## Report #1

Suggestions for revision or reasons for rejection (will be published if the paper is accepted for final publication).

Overall, the authors addressed the main issues, but in a few cases I still feel some more clarity should be added, this should not be much work, see below for details.

Dear reviewer, thank you very much for appreciating our revisions of the manuscript based on your constructive comments. In addition, we are grateful to you for clearly pointing out the few areas that require more clarification in order to further improve the manuscript. In the following specific sections, we pay attention to address those concerns. The corrections are marked in the manuscript using tracked changes.

**Revised version, L 146-147:** "For measurement of  $\delta^{13}$ C values, the standard used was VPDB (Vienna Pee Dee Belemnite), while  $\delta^{15}$ N values were measured in reference to air": these are just the references values to define the scale – mention which actual standards you measured to calibrate the data.

**Author reply:** We realized that the description of the isotopic data was inadequate, and calibration information was missing, thank you for this remark. We reformulated as follows:

1) On the EA-IRMS measurements we reformulated as:

"The  $\delta^{13}$ C and  $\delta^{15}$ N values reported were normalized on the Vienna Pee Dee Belemnite (VPDB) and AIR scales using USGS90-milet flour (accepted  $\delta^{13}$ C and  $\delta^{15}$ N: -13.75 ± 0.06 ‰ vs. VPDB and +8.84 ‰ ± 0.17 vs. AIR, respectively) and USGS91-rice flour (accepted  $\delta^{13}$ C and  $\delta^{15}$ N: -28.28 ± 0.08 ‰ vs. VPDB and +1.78 ± 0.12 ‰ vs. AIR, respectively), High Organic Content Soil – 162517 (-26.27 ± 0.15 ‰ vs. VPDB and + 4.42 ± 0.29 ‰ vs. AIR, calibrated toward IAEA-CH-6 and IAEA-N-1 by Elemental Microanalysis Ltd) was used for quality analysis (QA). Standard deviation on replicate analyses of QA was better than 0.2 ‰ for both <sup>13</sup>C and <sup>15</sup>N, and deviation from certified value better than 0.2 and 0.3 ‰ for <sup>13</sup>C and <sup>15</sup>N respectively." (See line 118-125).

 <u>Thanks to your remark we realized that normalization of the <sup>13</sup>C measurement in CO<sub>2</sub></u> using a laser based instrument was also not mentioned, therefore we added to that <u>section:</u>

<u>"…</u>analysed using Cavity Ring-Down Spectrometer, (G2113-I, CRDS CO<sub>2</sub> analyser, Picarro, United States, normalization toward VPDB scale was done using a dilution in zero air of a 5 % CO<sub>2</sub> ref gas calibrated by ISO ANALYTICAL toward IA-CO2 -3 ( $\delta^{13}C = -33.68$  ‰ vs. VPDB) traceable to NBS-19) at starting condition."(See line 162-163).

3) We also noted some discrepancies in the formulation of the equations 1) and 2) which were corrected.

**Original comment: section 2.3:** mention how you coped with removing a 45 mL gas sample from your incubation jars: was this volume replaced with air while taking these samples, if not how was the pressure difference accounted for in your measurements?

**Author reply:** We agree that this is needed. [...] While for laboratory incubation, headspace gas sampling for the determination of soil CO<sub>2</sub> fluxes was done twice, directly at the beginning before fully closing the jar and at the end of the incubation period. For gas sampling a gas tight syringe (45 mL) was used, which was immediately gas-tightly closed after taking the gas sample. Gas samples were analyzed immediately using Cavity Ring-Down Spectrometer, (G2113-I, CRDS CO2 analyser, Picarro, UnitedStates). Thus, during incubation the pressure in the jar was not affected. Moreover, immediately after the second sampling, the jars were opened and only covered with parafilm to equilibrate until the following day.

**New comment:** This does not really answer the question – when you take 45 mL out of the incubation jar with a gas-tight syringe, you expand the volume hence the pressure decreases. Did you correct the data for the increase in volume, or did you replace the headspace volume while sampling.

Thank you for this inquiry. As we mentioned in the methodology section 2.3, the first sample was taken before closing the jar, so no problem of pressure change here. The second sample was taken at the end of the incubation in which during the withdrawal of the sample, the pressure will drop indeed, however, once the syringe is filled with 45mL of headspace, a valve on the syringe is closed so the lower pressure in the syringe will not induce an aspiration of lab air into the sample (actually the pressure in the syringe will immediately go back to atmospheric pressure by free movement of the plunger). As both Air  $(N_2/O_2)$  and CO<sub>2</sub> can be considered as ideal gasses they will expand in the same manner, and the change in pressure during the sampling will thus not affect the concentration (the measurement by the CRDS is based on IR absorption of CO<sub>2</sub> isotopologues present in a measuring cell at a fixed 'low' pressure and is thus only dependent on the concentration and not on the amount present in the syringe (the latter would be the case for a GC or a gas bench measurement). After the sample was taken, the jar was immediately reopened, and kept open (covered with a parafilm) until the next measurement moment. The lowering of the pressure in the headspace during the sampling could induce a certain outgassing of the soil however, seeing the very short time of sampling (couple of seconds) and the limited pressure drop (i.e. c.a. 4.5 %) we are convinced that this can easily be ignored.

**Original comment:** -L403-404: the data presented in Figure 4f show a surprisingly large difference in  $\delta^{13}$ C values, albeit with relatively high standard deviations. It would be good to provide statistics for this: for which elevations are these differences significant or not ? Again, I assume that for the high elevation sites, they are not statistically different – which should not be a surprise, as you have very organic soils here for which you would need to have a very high turnover rate to see any differences in  $\delta^{13}$ C of the SOC pool after 2 years. You could likely do some back-of-the envelope calculations here.

**Author reply:** Thank you for this insight. We agree with this comment. As mentioned earlier, L402-403 and L484, there were no significant differences between control and warmed samples

for a given elevation, while using linear mixed effect model regression analysis across entire elevational gradient allowed to show a trend between warmed and control treatments. As such we agree to clarify in the new version that "the remaining SOC tended to be more enriched in <sup>13</sup>C in warmed than in control, and the SOC tended to be lower in warmed than in control treatment".

We agree to also add the statistics for SOC and  $\delta^{13}$ C in SI, Table 3 along with the slopes and intercepts of the linear mixed effect model regression fit in the new version of the manuscript.

**New comment:** From the statistics, it appears none of these differences are significant, although some close to. While this is acknowledged in the text, it is still phrased as "Finally, the  $\delta^{13}$ C values of the SOC showed that warmed soil became relatively more enriched in  $^{13}$ C as compared to control soil (Figure 4(f))." – that sort of statement suggests that your results are robust and significant, which they are not. Again, a quick back-of-the-envelope calculation could give you an indication of the turn over required to see the changes in d<sup>13</sup>C-SOC you report.

Thank you for this observation. We have now revised the statement to consistently state that there is only a trend but no significant difference.

i.e. "Finally, though not statistically significant, the  $\delta^{13}C$  values of the SOC showed a trend that warmed soil tended to become relatively more enriched in  $^{13}C$  as compared to control soil (Figure 4(f))."

Further, taking example of two elevation clusters at 1750-1850 and 2500-2600 m a.s.l, we applied the Rayleigh equation to estimate amount of SOC that should be respired for the observed changes in  $\delta^{13}C$  signature.

Fraction of SOC remaining =  $(\delta^{13}C \text{ warmed } + 1000) / (\delta^{13}C \text{ control } + 1000)^{(1/(alpha-1))}$ 

*Therefore, the fraction of SOC respired = 1-( fraction of SOC remaining).* 

From this we saw that at 1750-1850 m a.s.l, up to ca. 44 % of SOC is needed to be respired in the two years of warming, while up to 81 % SOC is needed to be respired at 2500-2600 m a.s.l. The high SOC combined with low fractionation at the higher elevation (2500-2600 m a.sl), would require a high rate of SOC loss in order to observe changes  $\delta^{13}$ C. On the other hand, at the lower elevation where SOC content was lower and there is higher fractionation, a relatively smaller fraction is needed to be respired to observe the changes.

Nonetheless, we agree with you that these seem indeed very high turnover. The high turnover estimates may be a result of yet unexplained error.

We did not add these back-of-the envelope calculation to the MS as not to over-reach our available data.

**Original comment:** Throughout the manuscript, the terminology related to stable isotopes is really not OK. For example, the authors refer to "the <sup>13</sup>C depletion factor" or "isotopic depletion factor" (L167) – that is not an accepted term in the literature, what you are referring to is termed fractionation (epsilon).

## **New comment:** SI, Table 3 still mentions " $\delta^{13}$ C depletion factor »

Thank you for this observation. We have corrected this to  ${}^{13}C$  isotopic fractionation during heterotrophic  $CO_2$  respiration (epsilon).

**Comments on Discussion, section 4.1.** I appreciate the changes made in the Discussion here; however given the non-significant differences I really would phrase this more cautiously. The text currently reads:

"Generally, after two years of in situ warming,  $\delta^{13}$ C values of SOC revealed a relative enrichment in <sup>13</sup>C in warmed soil as compared to the control (Figure 4(f)). This is consistent with the observation of <sup>13</sup>C depleted C losses during microbial CO<sub>2</sub> respiration (Figure 3(c). The relative enrichment in <sup>13</sup>C in warmed soil as compared to the control is likely due to enhanced mineralisation rates in the warmed soil. Higher mineralisation causes a change in <sup>13</sup>C fractionation due to change in C substrate(following depletion of most labile C) and/or microbial discrimination against <sup>13</sup>C during C transformation processes (Andrews et al., 2000; Ehleringer et al., 2000;Natelhoffer and Fry, 1988)."

-results on  $\delta^{13}$ C in warming vs control were not significant – I agree there is a tendency, but phrase it explicitly as such.

Thank you for your appreciation and for this insights. We have now rephrased the statement to only point out the depicted tendency or trend in  $\delta^{13}C$  in warming vs. control.