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Interactive comment on "Responses of soil microbial communities and enzyme activities to nitrogen and phosphorus additions in Chinese fir plantations of subtropical China" *by* W. Y. Dong et al.

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Comments in response to Referee 3

Dong and coauthors present a nice study investigating the effects of nitrogen and phosphorus addition to Chinese fir forest soils, focusing on soil enzyme activities and microbial community structure based on PLFA analyses. This paper is well written and clearly organized. The introduction nicely lays out the importance of understanding how nutrient additions affect soil enzyme and microbial activity, and the how previous

studies have found a range of effects, leaving a complex puzzle of which studying nutrient effects in coniferous forest soils is an important piece. Overall, this study shows differential effects of different nutrient additions on soil chemistry and microbial enzyme activities in coniferous soils, with clear differences in enzyme activities and microbial communities between nitrogen and phosphorus addition regimes. While this paper is strong overall, I recommend a number of minor revisions prior to publication. Most important is justification of some of the methods and brief discussions of drawbacks or assumptions of the PLFA analyses. Additionally, some of the conclusions may be overstated, overemphasizing the role of Gram-positive bacteria in controlling soil enzyme activities. Provided the authors address these points I believe this manuscript will be appropriate for publication in Biogeosciences upon revision. Response:We are very grateful for your encouraging comments and look forward to publishing our manuscript in this international journal after minor revision. We have revised our manuscript according to the comments. Please refer to the following response for details. 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.

SPECIFIC COMMENTS (1).-p. 6 lines 7-9: Why were these three enzymes selected for enzyme activities? How are they important? Make sure a reader not intimately familiar with the PLFA literature understands why these three enzymes were selected for activity measurements and what these enzymes actually do. Response: Extracellular enzymes catalyze the initial, rate-limiting step of decomposition and nutrient mineralization. In this study, we chose three important hydrolytic enzymes: one carbon-acquiring enzyme (βG), one nitrogen-acquiring enzyme (NAG) and one organic phosphorus-acquiring enzyme (aP). In the "Introduction", we have added the functions of the three enzymes on P3, L27 to P4,L2 as follows: "Hydrolytic enzymes control the decomposition of many biological macromolecules that are abundant in plant litter and soil such as cellulose, hemicellulose, chitin, and protein (Allison et al., 2007). For our study we chose three enzymes that are related to the soil oganic carbon cycle, β-Glucosidase (βG) mainly releases glucose from cellulose and plays an important role in C cycling.

N-acetyl-β-D-glucosaminidase (NAG) mainly releases N-acetyl-β-D-glucosamine from the terminal non-reducing ends of chitooligosaccharides and plays an important role in N cycling. Acid phosphatase (aP) mainly releases phosphate groups, and plays an essential role in P cycling (Stone et al., 2012). The production of such enzymes by microbes is closely related to the balance between the availability of and the demand for nutrients."

(2).-p. 10 lines 24-28: This paragraph is vague and should be removed. True, G+ bacteria outnumbered G- bacteria in fertilized treatments, but also in controls, and this ratio does not appear to change with fertilization (Fig. 3 e,f and p. 8 lines 6-7). So there does not seem to be any effect on G+ bacteria over G- bacteria, but rather effects on both populations as the whole bacterial population increases. This does not seem like evidence that G+ bacteria have "stronger environmental adaptability" than G- bacteria, a phrase that does not say anything specific anyway. Response: Thank you for comments. We have deleted the paragraph.

(3).-p. 11 lines 20-23, p. 12 lines 11-12: Be careful of overinterpreting these data. True, all three enzyme activities are correlating with G+ PLFAs, but Table 3 also shows at least 2 of the enzyme activities are also correlated with total PLFAs and strongly correlated with bacterial PLFAs and actinomycete PLFAs in addition to G+ PLFAs. Therefore it does not seem reasonable to conclude the correlation between enzyme activities and G+ PLFAs means enzyme activities are regulated by G+ biomass, as much of the activity could just as likely be regulated by other bacterial (including actinomycete) biomass as well. -In general, how are PLFA profiles or abundance related to other techniques commonly used to measure microbial diversity and abundance? Is it possible to infer any taxonomic information from PLFAs other than G+, G-, and actinomycetes? These are very broad groups of bacteria. If not, the conclusions of the study are not affected, but this should be noted as a drawback of this method somewhere in the introduction or discussion. Response: Thank you for the comments. We have deleted the sentence to avoid over interpreting our study results. Additionally, the biomass of groups such as

gram-negative bacteria, gram-positive bacteria, actinomycetes, and fungi can be estimated by determining the concentration of so-called signature fatty acids, which are specific for a given group. Therefore, the fatty acid pattern is used to determine community composition and is quantitative. Unfortunately, the PLFAs analysis provides no information on species composition. As you said, this is a drawback of PLFA analysis method, and we have added the limitation of this method on P13, L16 to L20 as follows: "Nevertheless, there are a few limitations with PLFA analysis, which cannot reveal species-level information and archae cannot be determined using this method. The abundance and diversities of some functional genes of C, N, and P cycling can be analyzed by molecular biology technique. It will present detail information about the relationships between soil microbial diversities and enzyme activities. "

(4).-Finally, many studies of soil microbiology have found archaea to be an important and active component of soil microbial communities. Is there a reason archaea are not included in this study? Has previous work suggested they are not important members of coniferous soil communities, or did the methods employed here simply not allow for their detection? Again, if this is a methodological issue, it does not discount the results of this manuscript, but this is a potentially important caveat to the data present here and should be mentioned somewhere in the manuscript. Response: The polar lipid of archaea was in the form of ethers rather than lipid and so it cannot be analysis by PLFA method. Therefore, the use of biomarkers to indicate archaea is limited by the difficulty of testing. We have added the limitation of PLFA method on P13, L16 to L20 as follows: "Nevertheless, there are a few limitations with PLFA analysis, which cannot reveal species-level information and archae cannot be determined using this method. The abundance and diversities of some functional genes of C, N, and P cycling can be analyzed by molecular biology technique. It will present detail information about the relationships between soil microbial diversities and enzyme activities.". As you mentioned, archaea has been found not only in extreme environments such as high-temperature, high-saline, but also in soil, which implies that archaea may contribute greatly to various ecosystems. We are studying about the effects of N and P

applications on functional genes of N dynamics. Abundance and diversities of archaea and some other bacteria were analyzed by molecular biology technique. We hope to publish the results in the near future.

TECHNICAL COMMENTS (1).-p. 1 line 26: Define “PLFA” in the Abstract. Response: Revised as recommended.

(2).-p. 3 line 11, p. 11 line 3: Is “gram bacteria” supposed to be either gram-negative or gram-positive? All bacteria are either gram positive or gram negative, so does “gram bacteria” just mean “bacteria”? Response: We have revised “gram bacteria” to “bacteria”.

(3).-p. 8 line 10: Assuming “F/B ratio” is fungal/bacteria ratio; define in line 5 if so. Response: Revised as recommended.

(4).-Table 1: For ease of reading, include brief descriptions of treatments in the table legend. While they are defined in the text, it is a lot of acronyms to keep straight, and a one sentence reminder in the legend will make this table much clearer. Response: Revised as recommended.

(5).-Figure 2,3,4: In the legends, make a note about what the lowercase letters mean (as is done for Table 1 legend, for instance) so a reader glancing only at one specific figure will know what these letters represent. Response: Revised as recommended.

Please also note the supplement to this comment:
http://www.biogeosciences-discuss.net/12/C4352/2015/bgd-12-C4352-2015-supplement.zip

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