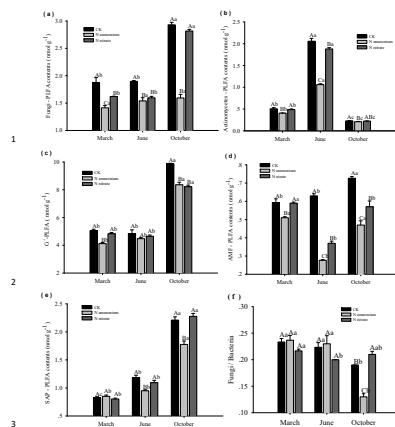
C6



3 Fig.3 The effects of ammonium and nitrate additions on Total PLFAs, PLFA contents of bacteria, G<sup>+</sup> and G-/G<sup>-</sup>. Small letters represent significant differences between treatments ( $P < 0.05$ ), error bars represent means  $\pm$  standard errors (n=9). G<sup>+</sup> represents gram positive bacteria and G<sup>-</sup> represents gram negative bacteria.

Fig. 3.

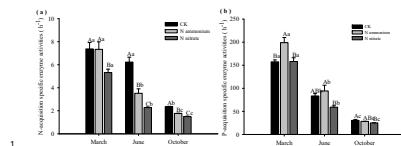
C7



4 Fig. 4 The effects of ammonium and nitrate additions on PLFA contents of fungi, actinomycetes, AMF, SAP, G<sup>+</sup>, and fungi:bacteria ratio for each sampling event. Capital letters represent significant differences between the treatments ( $P < 0.05$ ), and small letters represent significant differences between the sampling time ( $P < 0.05$ ), error bars represent means  $\pm$  standard errors (n=3). G<sup>+</sup> is gram positive bacteria, AMF is arbuscular mycorrhizal fungi, and SAP is saprophytic fungi.

Fig. 4.

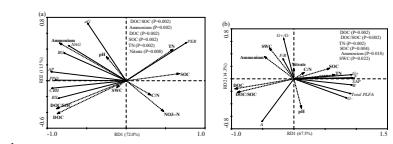
C8



2 **Fig. 5.** The effects of ammonium and nitrate additions on N, P-acquisition specific enzyme activities for  
3 each sampling event. Capital letters represent significant differences between the treatments ( $P < 0.05$ ).  
4 and small letters represent significant differences between the sampling time ( $P < 0.05$ ), error bars  
5 represent means  $\pm$  standard errors ( $n=3$ ).

Fig. 5.

C9



1  
2 Fig. 6. Redundancy analyses between (a) soil properties and enzyme activities, and (b) soil properties  
3 and PLFA-biomarker contents.

Fig. 6.

C10