

## ***Interactive comment on “Millennial-age GDGTs in forested mineral soils: $^{14}\text{C}$ -based evidence for stabilization of microbial necromass” by Hannah Gies et al.***

**Hannah Gies et al.**

[hannah.gies@erdw.ethz.ch](mailto:hannah.gies@erdw.ethz.ch)

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We thank Referee #1 for their constructive comments and have implemented them to improve our manuscript. The Referee points out that they would like more discussion of potential causes for the differences in compound-specific radiocarbon values between the two sites and that the implications of the  $\text{MBT}'_{5\text{ME}}$  and  $\text{CBT}'$  indices are not sufficiently discussed. We agree that the referee's remarks are justified and addressed their suggestions as follows:

**Referee: The results from the individual sites are insufficiently discussed. For example, regarding Fig. 5, there is only one reference line for the turnover time**

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for bulk SOM even though your study features 2 separate sites. Both GDGT and additional data from Van der Voort et al. (2007) partially differ between the sites. Consider showing individual reference lines for bulk SOM and discuss how the differences between data from both sites do or do not affect your overall conclusion.

**Authors' response:** The dotted line is the 1:1 line for bulk  $^{14}\text{C}$  on the x-axis and compound  $^{14}\text{C}$  on the y-axis, thus the line is the same for both sites, which are distinguishable by the filled and open symbols. A "1:1" label was added to Figure 5 to avoid confusion. We agree that the discussion would benefit from elaborating on the differences between both sites, and have hence modified the paragraph at the end of section 4.2:

*"[. . .] The older GDGT  $^{14}\text{C}$  age in the lowest depth interval of the Cambisol at Lausanne compared to the subalpine Podzol at Beatenberg with a bleached eluvial horizon also supports this conclusion. The Lausanne soil has higher contents of clay and highly reactive amorphous Fe and Al-oxides and hydroxides (Table 2) which are known to play a key role in the sorptive stabilization of SOM (Kaiser and Guggenberger 2003; Kleber et al., 2007). The  $^{14}\text{C}$  signatures of GDGTs are similar in the top 20 cm at both locations (Figure 4), however, in the Lausanne soil, the alkanes and fatty acids are less depleted in  $^{14}\text{C}$  compared to Beatenberg resulting in an offset between GDGTs and the plant-derived compounds. One explanation could be the different thicknesses of the organic layer at the two sites (Walthert et al., 2003.) The 20 cm thick organic layer at Beatenberg retards the inputs of plant-derived C into the mineral soil and thus leads to longer turnover times of bulk OC in the topsoil compared to Lausanne, where the organic layer is only 2 to 3 cm thick. Contrary to the plant wax components, the turnover of isoGDGTs and brGDGTs does not seem to be affected by the thickness of the organic layer, resulting in the greater age offset observed in the Lausanne soil."*

**Referee:** There is no explanation of the MBT' $_{5ME}$  and the CBT' ratio. The results for the ratios are currently not discussed and do not contribute to the overall

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## conclusion.

**Authors' response:** The primary focus of this manuscript is on the use of GDGTs as tracers of soil microbial bio-/necro-mass, rather than the proxy information residing in their molecular distributions. Nevertheless, we agree that the implications of the MBT'<sub>5ME</sub> and CBT' ratio should be discussed in more detail. The equations how to calculate the indices are added to Figure A1, additionally we have expanded the paragraph at the end of section 3.2:

*“Changes in the relative abundance of the individual brGDGTs (Figure A1) are reflected in the Methylation of Branched Tetraethers (MBT'<sub>5ME</sub>) index and in the Cyclisation of Branched Tetraethers (CBT') index, with these parameters increasing with higher proportions of methyl groups and cyclopentane moieties, respectively, in the brGDGT structures (de Jonge et al. 2014). In the Lausanne soil, the MBT'<sub>5ME</sub> is largely invariant with depth, while the CBT' increases from -1.59 to -0.93 (Figure 3), indicating a higher proportion of GDGTs with cyclopentane moieties in the subsoil. In the Beatenberg soil, a more modest increase of the CBT' index is evident, with a change from -1.46 to -1.38, while the MBT'<sub>5ME</sub> decreases slightly from 0.63 to 0.58.”*

The implications of these results are further discussed in a paragraph in section 4.3:

*“The relative abundance of different brGDGTs as expressed in the MBT'<sub>5ME</sub> and CBT' index values correlates with MAT and soil pH (Weijers et al., 2007). The changing proportions of the individual brGDGTs reflected in both soils in the increasing CBT' index and, in the Beatenberg soil, decreasing MBT'<sub>5ME</sub> index with depth correspond to a pH change from 4.6 to 5.7 in Lausanne and 4.8 to 5 in Beatenberg, while in the latter the reconstructed MAT decreases from 10.9°C to 9.5°C. In Beatenberg, these reconstructed values do not match the measured pH change from 3.7 to 4.4 with depth, or reflect the MAT of 4.6°C. Nevertheless, the direction of the changes, the increase of pH and the decrease of temperature with depth, is echoed in the relative abundance of GDGTs. In Lausanne, however, the brGDGT-based increase in pH is not observed*

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*in the soil values, with measured pH remaining largely invariant (4.6 to 4.5) throughout the profile. Therefore, the significant increase of the CBT' index and hence the higher relative proportion of brGDGTs with cyclopentane moieties might reflect preferential association of these compounds to mineral surfaces compared to those without cyclisation. Further work is needed to ascertain whether some GDGT structures are more prone to protection by mineral association than others, as a change in relative abundance of brGDGTs with time due to different turnover of individual GDGTs would need to be considered when using brGDGTs to reconstruct environmental conditions."*

### **Additional minor suggestions from the Referee:**

**Line 97: How exactly was the pooling performed? What compounds were pooled together?**

**Authors' response:** Instead of separating individual molecules, e.g., GDGT-0, GDGT-1, GDGT-2 etc. (see figure A1), we used a single time window for the collection of isoGDGTs and brGDGTs respectively. In this way, the molecules are pooled on the compound class level.

line 95-97 was changed to *"As sampling and extracting several kg of material is impractical, we did not attempt to isolate individual GDGT compounds (Figure A1), but focused instead on pooled compound class-level isolation and <sup>14</sup>C measurement of isoprenoid GDGTs and of branched GDGTs, respectively, based on the premise that there are common putative biological precursors and biosynthetic formation pathways for each compound class (Schouten et al., 2013)"*

**All typing errors, formatting suggestions and bulky sentences highlighted by the referee have been addressed.**

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