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

Anonymous Referee #3

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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 chlorophyll-a 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.

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 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.

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 (Bloemen 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.

Minor comments P11090: Please consider describing the rationale of this study in the first sentence of Abstract. P11090L10, P11098L15: The authors stated that 13C of periphyton do not trace carbon transfer between primary producers and primary con-

sumers. 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. P11090L15: mixture of only two sources (carbonates and atmospheric CO2)? What about CO2 derived from aquatic and terrestrial organic matter? P11091L26: Peripyton 14C is “often” derived P11094L23: washed with H2O after HCl treatment? P11094L24: when was the periphyton sample collected? P11095L8: Please describe briefly how to confirm that the product was phaeophytin-a. 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. P11099L5: proxy for “13C” of bulk algae. P11101L3: It is a great idea. But are there any studies demonstrating that an algae can collect phytol from DOC or POC? Fig.1 and 2.: Please indicate what the error bars stand for.

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