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***Interactive comment on “Application of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  isotopic signatures of organic matter fractions sequentially separated from adjacent arable and forest soils to identify carbon stabilization mechanisms” by Z. E. Kayler et al.***

**Anonymous Referee #2**

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General comment

In this paper, the authors aimed to gain insights into the level of microbial transformation of stable SOM and its type of interaction with minerals. To do so, they explored the natural abundances in  $^{13}\text{C}$  and  $^{15}\text{N}$  from a range of soils under arable and forest land use, after isolation of the fraction assumed to contained the stable OM by

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removal of particulate OM and water extractable OM. In this fraction they separated OM extractable by Na-pyrophosphate from OM non extractable by Na-pyrophosphate as they expected different stabilisation mechanisms to operate in these two fractions. They treated their data with partial least square regression and interpreted their results using a model for OM interaction with mineral surfaces.

The approach of the authors is quite interesting. The paper is very well written and easy to follow. However, the dataset is not really appropriate to reach the goal of the authors. Indeed their initial assumption is that stable isotopes can be used to determine the fractions that are microbially processed, which is not exact. Neither enrichment in  $^{13}\text{C}$  or in  $^{15}\text{N}$  can alone indicate a microbial transformation of OM.  $^{13}\text{C}$  enrichment could reflect other processes such as the Suess effect (as mentioned by the authors), or an enrichment in molecules such as pectin (Glaser, 2005), whereas various  $^{15}\text{N}$  enrichment may reveal different patterns for nitrogen mineralisation (see for example *Stable Isotope in Ecology and Environmental Science* for a synthesis or the pioneer studies of Mariotti for  $^{15}\text{N}$ ). To conclude on the presence of microbial processed OM by the use of isotopes, both  $^{13}\text{C}$  and  $^{15}\text{N}$  must show the same trend, possibly confirmed by other proxies, such as the C:N ratio for example.

But of more concern is the interpretation of isotope trends in term of microbial processing for arable lands having being submitted to fertilizers input and crop rotation (Kaiser et al., 2011). A maize cropping in the rotation may strongly impact the  $\delta^{13}\text{C}$  value, whereas fertilizers exhibit a broad range of  $^{15}\text{N}$  composition depending on their nature (manure,  $\text{NH}_4\text{...}$ ) and impact the soil  $\delta^{15}\text{N}$  for decades, even centuries.

Taking this major issue into account, the paper is not currently acceptable for publication. As their approach is very interesting, I strongly encourage the authors to focus their study on forest sites, maybe adding a couple of sites for a more robust analysis. I recommend to complete their dataset with C:N ratio, and if possible some molecular characterisation of the fractions so as to confirm the microbial trend they suspect. I also recommend to not over interpret their data. In my opinion, the current dataset is

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too weak to provide any evidence or to contradict the model developed by Kleber et al., 2007.

Other specific comments:

### 1. Abstract

Some sentences in the abstract are a bit disconnected from the manuscript (eg p1986, l.17: undisturbed soil - l.22 pooled OM fractions) p1986, l.18: “The d15N signature of OM fractions served as a reliable indicator for microbial processed carbon in both arable and forest land use types” - It does for organic matter rather than for C; not really demonstrated in the manuscript.

### 2. Introduction:

p1987, l.10: C storage would indeed increase when MRT increases but also if the input increases. p1988, l.7: C3C4 experiments are design to interpret 13C composition in term of MRT. 15N analyses are complementary analyses, possibly used to infer mechanisms, but not to determine MRT. Huygens et al., 2008: Many others before him provide evidences of microbial processing of OM in microaggregate. It would be nice to cite some of them.

### 3. Material & Methods:

Step 1: A bit more details would be useful

Step 2: Not clear to me whether the data you present are from the Na-Py extract after particle removal and water extraction or after particle removal, water extraction and HCl extraction

PLS: very well explained.

### 4. Results and Discussion:

As previously mentioned: impossible to conclude anything with the current dataset.

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Remove the data from arable land sites

PLS: Why don't you show the 2 first components to demonstrate they are related to particle-size? I do not know anything about PLS, but as I follow your explanation, I understand that the 3rd component is orthogonal to the two first ones, and should allow investigating other relationships than particle-size. However, Fig 6 and 7 indicate a contribution of clay, silt and sand for component #3.

Is the patchy distribution of OM on mineral compatible with the zonal model? If you focus your interpretation on a molecular model, then try to get some data on the molecular nature of your fractions. The difference in isotopic composition between distinct specific compounds can be larger than the one related to microbial fractionation Outline inconsistent in section 4.

5. Tables and Figures:

Fig 1: Be consistent in the names in Fig 1 and Tab 3.

Fig 1: HCf is lacking.

Fig 2, 3, 4: Better use the histogram representation for quantity or proportion. For values, prefer dotplot.

Combine some figures, for example fig 2 and 3

Fig 4: Not clear to me what it is: forest, arable, both combined?

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Interactive comment on Biogeosciences Discuss., 8, 1985, 2011.

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