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> Interactive Comment

Interactive comment on "Stable soil organic carbon is positively linked to microbial-derived compounds in four plantations of subtropical China" *by* H. Wang et al.

H. Wang et al.

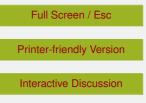
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Received and published: 27 January 2014

Interactive comment on "Stable soil organic carbon is positively linked to microbialderived compounds in four plantations of subtropical China" by H. Wang et al.

K. Fujii (Referee) fkazumichi@affrc.go.jp Received and published: 29 November 2013

We thank the reviewer for the critical but very constructive and helpful comments. We have thoroughly revised the manuscript and addressed all comments. The manuscript has been improved considerably and we hope that the revised manuscript has adequately addressed the reviewer's comments. In the following, we respond to all general and specific comments. The manuscript is attached with changes marked.





H. Wang et al. whui1919@gmail.com 27 January 2013

-This paper has dealt with relationships between soil organic matter composition and microbial composition. This is within the scope of Biogeoscience. Major discussions are based on the results of PLFAs and NMR. There are following three serious problems in this paper. 1. Absence of quantitative data and discussion including total soil organic C 2. Overestimation of chloroform-fumigation extracted microbial biomass-C 3. Low fungi/bacteria ratio, or failure to extract fungal PLFAs Regarding the above point (1), without quantitative analyses (discussion including SOC, SON data), the authors cannot discuss the source of SOM or accumulation of stable SOC. Even if there is no correlation between microbial parameters and SOC NMR index, microorganisms are always important precursors and producers of stable SOM, as mentioned by the authors.

Answer: We agree that "even if there is no correlation between microbial parameters and SOC NMR index, microorganisms are always important precursors and producers of stable SOM". We presented and discussed SOC and total N data in an earlier paper published in Ecological Research. In this study, we focused on the relationship between chemical compositions of SOC and plant litter and soil microbial community composition. In the revised manuscript, we added the SOC and total N data in Table 1, and added some discussion about the relationship between SOC, soil total N and other variables (e.g. fine root productivity and C/N ratio, and soil microbial biomass C and N) in the discussion. Please see the revised manuscript Page 13, line 2-5 and Page 13, line 12-16 and as detailed below.

"Soil organic C and soil total N in the 0-10 cm soil layer in the P. massoniana, C. hystrix, M. macclurei and M. laosensis plantations were reported in Wang et al. (2010) and they are also included in Table 1. Soil organic C stock was positively related with fine root production but negatively related with fine root C/N ratio (Wang et al., 2010)."

"There was a weak positive relationship between SOC concentration and soil MBC (R2

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= 0.22, p = 0.069). Soil total N concentration was positively correlated with soil MBN (R2 = 0.62, p < 0.05). In this study, we focused on the relationship between chemical compositions of SOC and plant litter and soil microbial community composition."

-In addition, necromass of microorganisms are more or less decomposed during humification processes. Therefore, similarity or correlation between microbial parameters and SOC NMR index cannot support their direct cause-and-effect link.

Answer: We agree with the reviewer that microbial processes are just one of the major factors affecting SOC composition. Thus, we revised the description of results in the revised manuscript. "We thus suggest that the stable SOC composition would be linked to microbial composition" and "soil microbial processes are one of the major factors affecting stable SOC composition." Please see the revised manuscript Page 2, line 15-16 and Page 17, line 7-8.

-The above point (2) can be a fatal flaw of this study. Soil sampling should be conducted to meet preconditions for analytical methods. Chloroform fumigation technique destructs cell walls of microorganisms, however, it also destructs cell walls of plant materials. This generally leads to overestimation of microbial biomass when samples contain the large amounts of organic materials. Thus, this method is not applicable to soil samples including organic horizons. The high microbial biomass in this study may be related to this problem. Please calculate the percentages of microbial biomass-C relative to SOC to confirm validity of the data.

Answer: we rewrote the description on soil sampling in the revised manuscript. And we compared our MBC data with other data in subtropical forest soils in the literature and calculated the percentages of microbial biomass-C relative to SOC to confirm the validity of our data in the revised manuscript. Please see the revised manuscript Page 8, line 3-8 and Page 13, line 5-12 and as detailed below.

"Mineral soil samples (0-10 cm) were collected in August 2008. The top very thin layer (\sim 1 cm) of the 0-10 cm mineral soil was somewhat contaminated with O horizon ma-

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terial when the thin O horizon material was removed before the mineral soil sampling. This ca. 1 cm O layer was not possible to be separated from the mineral soil. When the soil was thoroughly mixed before analysis, this O horizon material did not markedly increase the organic matter content in the 0-10 cm mineral soil sample."

"We compared our soil MBC data and MBC as a percent of SOC with those reported for subtropical forest soils in Dinghushan and Heshan in southern China. In Dinghushan, soil MBC and SOC was 320-566 mg kg-1 and 37-73 g kg-1, respectively, that resulted in a MBC/SOC ratio of 0.8-0.9 % (Liu et al., 2012). In Heshan, soil MBC and SOC was 190-220 mg kg-1 and 7.5-13.5 g kg-1, respectively, and that resulted in a MBC/SOC ratio of 1.6-2.5 % (Chen et al., 2010). In our study, soil MBC, SOC and MBC/SOC ratio was 270-438 mg kg-1, 26-31 g kg-1, and 0.9-1.4%, respectively. Therefore, soil MBC in this study was within the range reported for sites with similar climatic conditions."

-Regarding the above point (3), F/B ratio is very low in this study. The data are very unique for results from the forest soils. Please compare with the previous data and add explanation. I am afraid that the authors failed to extract or determine fungal PLFAs. In this case, the following discussions of this paper would be meaningless.

Answer: We reviewed recent publications and found that the $18:1\omega$ 9c PLFA fraction was used to calculate fungal biomass (Cusack et al., 2011; Thoms and Gleixner, 2013). Therefore, the ratio of fungi: bacteria was recalculated as the ratio of ($18:2\omega$ 6,9c and $18:1\omega$ 9c) : (gram-positive + gram-negative bacteria) in the revised manuscript. Please see the revised manuscript Page 10, line 9-10 and Page 15, line 20 to Page 16, line 2 and as detailed below.

"We compared our data of the fungi: bacteria PLFAs ratio with other published data in subtropical or tropical forest soils. Fungi: bacteria ratio was 0.16-0.18 in some tropical forest soils (Cusack et al., 2011) and 0.14-0.18 in some subtropical forest soils (Sun et al., 2011). In our study, fungi: bacteria ratio was 0.15-0.25, which is thus within the limits of data reported in the literature."

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-Specific comments: The title is ambiguous. What aspects of "Stable soil organic carbon" are linked to what of "microbial-derived compounds"? Based on the manuscript, chemical composition of stable soil organic carbon is linked to microbial composition.

Answer: We agree with your suggestion. We changed the title to "Chemical composition of soil organic carbon is linked to microbial composition in four plantations in subtropical China". Please see the revised manuscript Page 1, line 1-2.

-P18095 L22-23 Please clarify the definition of "labile". Some of polysaccharides are easily solubilized to monomers (labile to microorganisms), but the others form complexes with aromatic compounds (recalcitrant).

Answer: We rewrote these sentences in the revised manuscript. Please see the revised manuscript Page 3, line 3-12 and as detailed below.

"Chemical recalcitrance maybe an inherent property of organic molecules. For example, alkyl C chains in lipids and aromatic structures in aromatics and phenolics are more recalcitrant (Lorenz et al., 2007). More labile carbohydrates such as cellulose contain abundant O-alkyl groups (Baldock et al., 1992). A higher alkyl C/O-alkyl C ratio indicates a loss of more labile C relative to poor-quality C compounds (Cusack et al., 2011). Recalcitrant lignin-derived aromatics are highly refractory during litter decay (Berg and Meentemeyer, 2002). However, they do not always appear as recalcitrant in soils as initially thought (Kiem and Kögel-Knabner, 2003; Dignac and Rumpel, 2006). Several studies showed that aliphatic compounds often accumulate in the soil, thus contributing to the increased stable soil organic C (SOC) pools (Nierop, 1998; Mikutta et al., 2006; Lorenz et al., 2007)."

-L26 1998 is not recent. 15 years ago. Stable soil organic carbon is not composed only of "aliphatic" compounds.

Answer: We agree with your suggestion. We rewrote this sentence in the revised manuscript. Please see the revised manuscript Page 3, line 4-12 and as detailed

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below.

For example, alkyl C chains in lipids and aromatic structures in aromatics and phenolics are more recalcitrant (Lorenz et al., 2007). More labile carbohydrates such as cellulose contain abundant O-alkyl groups (Baldock et al., 1992). A higher alkyl C/O-alkyl C ratio indicates a loss of more labile C relative to poor-quality C compounds (Cusack et al., 2011). Recalcitrant lignin-derived aromatics are highly refractory during litter decay (Berg and Meentemeyer, 2002). However, they do not always appear as recalcitrant in soils as initially thought (Kiem and Kögel-Knabner, 2003; Dignac and Rumpel, 2006). Several studies showed that aliphatic compounds often accumulate in the soil, thus contributing to the increased stable soil organic C (SOC) pools (Nierop, 1998; Mikutta et al., 2006; Lorenz et al., 2007).

-P18096 L25-27 There appears to be misconceptions regarding the roles of microorganisms in SOM formation. As the authors mentioned, most of SOM is microbially processed compounds. However, this does not always mean that necromass of microorganisms are simply sequestered and that this process accounts for 80% of SOM. The ultimate source of SOM is plant materials and SOM is formed through microbial processing. To state that necromass of microorganisms account for 80% of SOM accumulation, please confirm whether the origin of aromatic compounds in SOM is microorganisms or not.

Answer: We have deleted this citation in the revised manuscript. Please see the revised manuscript Page 4, line 12.

-P18098 L5 Revise "controlling factors driving" to "factors controlling".

Answer: We changed "controlling factors driving" to "factors controlling" in the revised manuscript. Please see the revised manuscript Page 5, line 21.

-P18100 L26-27 "The organic horizons were not separately analyzed:." This is a fatal flaw of this study. Soil sampling should be conducted to meet preconditions for analyt-

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ical methods. Chloroform fumigation technique destructs cell walls of microorganisms, however, it also destructs cell walls of plant materials. This generally leads to overestimation of microbial biomass when samples contain plant materials. Thus, this method is not applicable to soil samples including organic horizons.

Answer: We rewrote the description of the soil sampling in the revised manuscript. The soil studied was mineral soil samples, not including organic horizons. We also compared our MBC data with other data in subtropical forest soils in the literature and calculated microbial biomass-C as a percentage of SOC to confirm the validity of the data in the revised manuscript. Please see the revised manuscript Page 8, line 3-10 and Page 13, line 5-12 and as detailed below.

"Mineral soil samples (0-10 cm) were collected in August 2008. The top very thin layer (\sim 1 cm) of the 0-10 cm mineral soil was somewhat contaminated with O horizon material when the thin O horizon material was removed before the mineral soil sampling. This ca. 1 cm O layer was not possible to be separated from the mineral soil. When the soil was thoroughly mixed before analysis, this O horizon material did not markedly increase the organic matter content in the 0-10 cm mineral soil sample." And "Soil samples were passed through a 2 mm sieve to remove plant roots and gravel."

"We compared our soil MBC data and MBC as a percent of SOC with those reported for subtropical forest soils in Dinghushan and Heshan in southern China. In Dinghushan, soil MBC and SOC was 320-566 mg kg-1 and 37-73 g kg-1, respectively, that resulted in a MBC/SOC ratio of 0.8-0.9 % (Liu et al., 2012). In Heshan, soil MBC and SOC was 190-220 mg kg-1 and 7.5-13.5 g kg-1, respectively, and that resulted in a MBC/SOC ratio of 1.6-2.5 % (Chen et al., 2010). In our study, soil MBC, SOC and MBC/SOC ratio was 270-438 mg kg-1, 26-31 g kg-1, and 0.9-1.4%, respectively. Therefore, soil MBC in this study was within the range reported for sites with similar climatic conditions."

-P18100 L20-21 The authors specified "Fe3+" and "Mn2+". Did you confirm that the Fe and Mn status of the soil are "Fe3+" and "Mn2+", not Fe2+ or Mn4+?

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Answer: We agree with your suggestion, and we changed "Fe3+" and "Mn2+" to Fe and Mn in the revised manuscript. Please see the revised manuscript Page 9, line 3.

-P18103 L23 What is "stable SOC content"? Please define.

Answer: we agree with your good suggestion, and we have changed "stable SOC content" to "soil alkyl content or alkyl C/O-alkyl C ratio". Please see the revised manuscript Page 12, line 13.

-P18104 L9-10 There is no discussion on concentrations of total SOC, although accumulation of stable SOC was discussed. This is one of major defects of this paper.

Answer: We presented and discussed SOC and SON data in our earlier paper published in Ecological Research. In this study, we focused on the relationship between chemical compositions of SOC and plant litter and soil microbial community composition. Thus, we added the SOC and total N data in Table 1, and added some discussion about SOC in the revised manuscript. Please see the revised manuscript Page 13, line 2-5 and Page 13, line 12-16 and as detailed below.

"Soil organic C and soil total N in the 0-10 cm soil layer in the P. massoniana, C. hystrix, M. macclurei and M. laosensis plantations were reported in Wang et al. (2010) and they are also included in Table 1. Soil organic C stock was positively related with fine root production but negatively related with fine root C/N ratio (Wang et al., 2010)."

"There was a weak positive relationship between SOC concentration and soil MBC (R2 = 0.22, p = 0.069). Soil total N concentration was positively correlated with soil MBN (R2 = 0.62, p < 0.05). In this study, we focused on the relationship between chemical compositions of SOC and plant litter and soil microbial community composition."

-L12"from environmental changes" is ambiguous for scientific paper.

Answer: We changed "from environmental changes" to "climate change and disturbance". Please see the revised manuscript Page 13, line 20-21.

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-P18106 L10 "contribution of fungi" to what? Please clarify.

Answer: We revised it to the contribution of fungi to recalcitrant SOC forms was higher than that of bacteria. Please see the revised manuscript Page 16, line 3.

-L11-12 This study has dealt with "fungal abundance" using PLFAs, but not "fungi derived compounds". There is a great leap of logic. Is it possible to use PLFA as an index of "fungi-derived compounds" as well as "fungal abundance"? For this, PLFAs should not be degraded during humification process.

Answer: We changed "fungi derived compounds" to "fungal abundance" in the revised manuscript. Please see the revised manuscript Page 16, line 6.

-P18107 L12 "determining the stable SOC pool" was not dealt with in this study. There is a great leap of logic. The authors investigated the composition of SOC, not pool size of stable SOC.

Answer: We revised it to "soil microbial processes were one of the major factors affecting stable SOC composition" Please see the revised manuscript Page 17, line 7-8.

-I hope these comments will be helpful for improvement of the manuscript. Forestry and Forest Products Research Institute Kazumichi Fujii

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Please also note the supplement to this comment: http://www.biogeosciences-discuss.net/10/C8244/2014/bgd-10-C8244-201410, C8244–C8254, 2014

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