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Interactive comment on "Chlorophyll a specific Δ^{14}C, δ^{13}C and δ^{15}N values in stream periphyton: implications for aquatic food web studies" *by* N. F. Ishikawa et al.

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Dear the editors and referees,

We are grateful to the constructive comments from three anonymous referees on our paper. We also thank the associate editor Dr. Tom J. Battin for handling the manuscript. Below we responded to each of the referees' comments and described how we revised the manuscript. The numbers of page and line (e.g., P10L23) in our response are for the revised manuscript (please see also supplement file, the revised sentences are highlighted). We believe that the revised manuscript has been greatly improved in accordance with the referees' valuable suggestions. In case we disagree with a

specific recommendation, further explanations supporting our approach were made.

Sincerely,

Naoto F. Ishikawa

Responses to the comments from Referee #3

(RC: Referee comment; AC: Author comment)

(RC) This study investigated the chlorophyll-a specific isotopic compositions in stream periphyton to examine whether the bulk isotopic compositions of periphyton could be used as representative of aquatic producers. The results showed that periphyton chlorophylla exhibited 13C and 14C values similar to the bulk tissue, but had higher 15N value than the bulk sample. The difference in 15N value between chlorophyll-a and bulk sample was attributed to N isotopic fractionation during chlorophyll-a biosynthesis and incorporation of cyanobacteria tissue into periphyton. Because of the novelty of measurement on chlorophyll-a specific isotopic compositions of 13C, 14C and 15N of stream periphyton, I would like to recommend this manuscript for Biogeosciences. However, I think that there are some issues to be addressed before final publication.

(AC) Thank you for your valuable comments. Please see our responses to your comments below.

(RC) For example, the authors calculated the relative contribution of algal carbon and terrestrial organic carbon to periphyton based on 14C values of bulk periphyton, chlorophyll a, and terrestrial plant for each season (April and October). They concluded that the periphyton consisted of 89 – 95 % algal carbon. I wonder if this is a meaningful and reliable calculation. The algal portion of periphyton should consist of both alive and dead (aged) algal tissues. Further, 14C value in periphyton chlorophyll-a changed largely (ca. 60 permil) differed between April and October. Therefore, I suppose that the difference in 14C values of bulk periphyton and chlorophyll-a could be accounted for not only by terrestrial organic carbon incorporation but also by the seasonal variation in

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14C of chlorophyll-a. Actually, Cladophora sp., the aquatic primary producer, also presented a difference (ca. 10 permil) in 14C between bulk periphyton and chlorophyll-a. The difference is comparable to that in periphyton in October. I think that it would be necessary to consider more carefully about the premise of the calculation.

(AC) Thank you for this comment. Assuming that our April and October data represent seasonal variation, bulk periphyton Δ14C values in April and October can be explained by both seasonal variation in aquatic end member (as indicated by chlorophyll a Δ14C in periphyton) and relative contributions of the aquatic and terrestrial end members to periphyton bulk matrix. As you pointed out, chlorophyll a Δ14C in periphyton in April was largely different from that in October. However, our long-term monitoring indicates that frequent flooding renews benthic environment and causes rapid turnover of algal community in periphyton in this stream. Textbooks in this field (e.g., Allan and Castillo 2007 Stream Ecology) state that turnover of periphytic algae is generally 3-6 weeks. As far as we know, chlorophyll a in April periphyton should not be remained in October periphyton and chlorophyll a Δ14C value for living algae should not be greatly different from that for dead algae. We agree with your comment that the Cladophora sp. Δ14C difference between bulk and chlorophyll a (10‰ is comparable to that in periphyton in October (10‰. However, this result does not indicate that October periphyton is consisted of 100% aquatic carbon because bulk and chlorophyll a Δ14C values for October periphyton are different from those for Cladophora sp. As Δ14C value of terrestrial end member (Q. glauca) is fixed in this study, a separate two-source mixing model should be applied to each of April and October. Based on your comments, two assumptions in our model were added to text to validate our approach and we revised several sentences. Please see P11L9-26.

(RC) Additionally, 14C value of chlorophyll-a of terrestrial plant leaves (-10 permil) was much lower than that of bulk 14C (27 permil). The difference was considered to be because of use of old soil CO2 and soil organic carbon. It should be extremely interesting if the plant can have access to such an old carbon source. The two cited papers (Bloe-

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men et al. and Bruggemann et al.) indeed described the potential importance of these carbon sources for plant production, but these two references did not demonstrate that plants could use such an old carbon for primary production. To my knowledge, most of previous 14C studies have shown that respired soil CO2 and dissolved soil organic carbon have modern carbon. The recycle of phytol was also used to explain the 14C difference between chlorophyll-a and bulk plant leaves. I like this idea but it is difficult to believe that plant reuse such an old phytol to synthesize chlorophyll-a. Please consider presenting more convincing evidence to support the authors' idea.

(AC) We revised this paragraph explaining the differences in Δ14C between bulk and chlorophyll a in Q. glauca. To support our explanation, two references (Trumbore and Zheng 1996; Koarashi et al., 2009) showing that soil organic carbon does not necessarily have modern carbon were added. Furthermore, we discussed that carbon in chlorophyll a molecule may be originated from various sources because its biosynthesis has multiple channels to acquire carbon. Please see P10L23-P11L8.

Minor comments

(RC) P11090: Please consider describing the rationale of this study in the first sentence of Abstract.

(AC) We revised the first two sentences in Abstract as "Periphytic algae attached to a streambed substrate (periphyton) are an important primary producer in stream ecosystems. We determined the isotopic composition of chlorophyll a in periphyton collected from a stream flowing on limestone bedrock in the Seri River, central Japan.". Please see P1L13-15.

(RC) P11090L10, P11098L15: The authors stated that 13C of periphyton do not trace carbon transfer between primary producers and primary consumers. However, the 13C data clearly indicated that the mayfly larva did not subsist on C of periphyton that was investigated. Please clarify what kind of C flow the authors intended to mention.

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(AC) We deleted this statement in both Abstract and section 3.2. This deletion did not influence our conclusion. Please see P1L20 and P8L16.

(RC) P11090L15: mixture of only two sources (carbonates and atmospheric CO2)? What about CO2 derived from aquatic and terrestrial organic matter?

(AC) We added ", CO2 derived from aquatic and terrestrial organic matters (variable Δ14C)" after "weathered carbonates (Δ14C = –1000‰". Please see P1L24.

(RC) P11091L26: Peripyton 14C is "often" derived

(AC) We added "often" after "Periphyton Δ14C is". Please see P2L28.

(RC) P11094L23: washed with H2O after HCl treatment?

(AC) We added "washed and" after "carbonate and were". Please see P5L9.

(RC) P11094L24: when was the periphyton sample collected?

(AC) We added "(November 2008)" after "the same site". Please see P5L10.

(RC) P11095L8: Please describe briefly how to confirm that the product was phaeophytin-a.

(AC) We added "Absorption spectra of our laboratory standards were consistent with those reported in literatures (Chikaraishi et al., 2007; Tyler et al., 2010)." after "chlorophyll a standard.". Please see P5L22-23.

(RC) P11096: Please add more explanations about how to transfer the dried chlorophyll-a samples to tin capsules for 13C and 15N and quartz tubes for 14C measurement.

(AC) We added "The dried chlorophyll a and phaeophytin a were dissolved in dichloromethane and transferred to tin capsules for δ13C and δ15N measurements or to quartz tubes for Δ14C measurements. The tin capsules and quartz tubes were dried again prior to measurements." as the last sentence of section 2.2. Please see

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P6L20-22.

(RC) P11099L5: proxy for “13C” of bulk algae.

(AC) We added “δ13C of” after “reliable proxy for”. Please see P8L30.

(RC) P11101L3: It is a great idea. But are there any studies demonstrating that an algae can collect phytol from DOC or POC?

(AC) We deleted phytol recycling mechanisms due to the lack of convincing evidence and revised the sentences as “Secondly, heterotrophs such as fungi and bacteria in periphyton community consume ambient DOC and release CO2 during their respiration (Fischer 2003). The CO2 derived from heterotrophic respiration of DOC may be another 14C-depleted carbon source that is utilized by periphytic algae for photosynthesis.”. Please see P10L19-22.

(RC) Fig.1 and 2.: Please indicate what the error bars stand for.

(AC) We added “Error bars indicate standard deviation (N = 4).” in legends of Figures. 1 and 2. Please see P22L7 and P23L6.

End of responses to the comments from Referee #3

Please also note the supplement to this comment:
http://www.biogeosciences-discuss.net/12/C6284/2015/bgd-12-C6284-2015-supplement.pdf

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Interactive comment on Biogeosciences Discuss., 12, 11089, 2015.