Biogeosciences Discuss., 8, C22–C26, 2011
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Biogeosciences Discussions

Interactive comment on "Carbon isotope discrimination during litter decomposition can be explained by selective use of substrate with differing delta;¹³C" *by* J. Ngao and M. F. Cotrufo

Anonymous Referee #1

Received and published: 24 January 2011

The study tries to reveal mechanisms of changes of d13C during long term incubation of plant residues under controlled conditions, and so to evaluate the processes of isotopic discrimination. This is very important topic as many studies are based on application of various 13C natural abundance approaches, and the isotopic discrimination is commonly considered, but mechanisms remains not really known. Therefore, I think that the study may have high relevance. Despite the study climes to reveal mechanisms, the most description remains as possible mechanisms, as they are not rigorously proven by the conducted experiments. Before resubmission, the isotopic

discrimination by cellulose extraction should be proven, presented and considered by calculations. Additionally, many details of the incubation experiment are lacking. Therefore, I suggest major revision.

General comments - Abstract: it is not clear how it is possible that the isotopic discrimination varied between -2 and 0 (L10) and this was described well (L11), but it was necessary to consider discrimination of -1 to -4.6. As to me this is just adjustment of the model to the data with approaches which are not proven experimentally. The Abstract should be written more clearly. - One important shortcoming of the study that the authors described kinetic fractionation generally, means independent on time. However, when the reaction is finished the kinetic fractionation = 0 (here I accept that it is one reactant – plant residues, and one product – CO2). So, the time course of reaction should be considered and all presentations and discussions related to kinetic fractionation should be time related. - The experimental design is not clear from Sections 2.1. and 2.2. How many treatments were used? How frequently CO2 was sampled? Which controls/references were used? How was the fractionation by chemical extraction of cellulose tested? - During the extraction of cellulose from litter and its preparation for d13C measurement 13C discrimination is very probable. This is common by the most extraction and fractionation approaches. It is not presented anywhere, how strong was this discrimination. - The study was done with litter alone, means without soil. I guess that many processes including litter decomposition would be different (rates and directions of fluxes) in the presence of soil. As well as I understood, no control with soil was considered in experimental design. - In section 2.6. Statistical Analyses it should be mentioned how many variables were fitted in total, and how many measurements were done. As to me it looks suspicious if a three pool model will be fitted – means 6 parameters and if each pool has own isotopic fractionation – additionally at least 3 parameters of isotopic discrimination (may be some other; depending on the controls and references) are fitted. So, what is the interdependence of the many parameters by the fitting? - I think that the authors are not aware about the different meaning between ‘simulation’ and ‘fitting’. I have not find any simulations in the ms. All the lines

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calculated are based on a simple fitting of exponential curves on the measured points. This means fitting. - When the authors try to separate two processes, it should be clarified in the whole text, where they really measured discrimination, where apparent discrimination and where different utilization. - 3.3.2. 'Model simulations' It is obvious that fitting the 3 component curve on such a dynamics will lead to very good correspondence between measured and fitted data. However, the disagreement between measured and fitter d13C values clearly shows that the suggested 3 pool model does not well properly. - It was necessary to explain the isotopic composition of CO2 by addition of isotopic discrimination to the fast and slow pools. Is it possible to get the same results by addition of isotopic discrimination to the intermediate pool only? If the intermediate pool contribute at the start and at the end to the CO2, then it should be possible. - The background of the paper is based on the model 3 pools decomposing exponentially; and the authors assume that this is the only possibility. However, it is possible that decomposition don't follow the classical 1st order kinetics. Microorganisms decomposing the litter are completely disregarded by the 3 pool approach. It is obvious that the described isotopic discrimination between the litter and CO2 occurs not in one step, but with the 'help' of microorganisms. Therefore, the isotopic discrimination may follow other rules as suggested by the 3 pools model. - There are too many assumptions for the model (number of pools, their d13C values, etc.). As the authors have not proven it experimentally, the relevance of these assumptions is very weak; with consequences for conclusions of the study. - The Discussion should be split in 2-3 sections each with clear focus. May be these sections should be focuses on the 3 fractionation possibilities suggested in the last section. - Generally I agree with the three possibilities for d13C changes during incubation (P18). However, in my view not any of these possibilities were rigorously proven in the study.

Other remarks P2 L2 Decomposition in soil or without soil? L7 during which period? L8 probably fitted instead of simulated L8 the isotopic composition cannot be higher; the d13C value can be higher P3 L1-5 these 3 options are only a small part of the possible isotopic applications in soil C studies. So, it should be deleted or the really whole range

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should be presented. P3 Usually isotopic discrimination is considered by linear mixing models by correction of values of the end members. This will be done in the most studies. P4 top It should be considered however, that if the processes are complete (decomposition is finished) there is no discrimination. So, the further conclusions are relevant only for partial decomposition. P4 L11 and L13 . . . delete heterotrophic. The respiration is all times heterotrophic; therefore, it is not correct to link respiration and heterotrophic in one phrase. Correct at all places in text P5 L8 It cannot be different at any time: If the mass balance low is correct (and this is a fundamental rule) then it should be at least one time period during decomposition when d13C of litter is = d13C of CO2. P5 L18 further P6 L8 this is not clear: if the moisture is > 100% of gravimetric content, then it will be a puddle of water at the bottom of the jar. P8 Section 2.4. P8 L21 what are C6 and C8? P9 L11 not clear what are the 10 variables? This was not mentioned before. P10 L17 ANOVA analyses sounds not well P11 L4 How the incubation days under controlled conditions were recalculated for years? P11 L19 As well as I understood, the incubation was in closed jars. How leaching can occur is not clear. P11 L19 What kind of two sets? It was nothing written in M&M about. P14 L4 here and at other places: please use depleted (or enriched) when the product is compared to the substrate. Not on the reverse way. P17 L24 This is surely not correct. There were studies reported d13C changes of CO2 during incubation of plant residues, and this by incubation with soil. Also the other studies already referred in the ms, should be analyzed more carefully.

Fig 3 About one third of the points show the deviation from the 1:1 line more than 10%. It means that the measurement error is comparatively high. How to explain it? Are the points means of 5 replicates or individual measurements? Fig 4 Cellulose content where? The temporal variation of isotopic content is not presented here, as there are only 2 points. Fig 4 The change of d13C of cellulose is about 2 ‰ when about 25-30% of cellulose was decomposed. It means that the d13C of endmembers in the cellulose (I am not sure what it can be), is about 6-8‰. Is it possible? I think it is impossible. Fig 5 Start with the upper panel. The error bars of the difference should be

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calculated and presented. Fig 6 The regression line here depends on two points only. All other points are clumped together and no trend in obvious there. How the R2 was calculated: based on means or on individual replications? Fig 7 It is not clear how the discrimination factors were estimated.

Interactive comment on Biogeosciences Discuss., 8, 51, 2011.