Reviewer 1# Remarks to the Author

This manuscript describes attempts to calibrate a laser-based absorption instrument for use in high-frequency measurements of 13C and 18O of CO2 in soil depth profiles, and provides very brief field data from two sites. This type of work is useful in that many investigators use new instruments, such as the LGR instrument described here, without sufficient validation. However, I have some major concerns about the calibration method which the authors can hopefully address in a revised manuscript. In addition, the paper would be much stronger if additional field data were presented, especially along with atmospheric measurements at the soil surface, which are needed to calculate the isotope composition of soil respired CO2 (as opposed to soil profile CO2). At present, it appears that <24 hours of field data are shown.

1. The authors develop non-linear calibration functions to account for the concentration dependence of isotope ratios but it is not clear to me how these functions might also vary in isotope space (i.e., as a dual function of isotope composition and concentration). For example, fig. 5 shows the correction functions for concentration dependence but does not show how/if these varied as a function of the isotope ratios of the standard gasses, which should all be shown on this figure. Furthermore, Fig 6 and 7 show serious deviations of calibrated vs. true values for both 13C and 18O between circa 2000 – 10000 ppm, of as much as 2 permil, even though those differences disappear at higher values. This deviation is unacceptable given the requirements of analyzing soil CO2, where differences of 2 permil may be highly significant from an ecological perspective. I note that CO2 concentrations < 10,000 ppm are commonplace in most soil profiles, especially in shallow horizons that typically dominate CO2 production, such that capacity for accurate and precise measurements in this lower concentration range is really critical. Even greater variability is shown in Fig 7, which appears to reach 4 per mil. This is not acceptable for natural abundance work.

Response: As per the suggestions made by both of the referees, we have conducted another round of calibration by diluting CO2 gas using synthetic air instead of N2. Diluting the CO2 standard gas with N2 resulted in a standard deviation of 8.1(‰) for δ^{13}C values and 4.7 (‰) for δ^{18}O values respectively. Diluting CO2 standard gases with synthetic air resulted in a standard deviation of 6.44(‰) and 6.802(‰) for δ^{13}C and δ^{18}O respectively (see Fig.1a-b). With our new calibration curves (see Fig.1 c-d, &Table.1,2), we are able to bring down standard deviation to 0.08(‰) for δ^{13}C and 0.09(‰) for δ^{18}O (see Fig.2a-b (residual distribution), Fig.3a-d (Corrected δ^{13}C and δ^{18}O values). By introducing the new calibration correction (see.Fig.3) the values are very close to the target value across the whole concentration range and hence we are confident that the system is suitable for ecosystem studies based on measuring subtle changes in isotopic signature of CO2 across plant soil atmosphere continuum.

Fig.1

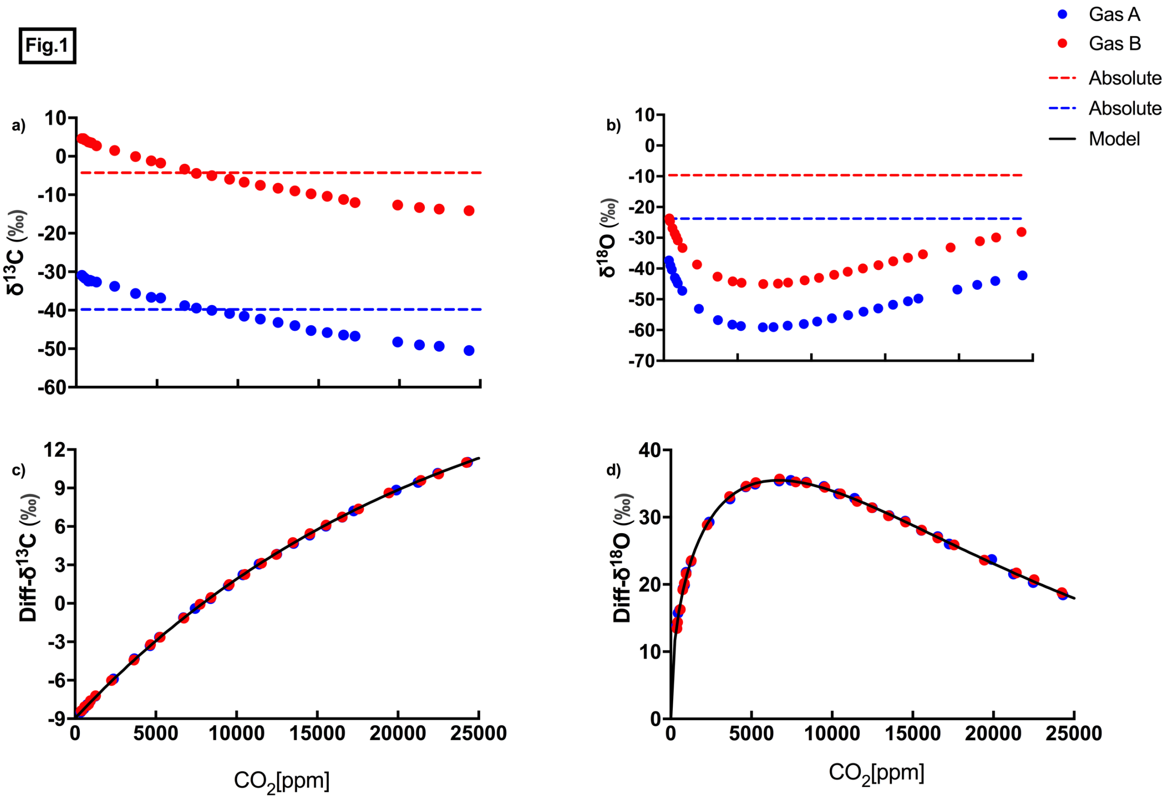


Figure.1: Deviation of measured $\delta^{13}C$ and $\delta^{18}O$ of CO2 (a,b) when diluted using synthetic air. (c-d) shows diff- $\delta^{13}C$, diff-$\delta^{18}O$ values across a concentration gradient. Red and Blue dots shows measured $\delta^{13}C$ & $\delta^{18}O$ values of two different gases with distinct isotopic signatures, red and blue dashed lines represents absolute $\delta^{13}C$ & $\delta^{18}O$ values of the respective gases. Black line denotes model fit for diff- $\delta^{13}C$, diff-$\delta^{18}O$ values across changing CO2 concentration (300 – 25000 ppm).

Fig.2

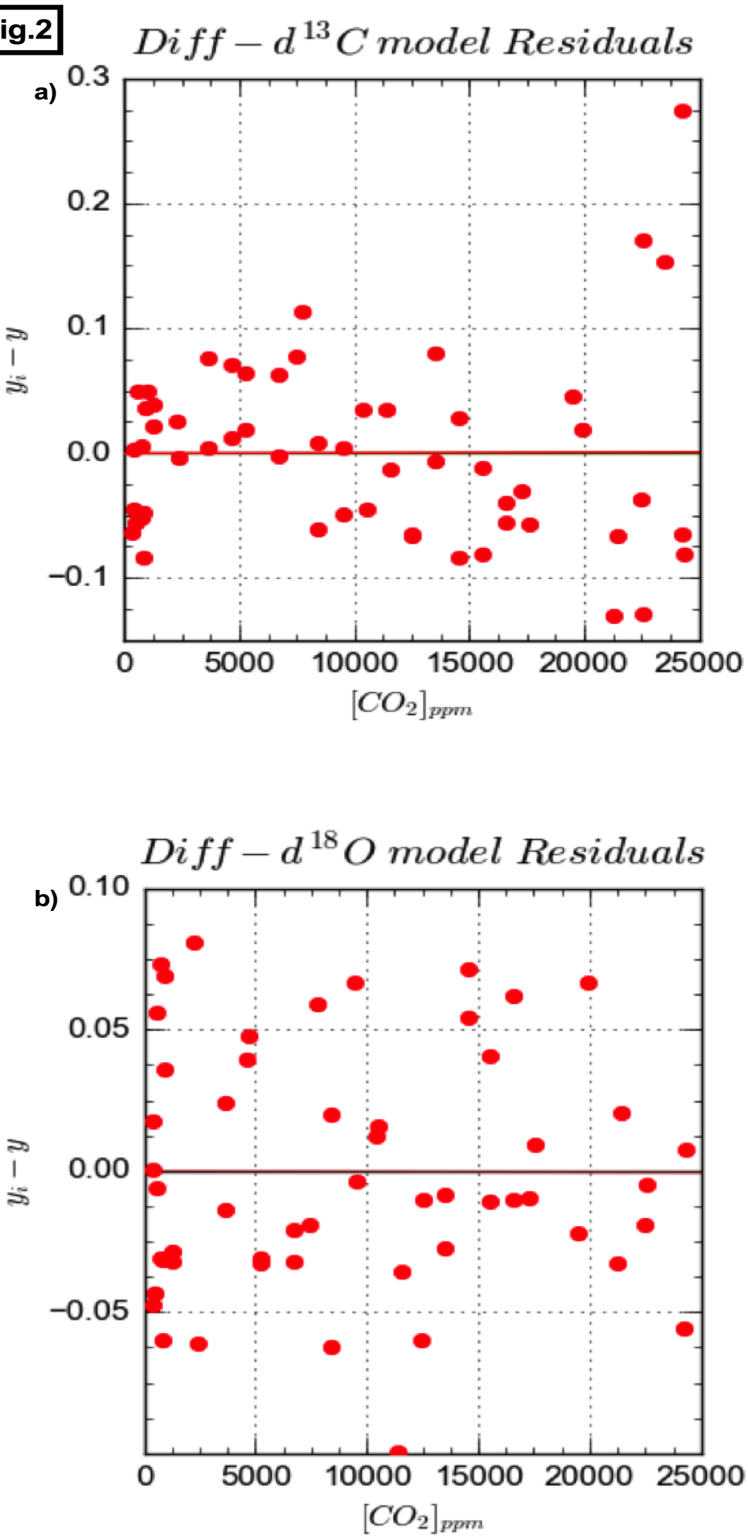


Figure 2: Residual distribution of modeled data for diff- $\delta^{13}C$, diff-$\delta^{18}O$ values across changing CO2 concentration (300 – 25000 ppm).

Fig.3

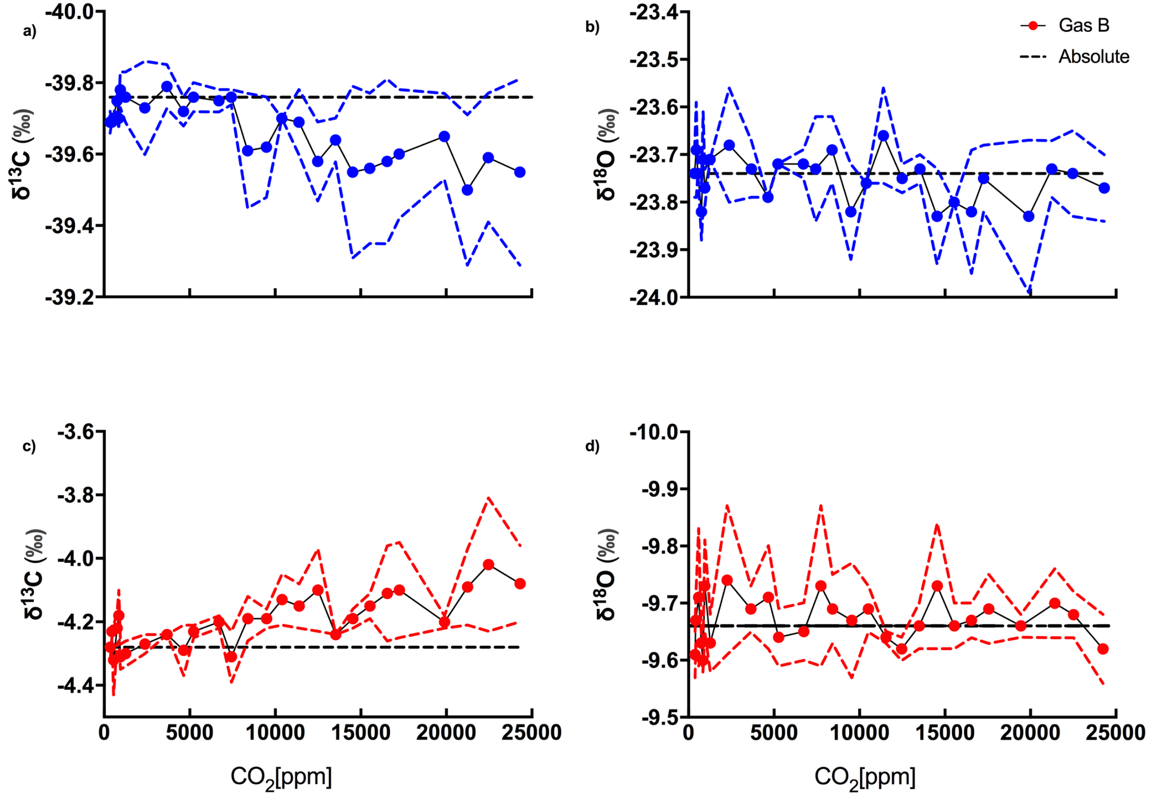


Figure 3: Corrected $\delta^{13}C$ & $\delta^{18}O$ values of two different standard gases measured after correcting for concentration dependent drift.

2. Also, basic details about the soils investigated are missing that are necessary to interpret the measured values of 13C and 18O of CO2. For example, what are the carbonate concentrations and isotope ratios in the calcareous soil, and how do they vary with depth? What are the 13C values of SOM? This is a prerequisite for interpreting the soil profile CO2 values. Also, to calculate the isotope ratios of soil-respired CO2, we need measurements of the atmospheric boundary condition. See Davidson 1995 GCA, doi:10.1016/0016-7037(95)00143-3. Note that several recent papers neglected have reported 13C of CO2 from soil profiles using high temporal-resolution optical measurements, these should be discussed or at least mentioned.

Fig.4

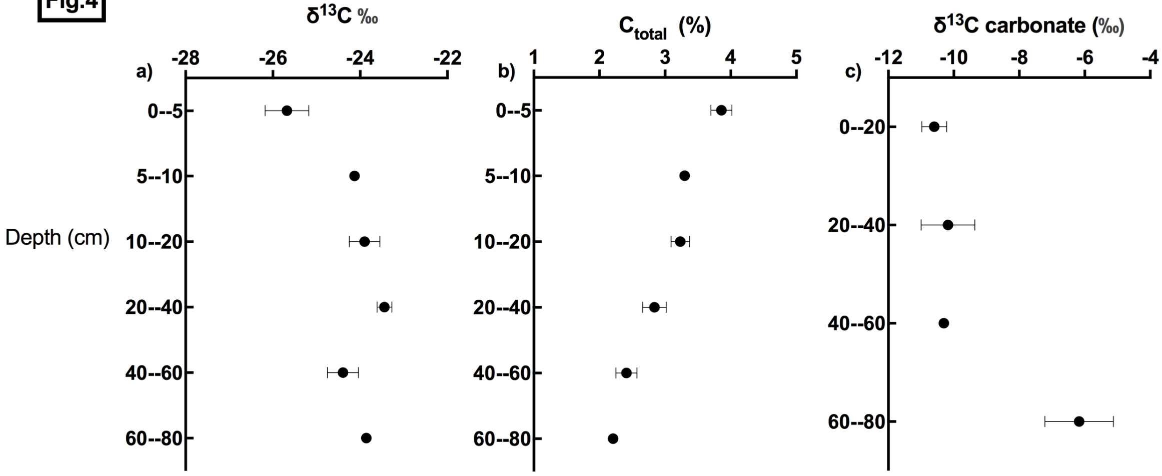


Figure.4: Bulk $\delta^{13}C$ (a), bicarbonate $\delta^{13}C$ (c) and % of carbon (b) in soil across a depth profile of (0-80 cm).

Fig.5

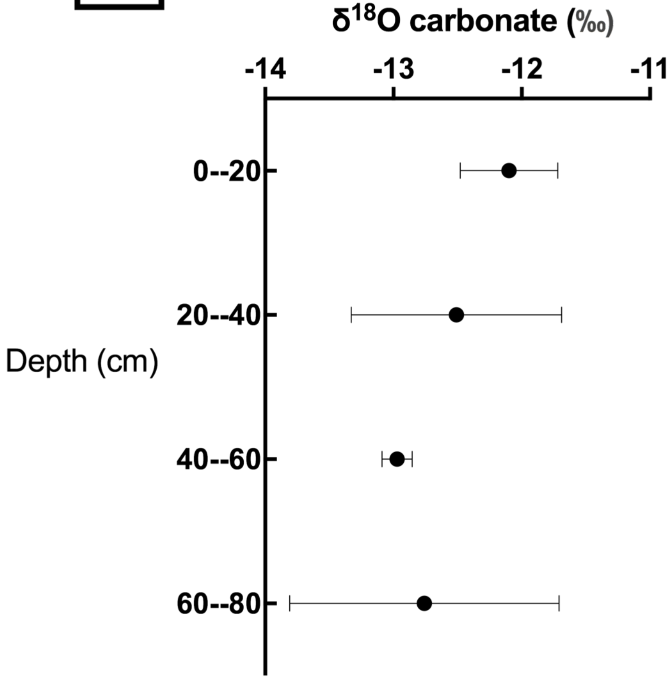


Figure.5: bicarbonate $\delta^{18}O$ in soil across a depth profile of (0-80 cm).

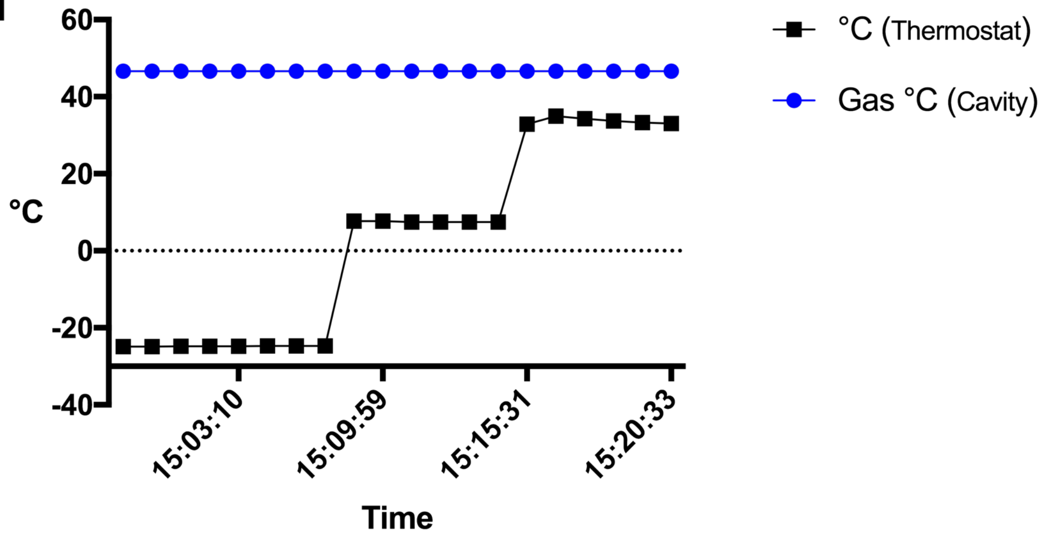
Response: We have measured soil samples for bulk $\delta^{13}C$, bicarbonate $\delta^{13}C$ & $\delta^{18}O$ values and also % carbon content in the soil across a depth profile of (0-80 cm) for the calcareous soil (See Fig.4a-c and Fig.5). We observed an increase in $\delta^{13}C$ values (of bulk soil and carbonate) in deeper soil layers (See Fig.4 a,c). This fits to our assumption of an increased contribution of bicarbonate derived δ^{13}C enriched CO2 in deeper soil layers.

Our aim is to establish a method which enables continuous online measurement of soil gas δ^{13}C & δ^{18}O values at different soil depths and hence calculating the isotope ratios of soil-respired CO2 is not done in this manuscript. This would be beyond the scope of a calibration focused paper – we however show the importance to properly calibrate laser-based systems to obtain valid measurements of d13C and d18O of soil CO2 which is a prerequisite for assessing the rate and isotopic composition of soil respiration.

3. I am skeptical as to the validity of the temperature tests employed. Note that we need to know the temperature of the analyte gas itself, which may be substantially different than the temperature of the water bath through which it circulates unless the residence time of the gas in the tubing and the heat transfer properties of the tubing allow for sufficiently rapid temperature equilibration, which may not completely occur if flow rates are high. For example, certain applications require heating of gasses at a sampling inlet to avoid condensation, yet the temperature of the gas at the point of the analysis may be substantially different (e.g. -4 – 40C) for some other optical gas analyzers, and should optimally be controlled within the analyzer cavity itself. Thus, unless the exact temperature of the gas at the point of measurement can be determined, I would not trust the results from the water bath experiment. Regardless, details of the analysis flow rate should be reported (and whether these rates were controlled during sample analysesâA˘TMFC’s are mentioned for standards only).

Response: The laser spectrometer was able to maintain the temperature inside the optical cavity quite stable at 46.61°C irrespective of the fluctuations in the gas temperature (See Figure attached below). It is clear that the temperature maintained in the water bath will not get directly reflected in the sample gas due to multiple reasons including diffusion barrier of the PTFE tubing and higher flow rates, nevertheless, there will be an increment or decrement in the gas temperature. The aim is to show that the system is also stable in field conditions where temperature fluctuation is happening. The system is running in a closed loop meaning there is enough time for the gas for heat exchange. We have adjusted the part where test for equipment stability under fluctuating temperature is done in the modified manuscript.

Fig.6



4. There are numerous issues with grammar, style, and errant capitalization throughout. The figures and tables have a strange mix of fonts (be consistent!) and the legends are compressed. Please follow standard procedures for presenting your MS (provide captions as text in the document, not as images). There is a significant typo in Table 1.

Response: This is addressed and rectified in the revised manuscript. We have also let a native speaker do the final editing of the manuscript.

5. Finally, it should be noted that the useful temporal resolution of the measurements will never actually be 1hz as reported given the Allan variance results.

Response: Not exactly clear what the reviewer meant by “it should be noted that the useful temporal resolution of the measurements will never actually be 1hz as reported given the Allan variance results”. It is always useful to get a better temporal resolution which can be used for identifying short term dynamics of CO2 efflux (e.g., diurnal pattern of soil CO2 efflux). Meaning more data points are available for taking an average across a time frame which is best for noise correction by using Allan deviation curves.

6. Was water vapor removed from the analyte gas, and if so, how?

Response: Yes, water vapor was removed using drierite desiccant cartridges. We have now add this information to the revised manuscript.

Table.1

Equation	y=a*(b-exp(-c*x))				
Standard Error	0.07468171				
Correlation Coeff.(r)	0.999941				
Coeff.of Determination (r^2)	0.99988246				
DOF	54				
AICC	-294.6349				
Parameters					
Value	Std	Err	Range	(95%	confidence)
a	31.007446	0.214984	30.576428	to	31.438463
b	0.713759	0.002376	0.708995	to	0.718522
c	0.000043	0	0.000042	to	0.000043
Covariance Matrix					
	a	b	c		
a	8.286768	0.088333	-0.000018		
b	0.088333	0.001012	0		
c	-0.000018	0	0		

Table.2

Equation	y=a*(b^x)*(x^c)				
Standard Error	0.04365503				
Correlation Coeff.(r)	0.999981				
Coeff.of Determination (r^2)	0.99996128				
DOF	51				
AICC	-337.04644				
Parameters					
	Value	StdErr	Err	Range (95% confidence)	
a	0.851623	0.003025	0.84555	to	0.857697
b	0.999928	0	0.999928	to	0.999928
c	0.477819	0.000472	0.476871	to	0.478767
Covariance Matrix					
	a	b	c		
a	0.004803	0	-0.000745		
b	0	0	0		
c	-0.000745	0	0.000117		

Reviewer 2# Remarks to the Author

1. **Preparation of the calibration gases:** You mixed the gases in N2. This will cause some shifts in your absorption spectra and will result in a shift of your isotopic values as it was shown in Bowling et al. (2003). Tuzon et al., (2008) address the calibration process in detail and it is recommended to consider this paper in this study. If the possibility is still given, it might be worth it produce new reference gases with synthetic CO2 free air (20% oxygen and 80% nitrogen) then repeat the calibration of the instrument, compare the results and reassess the results. I am aware that this is an unusual request and almost too much to ask for but it would be worth it.

   Response: As per the suggestions made by both of the referees, we have conducted another round of calibration by diluting the CO2 gas using synthetic air (20% oxygen and 80% nitrogen) instead of N2. Diluting the CO2 standard gas with N2 resulted in a standard deviation of 8.1(‰) for δ^{13}C values and 4.7 (‰) for δ^{18}O values respectively. Diluting CO2 standard gases with synthetic air resulted in a standard deviation of 6.44(‰) and 6.802(‰) for δ^{13}C and δ^{18}O respectively (see Fig.1a-b). With our new calibration curves (see Fig.1 c-d, &Table.1,2), we are able to bring down standard deviation to 0.08(‰) for δ^{13}C and 0.09(‰) for δ^{18}O (see Fig.2a-b (residual distribution), Fig.3a-d (Corrected δ^{13}C and δ^{18}O values). We now have restructured the respective section in the manuscript and have included the new calibration system in the revised version of the manuscript.

   "**S**tandard gases were released to a mass flow controller (ANALYT-MTC, series 358, MFC1) after passing through a pressure controller valve (See Figure. 1) with safety bypass (TESCOM, D43376-AR-00-X1-S; V5). A Swagelok filter, ((Stainless Steel All-Welded In-Line Filter (Swagelok, SS-4FWS-05; F1)) was installed at the inlet of the flow controller (ANALYT-MTC, series 358; MFC1). **Synth-air** was released and passed to another flow controller (ANALYT-MTC, series 358; MFC2) through a Swagelok filter (F2 in Figure. 1). CO_2 and **synth-air** leaving the flow controllers (MFC1 and MFC2 respectively) were then mixed and drawn through a ¼" Teflon tube (P8), which was kept in a **gas thermostat unit** (See Figure.1). *The **thermostat unit** contained, a) a thermostat-controlled water bath (Kottermann, 3082) and b) an Isotherm flask containing liquid nitrogen. The water bath was used to raise the temperature above room temperature and also to bring the temperature down to +5°C, by placing ice packs in the water bath. To reach low temperatures (-20°C), we immersed the tubes in the isotherm flask filled with liquid N_2.* Leaving the **thermostat unit**, the gas was directed to the multiport inlet unit of the OA-ICOS. By using the thermostat unit, we introduced a shift in the reference gas temperature and the aim was to test the temperature sensitivity of the OA-ICOS in measuring δ^{13}C and δ^{18}O values. The third CO_2 standard gas (which is used for validation) was produced by mixing the other two gas standards in equal molar proportions in a 10L volume plastic bag with inner aluminum foil coating and welded seams (CO_2 mix: Linde PLASTIGAS®) under 0.03 MPa pressure by diluting to the required concentration using **synth-air**. The mixture was then temperature adjusted and delivered to the multiport inlet unit (MIU) by using a ¼" Teflon tube (P10). From the multiport inlet unit, calibration gases were delivered into the OA-ICOS for measurement using a ¼" Teflon tube (P9) at a pressure < 0.0689 MPa, with a flow rate of 500 mL/min. The gas leaving the OA-ICOS through the exhaust was fed back to the ¼" Teflon tube (P8) by using a Swagelok pipe Tee (Stainless Steel Pipe Fitting, Male Tee, ¼". Male NPT), intersecting P8 line before entering the **thermostat unit**. Thus, the gas fed was looped in the system until steady values were reported by the OA-ICOS based on CO_2 [ppm], $\delta^{13}C$ and $\delta^{18}O$ measurements. *CO_2 gas standards were measured at 27 different CO_2 concentration levels ranging between 400 and 25,000 ppm.* Every hour before sampling, **synth-air** gas was flushed through the system to remove CO_2 to avoid memory effects. The calibration gases were measured in a sequence one after the other four times. During each round of measurement, every calibration gas was diluted to different concentrations of CO_2 (400 - 25,000 ppm) and the respective isotopic signature and concentration were determined."

Fig.1

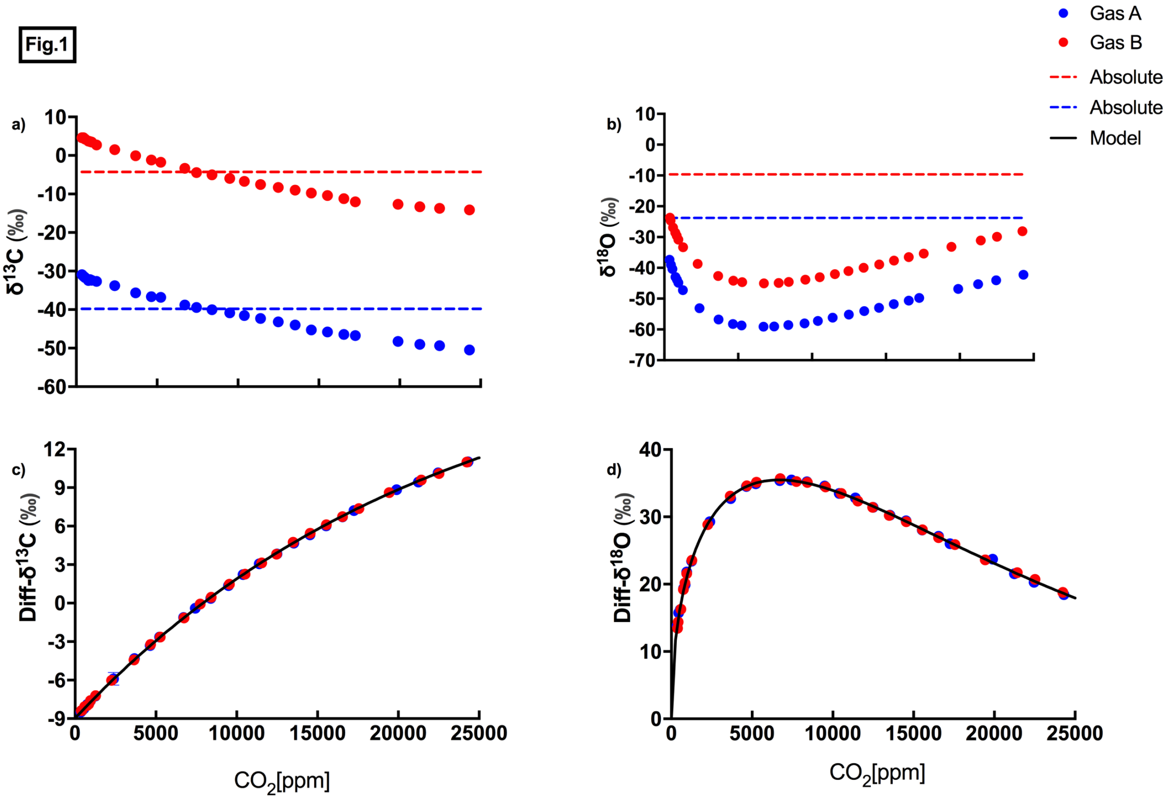


Figure.1: Measured $\delta^{13}C$ and $\delta^{18}O$ of CO_2 compared to the target values (a,b) when diluted using synthetic air. (c-d) shows the differences from the target values (diff- $\delta^{13}C$, diff-$\delta^{18}O$) across a concentration gradient. Red and Blue dots show measured $\delta^{13}C$ & $\delta^{18}O$ values of two different gases with distinct isotopic signatures, red and blue dashed lines represent the $\delta^{13}C$ & $\delta^{18}O$ target values of the respective gases calibrated independently by isotope ratio mass spectrometry. Black line denotes model fit for diff- $\delta^{13}C$, diff-$\delta^{18}O$ values across changing CO2 concentration (300 – 25000 ppm).

Fig.2

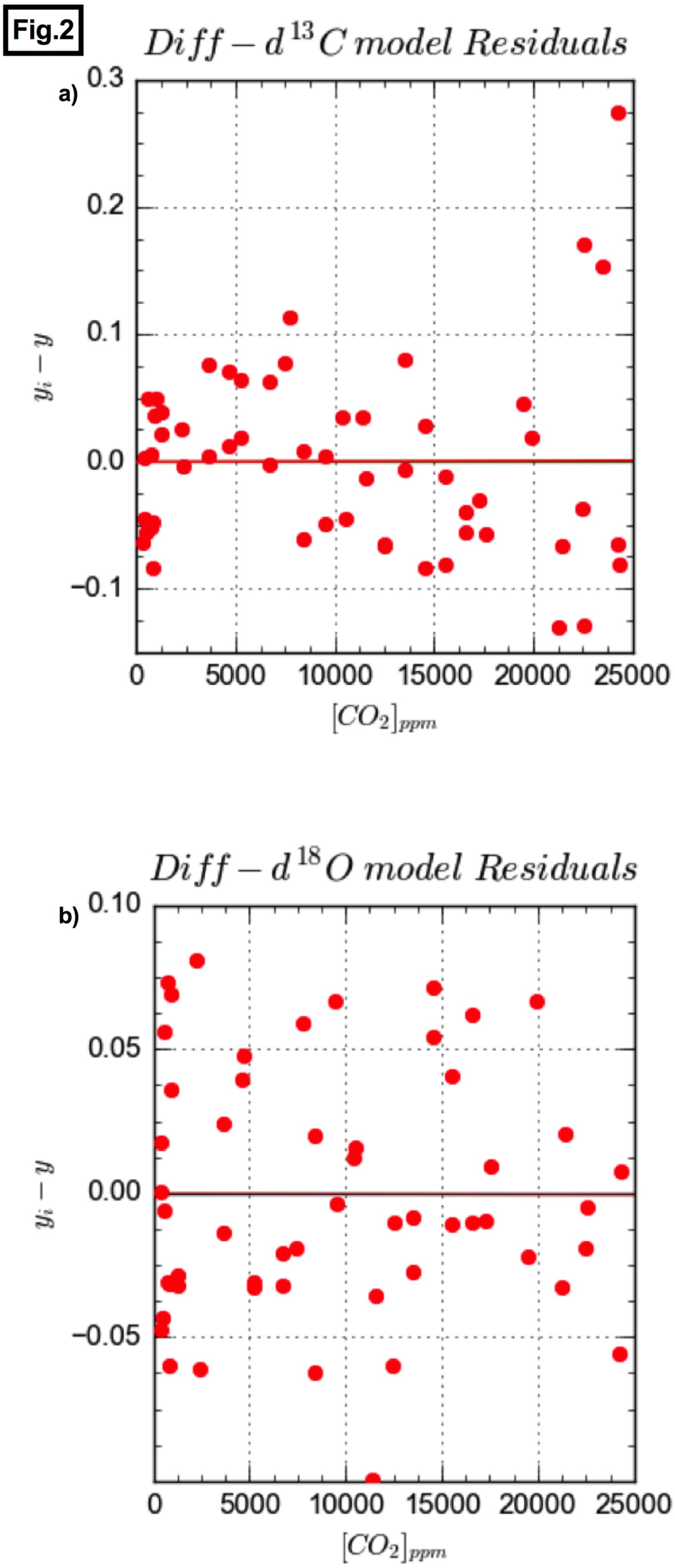


Figure 2: Residual distribution of modeled data for the differences in d18O between measured and target values (diff- δ^{13}C, diff-δ^{18}O) values across changing CO2 concentration (300 – 25000 ppm).

Fig.3

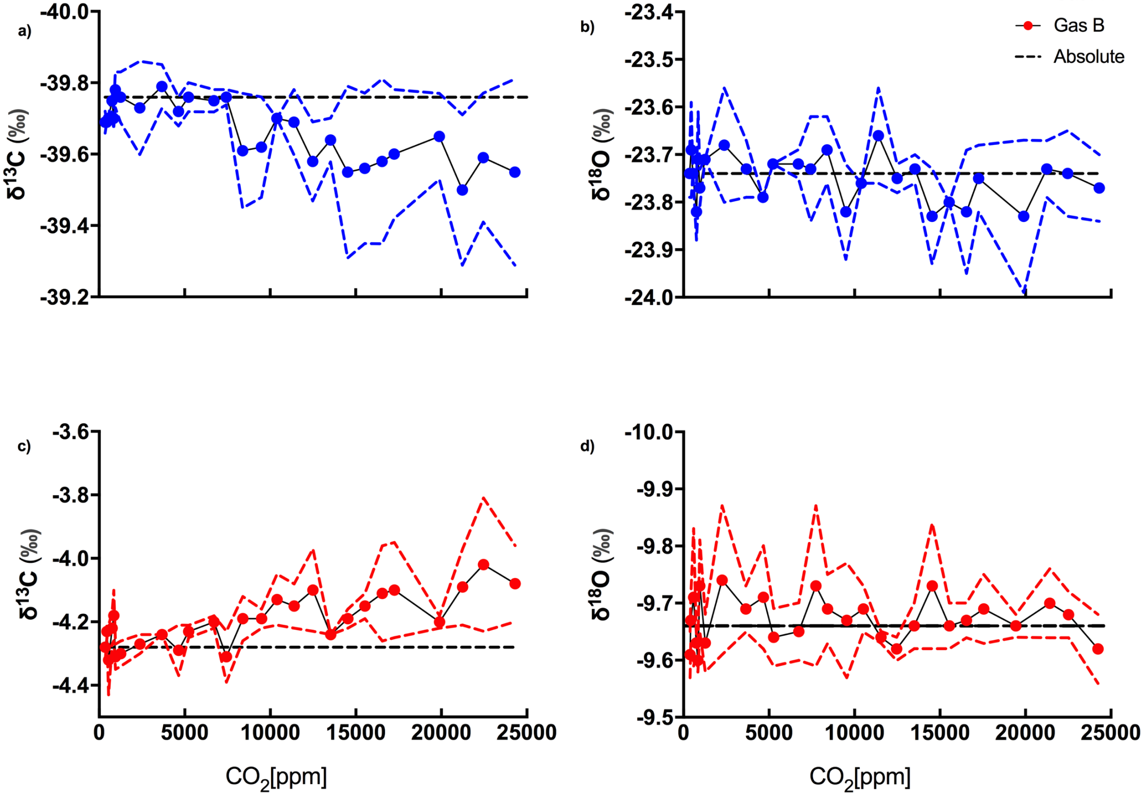


Figure 3: Corrected $\delta^{13}C$ & $\delta^{18}O$ values of two different standard gases measured after correcting for concentration dependent drift. The dashed lines indicated the target $\delta^{13}C$ and $\delta 1^{8}0$ target values calibrated independently by isotope ratio mass spectrometry.

2. How did you calibrate the gases, via gas bench-IRMS or via cryo extraction and Dual Inlet IRMS? If you used the gas bench method how did you handle the problem with the septa of the vacutainers leading to a large scatter for the 18O/16O ratio, in case you used this method?

   Response: The $\delta^{13}C$ and $\delta^{18}O$ values of our inhouse calibration gas standards were measured via cryo extraction and Dual Inlet IRMS. This information is now included in the revised version of the manuscript.

   "The $\delta^{13}C$ and $\delta^{18}O$ values of our inhouse calibration gas standards were measured via cryo-extraction and Dual Inlet IRMS"

3. It would be worth to insert subtitles in chapter 3: e.g. 3.1 Instrument calibration and correction (after Line 192) 3.2 Variation in soil CO2 concentration and its C and O isotope values (after line 241)

Response: Subtitles are added in the modified manuscript.

Specific comments

4. Line 140: PTFE or Swagelok filter? Clarify

   Response: Swagelok filter (Stainless Steel In-Line Particulate Filter, 6 mm Swagelok Tube Fitting, 15 Micron Pore Size); this information is added to the revised version.

   “A Swagelok filter, ((Stainless Steel All-Welded In-Line Filter (Swagelok, SS-4FWS-05; F1)) was installed at the inlet of the flow controller (ANALYT-MTC, series 358; MFC1). Synth-air was released and passed to another flow controller (ANALYT-MTC, series 358; MFC2) through a Swagelok filter (F2 in Figure. 1).”

5. Lines 141-142: what kind of a filter is this to prevent moisture from getting into the device? What device do you mean? Normally moisture isn´t captured with a filter but much rather with a water trap. But usually commercially available gas is very dry making a water trap dispensable.

   Response: The filter is a particulate matter filter and not a moisture filter. It can hold very little amount of liquid water and not water vapor. This is rectified in the revised manuscript.

6. Lines 145-146: If you intend to produce a gas with a temperature range from minus! -20°C to +40°C a water bath is certainly not the right choice. Please clarify. Either you used a different cooling liquid or you never went below 0°C.

Fig.6

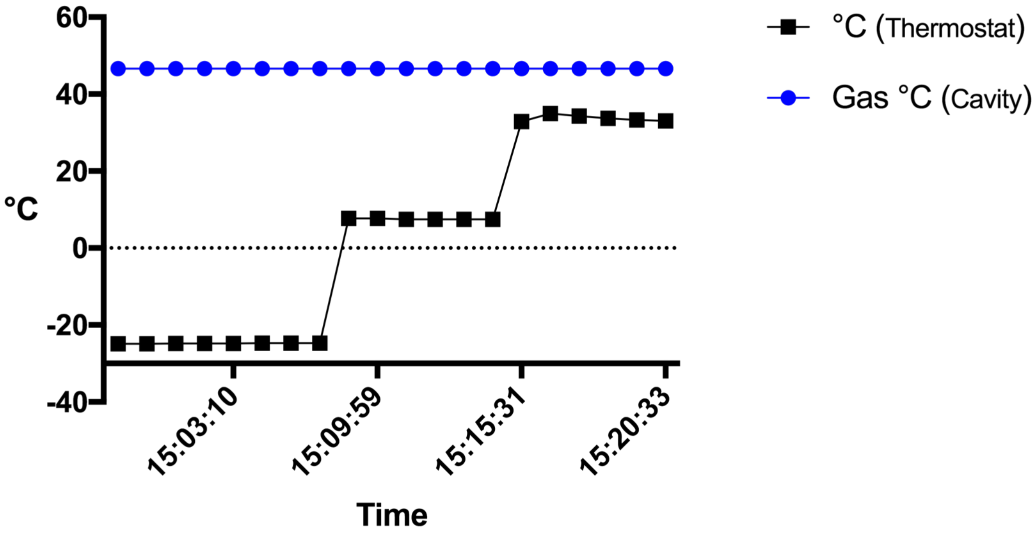


Fig.6: Gas temperature recorded inside the optical cavity (Blue line) & Temperature recorded in the thermostat system (black lines).

Response: The reviewer is right, it needs further clarification. We have used a water bath to increase the temperature to higher values than the room temperature. To reduce the temperature below, we immersed gas tubes in liquid Nitrogen kept in an isotherm flask. This information is included in the revised manuscript.

"*The **thermostat unit** contained, a) a thermostat-controlled water bath (Kottermann, 3082) and b) an Isotherm flask containing liquid nitrogen. The water bath was used to raise the temperature above room temperature and also to bring the temperature down to +5°C, by placing ice packs in the water bath. To reach low temperatures (-20°C), we immersed the tubes in the isotherm flask filled with liquid* N_2*.* Leaving the **thermostat unit**, the gas was directed to the multiport inlet unit of the OA-ICOS. By using the thermostat unit, we introduced a shift in the reference gas temperature and the aim was to test the temperature sensitivity of the OA-ICOS in measuring $\delta^{13}C$ and $\delta^{18}O$ values."

7. Line 156: Please indicate the concentration steps for the calibration.

   Response: We have used 27 concentration points across the range (300-25000 ppm). For more details see table.3.

Table.3

CO2 ppm	d13C	Stdev data
350.93	-31.28	0.04
453.32	-31.42	0.07
543.73	-31.54	0.07
755.35	-31.87	0.03
852.19	-31.94	0.03
951.99	-32.15	0.03
1257.59	-32.52	0.07
2377.12	-33.86	0.10
3670.33	-35.44	0.03
4651.48	-36.46	0.00
5230.98	-37.13	0.04
6718.14	-38.65	0.02
7441.17	-39.37	0.02
8396.27	-40.13	0.00
9491.37	-41.13	0.00
10390.11	-41.99	0.05
11402.32	-42.83	0.01

12488.75	-43.59	0.07
13531.13	-44.44	0.06
14532.92	-45.09	0.03
15534.13	-45.79	0.01
16547.02	-46.49	0.05
17255.32	-46.97	0.03
19893.50	-48.60	0.01
21237.86	-49.19	0.04
22462.06	-49.92	0.01
24313.08	-50.78	0.05

8. Line 187: How was the pressure regulated in this closed loop? For a proper operation of the laser instrument, the pressure in the cavity cell must be as constant as possible, since only slightest changes in pressure can mimic a change in concentration of all gas species.

Fig.7

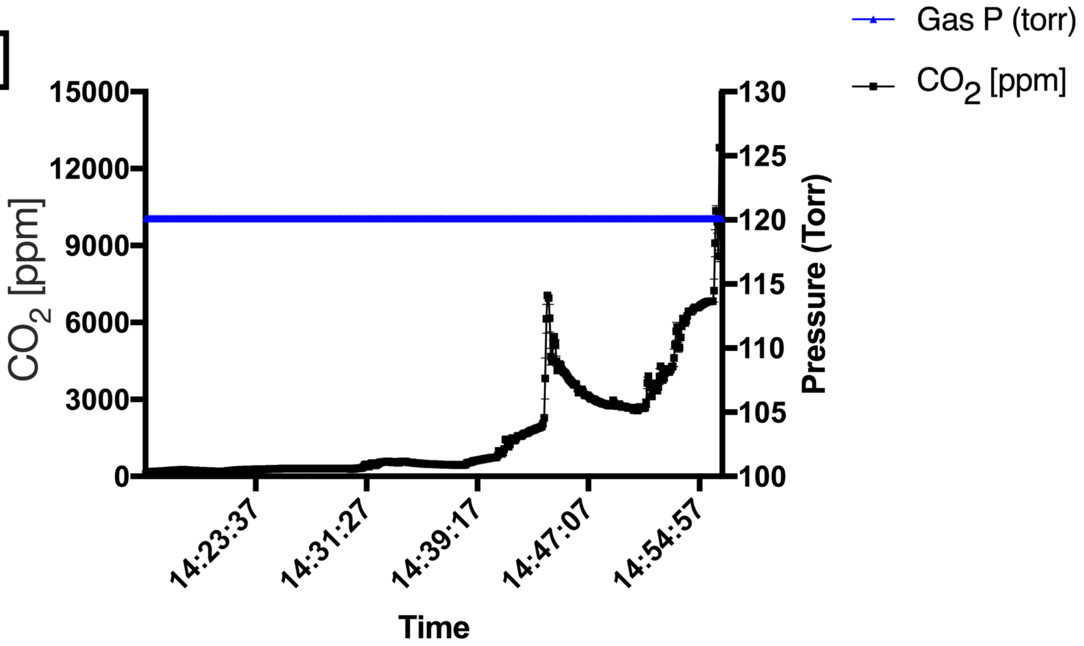


Figure.7: The figure shows pressure inside the optical cavity (blue line) plotted on right y axis and change in CO2 concentration (black lines) plotted on left y axis. Data was taken while the system is running in a closed loop system with periodic injections of CO2 gas.

Response: We did not encounter any pressure differences while maintaining a closed loop system. We have cavity pressure data monitored (see Figure.7). We have included this information in the revised version of the manuscript.

"We experienced no drop in cavity pressure while maintaining a closed loop (See Supplementary Figure S2)."

9. Line 204: To prevent misunderstandings it is better to write D-δ or Diff-δ instead of Δδ, since Δ is used for discrimination (fractionation) in the isotope literature.

   Response: We agree with the reviewer. It is corrected in the revised manuscript.

   "The dependency of $\delta^{13}C$ and $\delta^{18}O$ values on the CO_2 concentration was compensated by using a nonlinear model. The deviations (Diff-δ) of the measured delta values ($\delta_{\mathbf{(OA\text{-}ICOS)}}$) from the absolute value of the standard gas ($\delta_{\mathbf{(IRMS)}}$) at different concentrations of CO_2 were calculated (Diff-δ = $\delta_{\mathbf{(OA\text{-}ICOS)}}$ - $\delta_{\mathbf{(IRMS)}}$). Several mathematical models were then fitted on Diff-δ as a function of changing CO_2 concentration (See figure.5). The mathematical model with the best fit for Diff-δ data was selected using Akaike information criterion corrected (AICc) (Glatting et al., 2007; Hurvich and Tsai, 1989; Yamaoka et al., 1978). The non-linear model fits applied for Diff-$\delta^{13}C$, and Diff-$\delta^{18}O$ measurements are given in Tables 1 & 2, respectively. For Diff-$\delta^{13}C$, a three-parameter exponential model fitted best with $r^2 = 0.99$ (see Table 3 for the values of the parameters, see supplementary Figure S3 (a) for model residuals), and a three-parameter power function model (see Table 2) with $r^2 = 0.99$ showed the best fit for Diff-$\delta^{18}O$ (see Table 3 for the values of the parameters, see supplementary Figure S3 (b) for model residuals)."

10. Line 206: rewrite "… The mathematical model with the most fitting to…" write "…the mathematical model with the best fir for …"

    Response: Corrected in the revised manuscript.

    "The mathematical model with the best fit for Diff-δ data was selected using Akaike information criterion corrected (AICc)"

11. Line 211: replace "… most fitting model …" with "… best fit …"

    Response: Corrected in the revised manuscript.

    "The best fit was then introduced into the measured isotopic data ($\delta^{13}C$ and $\delta^{18}O$) and corrected for concentration-dependent errors (See figure. 6)"

12. Line 221: replace "… better…" with " … the needed…"

    Response: Corrected in the revised manuscript.

    "we found that routine calibration (Correction for concentration-dependent error plus three-point calibration) was inevitable for obtaining the required accuracy, in particular under fluctuating CO_2 concentrations."

13. Lines 223 – 231: A native English-speaking person should reassess these lines.

    Response: A native speaker has now seen the manuscript for final language editing.

14. Lines 226- 227: It would be more correct to say: " We assume that these deviations were instrument specific and the fitting parameters have to be adjusted for every single device.

    Response: Corrected in the revised manuscript.

    "We assume that these deviations are instrument specific and the fitting parameters need to be adjusted for every single device"

15. Lines 243-245. I can´t see that for the top 4 to 12cm. Clarify please.

    Response: Yes, for the calcareous soil there was no increase in CO2 concentration between 4 and 12 cm which is also related to the relative 13C depletion in 4 cm compared to 12 cm – both is assumed to be due to mixing in of atmospheric air (having lower CO2 concentrations and a d13C of approx. -8). We have clarified that in the revised version of the manuscript.

    "Relative ^{13}C enrichment of the CO_2 in the topsoil (4 cm) compared to 8 cm depth is probably due to the invasive diffusion of atmospheric CO_2 which has a $\delta^{13}C$ value close to -8‰ (e.g., (Levin et al., 1995))."

16. Line 246: …relative to what? Soil δ13CO2 was only slightly enriched, according to Fig. 8

    Response: The $\delta^{13}C$ signal of soil CO_2 at 4 cm depth is enriched compared to the one sampled from 8cm depth and this is visible in Figure.9. We see a constant depletion in 13C of soil CO2 from 80 to 8 cm soil depth – the 4 cm depth does not fit into that trend as we here see compared to 8 cm a slight enrichment.

17. Lines 242-272: For this whole paragraph it would be worth to read the paper of Cerling, 1984, and Bowen, 2004 (see recommended literature).

    Response: The whole paragraph is modified by including relevant information from Cerling, 1984, and Bowen, 2004.

    "The patterns observed for the $\delta^{13}C$ values of CO_2 in the calcareous soil with ^{13}C enrichment in deeper soil layers can be explained by a substantial contribution of CO_2 from abiotic origin to total soil CO_2 release as a result of carbonate weathering and subsequent out-gassing from soil water (Schindlbacher et al., 2015).

According to Cerling (1984), the distinct oxygen and carbon isotopic composition of soil carbonate depends on the isotopic signature of meteoric water and to the proportion of C_4 biomass present at the time of carbonate formation (Cerling, 1984). CO_2 released as a result from carbonates have a distinct $\delta^{13}C$ value close to 0‰ vs. VPDB, while CO_2 released during biological respiratory processes has usually $\delta^{13}C$ values around -24‰ as observed in the acidic soil (Figure 10 (e)). Even though the contribution of CO_2 from abiotic sources to soil CO_2 is often considered to be low, several studies have reported significant proportions ranging between (10 - 60%) emanating from abiotic sources (Emmerich, 2003; Plestenjak et al., 2012; Ramnarine et al., 2012; Serrano-Ortiz et al., 2010; Stevenson and Verburg, 2006; Tamir et al., 2011). Bowen and Beerling, (2004) showed that isotope effects associated with soil organic matter decomposition can cause a strong gradient in δ values of soil organic matter (SOM) with depth, but are not always reflected in the $\delta^{13}C$ values of soil CO_2. We have measured soil samples for bulk soil $\delta^{13}C$, bicarbonate $\delta^{13}C$ & $\delta^{18}O$ values and also determined the percentage of total carbon in the soil across a depth profile of (0-80 cm) (See Figure 8). We observed an increase in $\delta^{13}C$ values for bulk soil in deeper soil layers (See Figure 8 (a,c))."

18. Line 250: No specific pattern…Actually the pattern for δ18O is quite similar to that of the δ13C, except for this sharp decline at around 2:00, (which is less visible for the δ13C time course). The authors should comment that, what could be the cause?

    Response: We have now added the following section to explain this pattern: " For $\delta^{18}O$ values of calcareous soil, the depth profile showed no specific pattern except for the $\delta^{18}O$ values at 80 cm depth was found to be less negative. The $\delta^{18}O$ value in the top 4 cm was found to be slightly more enriched that the 8 cm depth and between 8 cm – 35 cm, $\delta^{18}O$ values showed little variation relative to each other. For the sub-daily measurements, we observed a sharp decline in $\delta^{18}O$ values at around 02:00, which is also observed but less pronounced for $\delta^{13}C$ signal. We assume that, the reason for such aberrant values is rather a technical issue than any biological process. It can be due to the fact that the internal pump in the OA-ICOS was not taking adequate amount of gas into the optical cavity, thereby creating a negative pressure inside the cavity resulting in the observed aberrant values."

19. Line 254: It would be highly beneficial for this statement if you had the δ values of the soil organic matter for the respective soil depths.

Fig.4

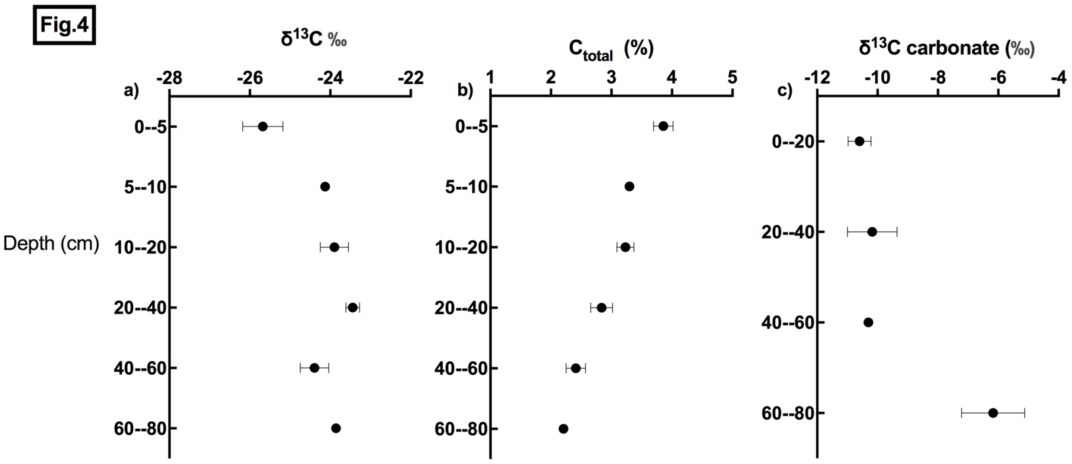


Figure.4: Bulk $\delta^{13}C$ (a), bicarbonate $\delta^{13}C$ (c) and % of total carbon (b) in soil across a depth profile of (0-80 cm).

Fig.5

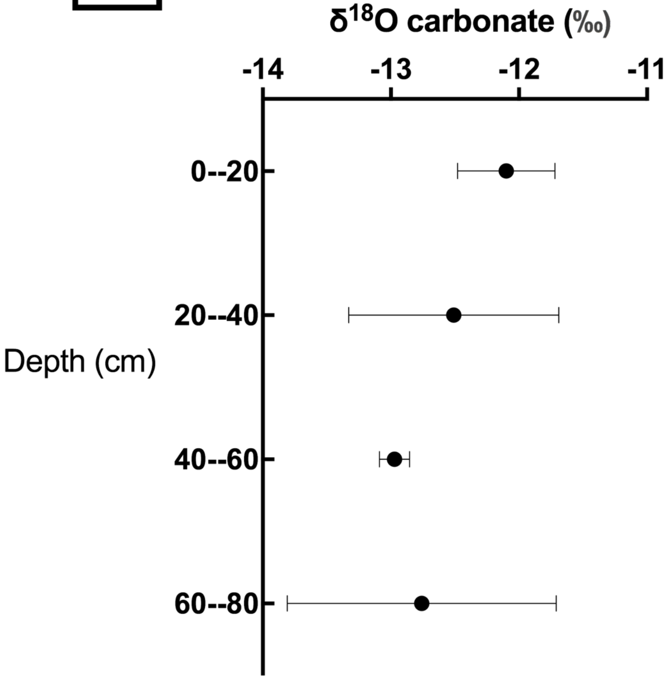


Figure.5: bicarbonate $\delta^{18}O$ in soil across a depth profile of (0-80 cm).

Response: We have measured soil samples for bulk $\delta^{13}C$, bicarbonate $\delta^{13}C$ & $\delta^{18}O$ values and also % total carbon in the soil across a depth profile of (0-80 cm) for the calcareous soil (See Fig.4a-c and Fig.5). We observed a slight increase in $\delta^{13}C$ values for bulk soil in deeper soil layers (See Fig.4 a,c). Moreover, also the carbonate d13C gets more positive in the 60-80 cm layer. Since total organic carbon content decreases with depth it can be assumed that CO2 derived from carbonate weathering having less negative d13C more strongly contributed to the soil CO2 in this depth (especially since we see an increase in soil CO2 concentration with depth). This is accordance with the laser-based measurements which shows a strong increase in d13C of soil CO2 in the deepest soil layer leading us to the hypothesis that this signal is indicating carbonate derived CO2.

20. Line 264: It would be more accurate to say: "…is assumed to be the dominating source of soil CO2…"

    Response: Corrected in the revised manuscript.

    "In contrast to the deeper soil layers, where the carbonate content is high, CO_2 from carbonate weathering is assumed to be a dominating source of soil CO_2"

21. Lines 269-272: Are you sure that the δ18O values of the soil CO2 are referred to VSMOW? It looks more like VPDB. Please check that! Then, compared to the δ18O values close to the soil surface CO2 the δ18O values in -80 cm depth are surprisingly high relative to the topsoil. Soil surface water is more prone to be enriched, due to soil surface evaporation processes, than water close to ground water. The authors should comment on that.

    Response: δ18O values are reported against VPDB and not VSMOW. This is corrected in the revised manuscript. When we assume that in 80 cm soil depth a relatively large part of the CO2 derives from carbonate this could explain the strongly enriched 18O signal. We, however, need then to assume that that the oxygen in the CO2 is not in full equilibrium with the precipitation influenced soil water. As mainly microbial carbonic anhydrase mediates the fast equilibrium between CO2 and water in the soil and the microbial activity is low in deeper soil layers (e.g. Schmidt MWI, Torn MS, Abiven S, et al. Persistence of soil organic matter as an ecosystem property. *Nature*. 2011;478(7367):49-56. doi:10.1038/nature10386.) we can speculate that in deep layers with a significant production of carbonate derived CO2 a lack of full equilibration might be the reason for the observed d18O values.

22. Lines 281-283: Here it would be valuable to have more information on the soil structure. Isn´t the acidic soil less compact and dense than the calcareous soil and therefore the diffusivity would be higher in the acidic soil. Its higher CO2 concentration could as well be a result of a higher microbial activity due to its higher organic content. It would be

interesting to see soil respiration data for these soils. Maybe the authors can comment on that

Response: Calcareous soil sampled from our study site was gravel rich and less compact. while the acidic soil was more fine, homogeneous and compact. It is sound to consider gas diffusivity in calcareous soil (in our study site) higher in comparison to the acidic soil. It is highly likely that it is due to an increased microbial activity in the acidic soil. We have soil respiration data for the acidic but not for the calcareous soil so we cannot make a comparison.

23. Lines 285-287: Again, are these δ18O values really referring to the VSMOW scale? Then somehow your calculation between the δ18O of the soil water and that of the CO2 is strange. If you add 41‰ (oxygen fractionation between water and CO2) to - 10‰ (δ18O of the soil water) that would result in ca. 31‰, but you indicate -10‰. Please clarify.

    Response: δ18O values are reported against VPDB and not VSMOW. This is corrected in the revised manuscript as follows:

    "Assuming an ^{18}O fractionation of 41‰ between CO_2 and water (Brenninkmeijer et al., 1983) this would result in an expected value for CO_2 of ≈ -10 ± 2‰ vs. VPDB-CO_2"

24. **Conclusion:** The first 8 lines are more a summary than a conclusion. Focus on the main outcome of your study, which is the non-linear response of the δ-values versus CO2 concentration. This is a strong demonstration for how essential a careful concentration vs. Isotope ratio calibration is especially when the system is used for such a wide concentration range. Then it would be interesting if your tube-soil-CO2-capture method is reliable and highlight the advantages and disadvantages versus other methods. You practically ignored this method in the discussion. It would be interesting to know more about your experience with it. In that light what do you conclude from your first results?

    Response: We agree with the reviewer regarding the fact that the calibration procedure is not well discussed and needs to shed more light into it. We have now rewritten the conclusion section to focus on the main outcome of the study.

    "During our preliminary tests with the OA-ICOS, we found that the equipment was highly sensitive to changes in CO_2 concentrations. We found a non-linear response of the δ^{13}C and δ^{18}O values against changes in CO_2 concentration. Given the fact that laser-based CO_2 isotope analyzers are getting deployed more commonly in tracing various ecosystem processes, we think that it is important to address this issue.

Therefore, we developed a calibration strategy for correcting errors introduced in $\delta^{13}C$ and $\delta^{18}O$ measurements due to the sensitivity of the device against changing CO_2 concentrations. We found that the OA-ICOS measures stable isotopes of CO_2 gas samples with a precision comparable to conventional IRMS. The method described in this work for measuring CO_2 concentration, $\delta^{13}C$ and $\delta^{18}O$ values in soil air profiles using an OA-ICOS and hydrophobic gas permeable tubes are promising and can be applied for soil CO_2 flux studies. As this set up is capable of measuring continuously for longer time periods at higher temporal resolution (1 Hz), it offers greater potential to investigate the isotopic identity of CO_2 and the interrelation between soil CO_2 and soil water. By using our measurement setup, we could identify abiotic as well as biotic contributions to the soil CO_2 in the calcareous soil. We infer that that degassing of CO_2 from carbonates due to weathering and evasion of CO_2 from groundwater may leave the soil CO_2 with a specific and distinct $\delta^{13}C$ signature especially when the biotic activity is rather low."

25. **Figures:** In all Figures, where you plot δ18O values, check whether you used the VSMOW or VPDB scale.

    Response: Yes, all the $\delta^{18}O$ values are expressed on VPDB scale.

26. Fig. 1: the expression "water bath" is misleading better to use an expression like "gas thermostat system" or something alike. Clarify whether you used PTFE (brand, type, producer etc.) or Swagelok filters.

    Response: "Expression "water bath" is changed to "Thermostat unit" in the revised manuscript.

    We used Swagelok filter (Stainless Steel In-Line Particulate Filter, 6 mm Swagelok Tube Fitting, 15 Micron Pore Size) and is corrected in the revised manuscript.
27. Fig 5 and Fig 6: it would be better to use D-δ or Diff-δ instead of Δδ

    Response: Diff-δ is used instead of Δδ in the revised manuscript.

28. Fig. 8: Indicate in the figure legend that this is a "… Time course of the evolution of …" with the specific time resolution.

    Response: In the revised manuscript, the mentioned figure number is changed to Figure 9.

    Legend is corrected to "Time course of the evolution of soil gas CO_2 [ppm], $\delta^{13}C$ and $\delta^{18}O$ in calcareous (a,c,e) and acidic (b,d,f) soils. Data collected continuously over a 12 hour time frame for the calcareous soil and a 14 hour time window with intermittent data collection for the acidic soil.

29. Fig. 9: Indicate in the figure legend that you display “…Daily? averages of CO2 concentration and isotope values in depth profiles…”

    Response: In the revised manuscript, the mentioned figure number is changed to Figure 10. Legend is corrected to “Daily average data of soil CO_2 [ppm], $\delta^{13}C$ and $\delta^{18}O$ in calcareous (a,b,c) and acidic (d,e,f) soils across soil depth profiles.”

Table.1

Equation	y=a*(b-exp(-c*x))				
Standard Error	0.07468171				
Correlation Coeff.(r)	0.999941				
Coeff.of Determination (r^2)	0.99988246				
DOF	54				
AICC	-294.6349				
Parameters					
Value	Std	Err	Range	(95%	confidence)
a	31.007446	0.214984	30.576428	to	31.438463
b	0.713759	0.002376	0.708995	to	0.718522
c	0.000043	0	0.000042	to	0.000043
Covariance Matrix					
	a	b	c		
a	8.286768	0.088333	-0.000018		
b	0.088333	0.001012	0		
c	-0.000018	0	0		

Table.2

Equation	y=a*(b^x)*(x^c)				
Standard Error	0.04365503				
Correlation Coeff.(r)	0.999981				
Coeff.of Determination (r^2)	0.99996128				
DOF	51				
AICC	-337.04644				
Parameters					
	Value	StdErr	Err	Range (95% confidence)	
a	0.851623	0.003025	0.84555	to	0.857697
b	0.999928	0	0.999928	to	0.999928
c	0.477819	0.000472	0.476871	to	0.478767
Covariance Matrix					
	a	b	c		
a	0.004803	0	-0.000745		
b	0	0	0		
c	-0.000745	0	0.000117		

Application of a laser-based spectrometer for continuous insitu measurements of stable isotopes of soil CO_2 in calcareous and acidic soils

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Abstract

The short-term dynamics of carbon and water fluxes across the soil-plant-atmosphere continuum are still not fully understood. One important constraint is the lack of methodologies that enable simultaneous measurements of soil CO_2 concentration and respective isotopic composition at a high temporal resolution for longer periods of time. $\delta^{13}C$ of soil CO_2 can be used to derive information on the origin and physiological history of carbon and $\delta^{18}O$ in soil CO_2 aids to infer interaction between CO_2 and soil water. We established a real-time method for measuring soil CO_2 concentration, $\delta^{13}C$ and $\delta^{18}O$ values across a soil profile at higher temporal resolutions up to 1Hz using an Off-Axis Integrated Cavity Output Spectrometer (OA-ICOS). We also developed a calibration method correcting for the sensitivity of the device against concentration-dependent shifts in $\delta^{13}C$ and $\delta^{18}O$ values under highly varying CO_2 concentration. The deviations of measured data were modelled, and a mathematical correction model was developed and applied for correcting the shift. By coupling an OA-ICOS with hydrophobic but gas permeable membranes placed at different depths in acidic and calcareous soils, we investigated the contribution of abiotic and biotic components to total soil CO_2 release. We found that in the calcareous Gleysol, CO_2 originating from carbonate dissolution contributed to the total soil CO_2 concentration at detectable degrees potentially due to CO_2 evasion from groundwater. Inward diffusion of atmospheric CO_2 was found to be rather pronounced in the topsoil layers at both sites. $\delta^{18}O$ values of CO_2 reflected most likely the $\delta^{18}O$ of soil water at the acidic soil site.

Key words: $\delta^{13}C$, $\delta^{18}O$, OA-ICOS, hydrophobic/gas permeable membrane.

1 Introduction

Global fluxes of CO_2 and H_2O are two major driving forces controlling earth's climatic systems. To understand the prevailing climatic conditions and predict climate change, accurate monitoring and modeling of these fluxes are inevitable (Barthel et al., 2014; Harwood et al., 1999; Schär et al., 2004). Approximately 30 - 35% of the global CO_2 flux is contributed by soil CO_2 efflux, which is, therefore, a significant determinant of the net ecosystem carbon balance (Schlesinger and Andrews, 1999). The long-term dynamics of CO_2 release on a seasonal scale are reasonably well understood (Satakhun et al., 2013), whereas less information on CO_2 dynamics and isotopic composition are available for short-term variations on a diurnal scale (Werner and Gessler, 2011). The lack of proper understanding of the diurnal fluctuations in soil CO_2 release might introduce uncertainty in estimating the soil carbon budget and the CO_2 fluxes to the atmosphere. The isotopic composition of soil CO_2 and its diel fluctuation can be a critical parameter for the partitioning of ecosystem gas exchange into its components (Bowling et al., 2003; Mortazavi et al., 2004) and for disentangling plant and ecosystem processes (Werner and Gessler 2011). By assessing $\delta 1^{3}C$ of soil CO_2, it is possible to identify the source for CO_2 (Kuzyakov, 2006) and the coupling between photosynthesis and soil respiration when taking into account post-photosynthetic isotope fractionation (Werner et al., 2012; Wingate et al., 2010). $\delta^{13}C$ soil CO_2 reflects, however, not only microbial and root respiration but also abiotic sources from carbonate weathering (Schindlbacher et al., 2015).

Soil water imprints its $\delta^{18}O$ signature on soil CO_2 as a result of isotope exchange between H_2O and CO_2 (aqueous). The oxygen isotopic exchange between CO_2 and soil water is catalyzed by microbial carbonic anhydrase (Sperber et al., 2015; Wingate et al., 2009). Thus, soil CO_2 can give information on the isotopic composition of both soil water resources and carbon sources. The oxygen isotope composition of plant-derived CO_2 is both, a tracer of photosynthetic and respiratory CO_2 and gives additional quantitative information on the water cycle in terrestrial ecosystems (Francey and Tans, 1987). To better interpret the $\delta^{13}C$ and $\delta^{18}O$ signals of atmospheric CO_2, the isotopic composition and its variability of the different sources need to be better understood (Werner et al., 2012; Wingate et al., 2010).

The conventional method to estimate $\delta^{13}C$ and $\delta^{18}O$ of soil CO_2 efflux is by using two end-member mixing models of atmospheric CO_2 and CO_2 produced in the soil (Keeling, 1958). The conventional methods for sampling soil produced CO_2 are chamber based (Bertolini et al., 2006; Torn et al., 2003), 'mini-tower' (Kayler et al., 2010; Mortazavi et al., 2004), and soil gas well (Breecker and Sharp, 2008; Oerter and Amundson, 2016) based methods. In conventional methods, air sampling is done at specific time intervals, and $\delta^{13}C$ and $\delta^{18}O$ are analyzed using Isotope Ratio Mass Spectrometry (IRMS) (Ohlsson et al., 2005). Such offline methods have several disadvantages like high sampling costs, excessive time consumption for sampling and analysis, increased sampling error and low temporal resolution. Kammer et al. (2011), showed how error-prone the conventional methods could be while calculating $\delta^{13}C$ and $\delta^{18}O$ (up to several per mil when using chamber and mini tower-based methods) (Kammer et al., 2011). In chamber-based systems, non-steady-state conditions may arise within the chamber due to increased CO_2 concentrations which in turn hinders the diffusion of $^{12}CO_2$ more strongly than that of heavier $^{13}CO_2$ (Risk and Kellman, 2008). Moreover, it has been found that $\delta^{18}O$ of CO_2 inside a chamber is significantly influenced by the $\delta^{18}O$ of the surface soil water as an equilibrium isotopic exchange happens during the upward diffusive movement of soil CO_2 (Mortazavi et al., 2004).

The advent of laser-based isotope spectroscopy has enabled cost-effective, simple, and high precision real-time measurements of $\delta^{13}C$ and $\delta^{18}O$ in CO_2 (Kammer et al., 2011; Kerstel and Gianfrani, 2008). This technique opened up new possibilities for faster and reliable measurements of stable isotopes, based on the principle of light absorption, using laser beams of distinct wavelengths in the near and mid-infrared range (Bowling et al., 2003).

In 1988, O'Keefe and Decon introduced the Cavity Ring-Down Spectroscopy (CRDS) for measuring the isotopic ratio of different gaseous species based on laser spectrometry (O'Keefe and Deacon, 1988). With the laser-based spectrometry techniques, measuring sensitivities up to parts per trillion (ppt) concentrations are achieved (von Basum et al., 2004; Peltola et al., 2012). In CRDS, the rate of change in the absorbed radiation of laser light that is temporarily "trapped" within a highly reflective optical cavity is determined. This is achieved using resonant coupling of a laser beam to the optical cavity and active locking of laser frequency to cavity length (Parameswaran et al., 2009). Another well-established technique similar to CRDS is Off-Axis Integrated Cavity Output Spectroscopy (OA-ICOS). It is based on directing lasers with narrowband and continuous-wave in an off-axis configuration to the optical cavity (Baer et al., 2002).

Even though OA-ICOS can measure concentration and isotope signature of various gaseous species at high temporal resolution, we found pronounced deviations in $\delta^{13}C$ and $\delta^{18}O$ measurements from the absolute values when measured under changing CO_2 concentrations. So far to our knowledge, no study has been made available detailing the calibration process of OA-ICOS CO_2 analyzers correcting for fluctuations of $\delta^{13}C$ and $\delta^{18}O$ values under varying CO_2 concentrations. Most of the OA-ICOS CO_2 analyzers are built for working under stable CO_2 concentrations, so that periodical calibration against in-house gas standards at a particular concentration is sufficient. However, as there are pronounced gradients in CO_2 levels in soils (Maier and Schack-Kirchner, 2014), CO_2 concentration depending shifts in measured isotopic values have to be addressed and corrected. Such calibration is, however, also relevant for any other OA-ICOS application with varying levels of CO_2 (e.g., in chamber measurements). Hence the first part of this work comprises the establishment of a calibration method for OA-ICOS. The second part describes a method for online measurement of CO_2 concentrations and stable carbon and oxygen isotope composition of CO_2 in different soil depths by coupling OA-ICOS with gas permeable hydrophobic tubes (Membrane tubes, Accurel®). The use of these tubes for measuring soil CO_2 concentration (Gut et al., 1998) and $\delta^{13}C$ of soil CO_2 (Parent et al., 2013) has already been established, but the coupling to an OA-ICOS system has not been performed, yet.

We evaluated our measurement system by assessing and comparing the concentration, $\delta^{13}C$ and $\delta^{18}O$ of soil CO_2 for a calcareous and an acidic soil system. The primary foci of this study are to (1) introduce OA-ICOS in online soil CO_2 concentration and isotopic measurements; (2) calibrate the OA-ICOS to render it usable for isotopic analysis carried out under varying CO_2 concentrations; and (3) analyze the dynamics of $\delta^{13}C$ and $\delta^{18}O$ of soil CO_2 at different soil depths in different soil types at a higher temporal resolution.

2 Materials and Methods

2.1 Instrumentation

The concentration, $\delta^{13}C$ and $\delta^{18}O$ values of CO_2 were measured with an OA-ICOS, as described in detail by (Baer et al., 2002; Jost et al., 2006). In this study, we used an OA-ICOS, (LGR-CCIA 36-d) manufactured by Los Gatos Research Ltd, San-Francisco, USA. LGR-CCIA 36-d measures CO_2 concentration, and $\delta^{13}C$ and $\delta^{18}O$ values at a frequency up to 1 Hz. The operational CO_2 concentration range was 400 to 25,000 ppm. Operating temperature range was +10 - +35°C, and sample temperature range (Gas temperature) was between -20°C and 50°C. Recommended inlet pressure was < 0.0689 MPa. The multiport inlet unit, an optional design that comes along with LGR-CCIA 36-d, had a manifold of 8 digitally controlled inlet ports and one outlet port. It rendered the user with an option of measuring eight different CO_2 samples at the desired time interval. Three standard gases with distinct $\delta^{13}C$ and $\delta^{18}O$ values were used for calibration in this study (See Supplementary Table.1). The standard gases used in this study were analyzed for absolute concentration and respective $\delta^{13}C$ and $\delta^{18}O$ values. δ-values are expressed based on Vienna Pee Dee Belemnite (VPDB)-CO_2 scale, and were determined by high precision IRMS analysis.

2.2 Calibration setup and protocol

We developed a two-step calibration procedure to; a) correct for concentration-dependent errors in isotopic data measurements, and b) correct for deviations in measured δ-values from absolute values due to offset (other than concentration-dependent error) introduced by the laser spectrometer. Also, we used Allan variance curves for determining the time interval to average the data (Nelson et al., 2008) to achieve the highest precision that can be offered by LGR-CCIA 36-d (Allan et al., 1997).

The first part of our calibration methodology was developed to correct for the concentration-dependent error observed in preliminary studies for $\delta^{13}C$ and $\delta^{18}O$ values measured using OA-ICOS. Such a calibration protocol was used in addition to the routine three-point calibration performed with in-house CO_2 gas standards of known $\delta^{13}C$ and $\delta^{18}O$ values. We developed a CO_2 dilution set up (See Figure. 1), with which each of the three CO_2 standard gases was diluted with synthetic CO_2 free air (synth-air) to different CO_2 concentrations. By applying a dilution series, we identified the deviation of the measured (OA-ICOS) from the absolute (IRMS) $\delta^{13}C$ and $\delta^{18}O$ values depending on CO_2 concentration (See Figure.4). The $\delta^{13}C$ and $\delta^{18}O$ values of our inhouse calibration gas standards were measured via cryo-extraction and Dual Inlet IRMS. $\delta^{13}C$, and $\delta^{18}O$ of the standard gases (See Supplementary Table.1) across a wide range of CO_2 concentrations are measured using OA-ICOS. The deviation of the measured $\delta^{13}C$, and $\delta^{18}O$ from absolute values with respect to changing CO_2 concentrations was mathematically modeled and later used for data correction (See Figure.5). A standard three-point calibration was then applied correcting for concentration-dependent errors (See Figure.7). The standards used covered a wide range of $\delta^{13}C$ and $\delta^{18}O$, including the values observed in the field of application.

Standard gases were released to a mass flow controller (ANALYT-MTC, series 358, MFC1) after passing through a pressure controller valve (See Figure. 1) with safety bypass (TESCOM, D43376-AR-00-X1-S; V5). A Swagelok filter, ((Stainless Steel All-Welded In-Line Filter (Swagelok, SS-4FWS-05; F1)) was installed at the inlet of the flow controller (ANALYT-MTC, series 358; MFC1). Synth-air was released and passed to another flow controller (ANALYT-MTC, series 358; MFC2) through a Swagelok filter (F2 in Figure. 1). CO_2 and synth-air leaving the flow controllers (MFC1 and MFC2 respectively) were then mixed and drawn through a ¼" Teflon tube (P8), which was kept in a gas thermostat unit (See Figure.1). The thermostat unit contained, a) a thermostat-controlled water bath (Kottermann, 3082) and b) an Isotherm flask containing liquid nitrogen. The water bath was used to raise the temperature above room temperature and also to bring the temperature down to +5°C, by placing ice packs in the water bath. To reach low temperatures (-20°C), we immersed the tubes in the isotherm flask filled with liquid N_2. Leaving the thermostat unit, the gas was directed to the multiport inlet unit of the OA-ICOS. By using the thermostat unit, we introduced a shift in the reference gas temperature and the aim was to test the temperature sensitivity of the OA-ICOS in measuring $\delta^{13}C$ and $\delta^{18}O$ values. The third CO_2 standard gas (which is used for validation) was produced by mixing the other two gas standards in equal molar proportions in a 10L volume plastic bag with inner aluminum foil coating and welded seams (CO_2 mix: Linde PLASTIGAS®) under 0.03 MPa pressure by diluting to the required concentration using synth-air. The mixture was then temperature adjusted and delivered to the multiport inlet unit (MIU) by using a ¼" Teflon tube (P10). From the multiport inlet unit, calibration gases were delivered into the OA-ICOS for measurement using a ¼" Teflon tube (P9) at a pressure < 0.0689 MPa, with a flow rate of 500 mL/min. The gas leaving the OA-ICOS through the exhaust was fed back to the ¼" Teflon tube (P8) by using a Swagelok pipe Tee (Stainless Steel Pipe Fitting, Male Tee, ¼". Male NPT), intersecting P8 line before entering the thermostat unit. Thus, the gas fed was looped in the system until steady values were reported by the OA-ICOS based on CO_2 [ppm], $\delta^{13}C$ and $\delta^{18}O$ measurements. CO_2 gas standards were measured at 27 different CO_2 concentration levels ranging between 400 and 25,000 ppm. Every hour before sampling, synth-air gas was flushed through the system to remove CO_2 to avoid memory effects. The calibration gases were measured in a sequence one after the other four times. During each round of measurement, every calibration gas was diluted to different concentrations of CO_2 (400 - 25,000 ppm) and the respective isotopic signature and concentration were determined. For each measurement of $\delta^{13}C$ and $\delta^{18}O$ at a given concentration, the first 50 readings were omitted to avoid possible memory effects of the laser spectrometer and the subsequent readings for the next 256 seconds were taken and averaged to get maximum precision for $\delta^{13}C$ and $\delta^{18}O$ measurements. When switching between different calibration gases at the multiport inlet unit, synth-air was purged through the systems for 30 seconds to avoid cross-contamination.

2.3 Experimental Sites

In situ experiments were conducted to measure $\delta^{13}C$, $\delta^{18}O$ and concentrations of soil CO_2 in two different soil types (calcareous and acidic soil). The measurements in a calcareous soil were conducted during June 2014 in cropland cultivated with wheat (Triticum aestivum) in Neuried, a small village in the Upper Rhine Valley in Germany situated at 48°26'55.5"N, 7°47'20.7"E, 150 m a.s.l. The soil type described as calcareous fluvic Gleysol developed on gravel deposits in the upper Rhine valley. Soil depth was medium to deep, with high contents of coarse material (> 2 mm) up to 30 - 50%. Mean soil organic carbon (SOC) content was 1.2 - 2% and, SOC stock was ranging between 50 and 90 t/ha. The average pH was found to be 8.6. The study site receives an annual rainfall of 810 mm and has a mean annual temperature of 12.1°C.

In situ measurements in an acidic soil were conducted by the end of July 2014 in the model ecosystem facility (MODOEK) of the Swiss Federal Research Institute WSL, Birmensdorf, Switzerland (47°21'48" N, 8°27'23" E, 545 m a.s.l.). The MODOEK facility comprises 16 model ecosystems, belowground split into two lysimeters with an area of 3 m^2 and a depth of 150 cm. The lysimeters used for the present study were filled with acidic (haplic Alisol) forest soil and planted with young beech trees (Arend et al., 2016). The soil pH was 4.0 and a total SOC content of 0.8% (Kuster et al., 2013).

2.4 Experimental Setup

The OA-ICOS was connected to gas permeable, hydrophobic membrane tubes (Accurel® tubings, 8 mm OD) of 2 m length, placed horizontally in the soil at different depths. Tubes were laid in six different depths (4, 8, 12, 17, 35, and 80 cm) for calcareous soil and three (10, 30, and 60 cm) for acidic soil.

Technical details of the measurement setup are shown in Figure 2. Both ends of the membrane tubes were extended vertically upwards reaching the soil top by connecting them to gas impermeable Synflex® tubings (8 mm OD) using Swagelok tube fitting union (Swagelok: SS-8M0-6, 8 mm Tube OD). One end of the tubing system was connected to a solenoid switching valve (Bibus: MX-758.8E3C3KK) and by using a stainless-steel reducing union (Swagelok: SS-8M0-6-6M), to the outlet of the LGR CCIA 36-d by using ¼" Teflon tubing. The other end was connected via the multiport inlet unit to the gas inlet of the LGR CCIA 36-d.

This way, a loop was created in which the soil CO_2 drawn into the OA-ICOS was circulated back through the tubes and in and out of the OA-ICOS and measured until a steady state was reached. We experienced no drop in cavity pressure while maintaining a closed loop (See Supplementary Figure S2). Each depth was selected and continuously measured for 6 minutes at specified time intervals by switching to defined depths at the multiport inlet unit and also at the solenoid valve.

3 Results and Discussion

3.1 Instrument calibration and correction

The highest level of precision obtained for $\delta^{13}C$ and $\delta^{18}O$ measurements at the maximum measuring frequency (1Hz) were determined by using Allan deviation curves (see Figure 3). Maximum precision of 0.022‰ for $\delta^{13}C$ was obtained when the data were averaged over 256 seconds, and for $\delta^{18}O$, 0.077‰ for the same averaging interval as for $\delta^{13}C$.

To correct for CO_2 concentration-dependent errors in raw $\delta^{13}C$ and $\delta^{18}O$ data, we analysed data obtained from the OA-ICOS to determine the sensitivity of $\delta^{13}C$ and $\delta^{18}O$ measurements against changing concentrations of CO_2. We observed a specific pattern of deviance in the measured isotopic data from the absolute values (both for $\delta^{13}C$ and

$\delta^{18}O$) across CO_2 concentration ranging from 25,000 to 400 ppm (See Figure.4). Uncalibrated $\delta^{13}C$ and $\delta^{18}O$ measurements showed a standard deviation of 6.44 ‰ and 6.802 ‰ respectively, when measured under changing CO_2 concentrations.

The dependency of $\delta^{13}C$ and $\delta^{18}O$ values on the CO_2 concentration was compensated by using a nonlinear model. The deviations (Diff-δ) of the measured delta values ($\delta_{\mathbf{(OA\text{-}ICOS)}}$) from the absolute value of the standard gas ($\delta_{\mathbf{(IRMS)}}$) at different concentrations of CO_2 were calculated (Diff-δ = $\delta_{\mathbf{(OA\text{-}ICOS)}}$) - $\delta_{\mathbf{(IRMS)}}$). Several mathematical models were then fitted on Diff-δ as a function of changing CO_2 concentration (See figure.5). The mathematical model with the best fit for Diff-δ data was selected using Akaike information criterion corrected (AICc) (Glatting et al., 2007; Hurvich and Tsai, 1989; Yamaoka et al., 1978). The non-linear model fits applied for Diff-$\delta^{13}C$, and Diff-$\delta^{18}O$ measurements are given in Tables 1 & 2, respectively. For Diff-$\delta^{13}C$, a three-parameter exponential model fitted best with $r^2 = 0.99$ (see Table 3 for the values of the parameters, see supplementary Figure S3 (a) for model residuals), and a three-parameter power function model (see Table 2) with $r^2 = 0.99$ showed the best fit for Diff-$\delta^{18}O$ (see Table 3 for the values of the parameters, see supplementary Figure S3 (b) for model residuals). The best fit was then introduced into the measured isotopic data ($\delta^{13}C$ and $\delta^{18}O$) and corrected for concentration-dependent errors (See figure. 6). After correction, the standard deviation of $\delta^{13}C$ was reduced to 0.08 ‰ and of $\delta^{18}O$ to 0.09 ‰ for all measurements across the whole CO_2 concentration range.

After correcting the measured $\delta^{13}C$ and $\delta^{18}O$ values for the CO_2 concentration-dependent deviations, a three-point calibration (Sturm et al., 2012) was made by generating linear regressions with the concentration corrected $\delta^{13}C$ and $\delta^{18}O$ values against absolute $\delta^{13}C$ and $\delta^{18}O$ values (See Figure.7, see supplementary Figure S4 for linear regression residuals). Using the linear regression lines, we were able to measure the validation gas standard with standard deviations of 0.0826 ‰ for $\delta^{13}C$ and 0.0941 ‰ for $\delta^{18}O$.

For the LGR CCIA 36-d, we found that routine calibration (Correction for concentration-dependent error plus three-point calibration) was inevitable for obtaining the required accuracy, in particular under fluctuating CO_2 concentrations. The LGR CCIA-36d offers an option for calibration against a single standard, a feature which was already in place in a predecessor model (CCIA DLT-100) (Guillon et al., 2012). This internal calibration is sufficient, when LGR CCIA-36d is operated only under stable CO_2 concentrations. To correct for the concentration dependency, we introduced mathematical model fits, which corrected for the deviation pattern found for both $\delta^{13}C$ and $\delta^{18}O$. We assume that these deviations are instrument specific and the fitting parameters need to be adjusted for every single device. Experiments conducted to investigate the influence of external temperature fluctuations on OA-ICOS measurements did not show any significant changes in the temperature inside the optical cavity of OA-ICOS (See Supplementary Figure S1). The previous version of the Los Gatos CCIA was strongly influenced by temperature fluctuations during sampling (Guillon et al., 2012). The lack of temperature dependency as observed here with the most recent model can be mostly due to the heavy insulation provided with the system, which was not found in the older models.

Guillon et al. (2012) found a linear correlation between CO_2 concentration and respective stable isotope signatures with a previous version of the Los Gatos CCIA CO_2 stable isotope analyser. In our experiments with the OA-ICOS, best fitting correlation between CO_2 concentration and $\delta^{13}C$ and $\delta^{18}O$ measurements were exponential and power functions, respectively. We assume that measurement accuracy is influenced by the number of CO_2 molecules present inside the laser cavity of the particular laser spectrometer as we observed large standard deviation in isotopic measurements at lower CO_2 concentrations. This behavior of an OA-ICOS can be expected as it functions by sweeping the laser along an absorption spectrum, measuring the energy transmitted after passing through the sample. Therefore, energy transmitted is proportional to the gas concentration in the cavity. The laser absorbance is then determined by normalizing against a reference signal, finally calculating the concentration of the sample measured by integrating the whole spectrum of absorbance (O'Keefe et al., 1999).

3.2 Variation in soil CO_2 concentration, carbon and oxygen isotope values

Figures 9 and 10 show the CO_2 concentration, $\delta^{13}C$ and $\delta^{18}O$ measurements of soil CO_2 in the calcareous as well as in the acidic soil across the soil profile with sub-daily resolution and as averages for the day, respectively. We observed an increase in the CO_2 concentration across the soil depth profile for both, the calcareous and the acidic soil. Moreover, there were rather contrasting $\delta^{13}C$ values across the profile for the two soil types. In the calcareous soil, CO_2 was relatively enriched in ^{13}C in the surface soil (4 cm) as compared to the 8 cm depth. Below 8 cm down to 80 cm depth, we found an increase in $\delta^{13}C$ values. At 80 cm depth, the $\delta^{13}C$ in soil CO_2 ranged between -7.15 and -3.35 ‰ (See Figure. 9) with a daily average of -6.19 ± 1.45 ‰ (See Figure. 10) and hence clearly above atmospheric values (≈ -8.0 ‰). For $\delta^{18}O$ values of calcareous soil, the depth profile showed no specific pattern except for the $\delta^{18}O$ values at 80 cm depth was found to be less negative than the values of the other depths. The $\delta^{18}O$ value in the top 4 cm was found to be slightly more enriched that the 8 cm depth and between 8 cm – 35 cm, $\delta^{18}O$ values showed little variation relative to each other. For the sub-daily measurements, we observed a sharp decline in $\delta^{18}O$ values at around 02:00, which is also observed but less pronounced for $\delta^{13}C$ signal. We assume that, the reason for such aberrant values is rather a technical issue than a biological process. It could be due to the fact that the internal pump in the OA-ICOS was not taking adequate amount of gas into the optical cavity, thereby creating a negative pressure inside the cavity resulting in the observed aberrant values. The patterns observed for the $\delta^{13}C$ values of CO_2 in the calcareous soil with ^{13}C enrichment in deeper soil layers can be explained by a substantial contribution of CO_2 from abiotic origin to total soil CO_2 release as a result of carbonate weathering and subsequent out-gassing from soil water (Schindlbacher et al., 2015). According to Cerling (1984), the distinct oxygen and carbon isotopic composition of soil carbonate depends on the isotopic signature of meteoric water and to the proportion of C_4 biomass present at the time of carbonate formation (Cerling, 1984). CO_2 released as a result from carbonates have a distinct $\delta^{13}C$ value close to 0‰ vs. VPDB, while CO_2 released during biological respiratory processes has usually $\delta^{13}C$ values around -24‰ as observed in the acidic soil (Figure 10 (e)). Even though the contribution of CO_2 from abiotic sources to soil CO_2 is often considered to be low, several studies have reported significant proportions ranging between (10 - 60%) emanating from abiotic sources (Emmerich, 2003; Plestenjak et al., 2012; Ramnarine et al., 2012; Serrano-Ortiz et al., 2010; Stevenson and Verburg, 2006; Tamir et al., 2011). Bowen and Beerling, (2004) showed that isotope effects associated with soil organic matter decomposition can cause a strong gradient in δ values of soil organic matter (SOM) with depth, but are not always reflected in the $\delta^{13}C$ values of soil CO_2. We have measured soil samples for bulk soil $\delta^{13}C$, bicarbonate $\delta^{13}C$ & $\delta^{18}O$ values and also determined the percentage of total carbon in the soil across a depth profile of (0-80 cm) (See Figure 8). We observed an increase in $\delta^{13}C$ values for bulk soil in deeper soil layers (See Figure 8 (a,c)). Moreover, also the carbonate $\delta^{13}C$ values got more positive in the 60-80 cm layer. Since total organic carbon content decreases with depth it can be assumed that CO_2 derived from carbonate weathering having less negative $\delta^{13}C$ more strongly contributed to the soil CO_2 (especially since we see an increase in soil CO_2 concentration with depth). This is accordance with the laser-based measurements which showed a strong increase in $\delta^{13}C$ of soil CO_2 in the deepest soil layer leading us to the hypothesis that this signal is indicating a strong contribution of carbonate derived CO_2. Water content and soil CO_2 concentration are two major factors influencing carbonate weathering, and variations in soil CO_2 partial pressure, moisture, temperature, and pH can cause degassing of CO_2 which contributes to the soil CO_2 efflux (Schindlbacher et al., 2015). We assume that at our study site, the topsoil is de-carbonated due to intensive agriculture for a longer period and thus soil CO_2 there originates primarily from autotrophic and heterotrophic respiratory activity. In contrast to the deeper soil layers, where the carbonate content is high, CO_2 from carbonate weathering is assumed to be a dominating source of soil CO_2. Also, outgassing of CO_2 from the large groundwater body underneath the calcareous Gleysol might contribute to the inorganic CO_2 sources in the deeper soil as we found ground water table to be 1-2m below the soil surface. Relative ^{13}C enrichment of the CO_2 in the topsoil (4 cm) compared to 8 cm depth is probably due to the invasive diffusion of atmospheric CO_2 which has a $\delta^{13}C$ value close to -8‰ (e.g., (Levin et al., 1995)). The $\delta^{18}O$ patterns for CO_2 between 4 and 35 cm might reflect the $\delta^{18}O$ of soil water with stronger evaporative enrichment at the top and ^{18}O depletion towards deeper soil layers. In comparison, the strong ^{18}O enrichment of soil CO_2 towards 80 cm in the calcareous Gleysol very likely reflects the ^{18}O values of groundwater lending further support for the high contribution of CO_2 originating from the outgassing of groundwater. We, however, need then to assume that that the oxygen in the CO_2 is not in full equilibrium with the precipitation influenced soil water. As mainly microbial carbonic anhydrase mediates the fast equilibrium between CO_2 and water in the soil and the microbial activity is low in deeper soil layers (Schmidt et al., 2011), we speculate that in deep layers with a significant contribution of ground-water derived CO_2 to the CO_2 pool, a lack of full equilibration with soil water might be the reason for the observed $\delta^{18}O$ values.

Soil CO_2 concentration in the acidic soil showed a positive relationship with soil depth as CO_2 concentration increased along with increasing soil depth (Figs. 9 & 10). CO_2 concentrations were distinctly higher than in the calcareous soil, very likely due to the finer texture than in the gravel-rich calcareous soil. $\delta^{13}C$ values amounted to approx. – 26 ‰ in 30 and 60 cm depth indicating the biotic origin from (autotrophic and heterotrophic) soil respiration (Schönwitz et al., 1986). In the topsoil, $\delta^{13}C$ values did not strongly increase, pointing towards a less pronounced inward diffusion of CO_2 in the acidic soil site, most likely due to more extensive outward diffusion of soil CO_2 as indicated by the still very high CO_2 concentration at 10 cm creating a sharp gradient between soil and atmosphere. Moreover, the acidic soil was rather dense and contained no stones, strongly suggesting that gas diffusivity was rather small. $\delta^{18}O$ depths patterns of soil CO_2 in the acidic soil were most likely reflecting $\delta^{18}O$ values of soil water as CO_2 became increasingly ^{18}O depleted from top to bottom. $\delta^{18}O$ of deeper soil layers CO_2 (30 - 60 cm) was close to the values expected when full oxygen exchange between soil water and CO_2 occurred (Kato et al., 2004). Assuming an ^{18}O fractionation of 41‰ between CO_2 and water (Brenninkmeijer et al., 1983) this would result in an expected value for CO_2 of ≈ -10 ± 2‰ vs. VPDB-CO_2. Corresponding results had been shown for $\delta^{18}O$ of soil CO_2 using similar hydrophobic gas permeable membrane tubes used when measuring $\delta^{18}O$ of soil CO_2 and soil water *in situ* (Gangi et al., 2015).

4 Conclusions

During our preliminary tests with the OA-ICOS, we found that the equipment was highly sensitive to changes in CO_2 concentrations. We found a non-linear response of the $\delta^{13}C$ and $\delta^{18}O$ values against changes in CO_2 concentration. Given the fact that laser-based CO_2 isotope analyzers are getting deployed more commonly in tracing various ecosystem processes, we think that it is important to address this issue. Therefore, we developed a calibration strategy for correcting errors introduced in $\delta^{13}C$ and $\delta^{18}O$ measurements due to the sensitivity of the device against changing CO_2 concentrations. We found that the OA-ICOS measures stable isotopes of CO_2 gas samples with a precision comparable to conventional IRMS. The method described in this work for measuring CO_2 concentration, $\delta^{13}C$ and $\delta^{18}O$ values in soil air profiles using an OA-ICOS and hydrophobic gas permeable tubes are promising and can be applied for soil CO_2 flux studies. As this set up is capable of measuring continuously for longer time periods at higher temporal resolution (1 Hz), it offers greater potential to investigate the isotopic identity of CO_2 and the interrelation between soil CO_2 and soil water. By using our measurement setup, we could identify abiotic as well as biotic contributions to the soil CO_2 in the calcareous soil. We infer that that degassing of CO_2 from carbonates due to weathering and evasion of CO_2 from groundwater may leave the soil CO_2 with a specific and distinct $\delta^{13}C$ signature especially when the biotic activity is rather low.

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Figure 1

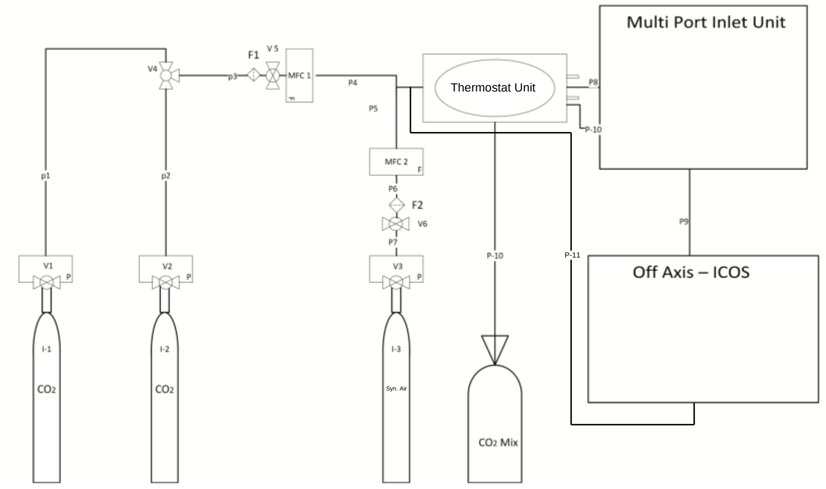

Figure 1: Setup made for calibration of OA- ICOS (LGR-CCIA 36-d). I(1,2): CO2 standards, CO2 Mix: Gas standards mixed in equal molar proportion, I3: Synthetic Air, MFC(1, 2): Mass Flow Controller, F(1, 2): PTFE filter, V(1, 2, 3): Pressure reducing Valves, V4: Three way ball valve, V(5,6): pressure controller valve with safety bypass , P (1-7): Steel pipes, P(8-11):Teflon tubing.

Figure 2

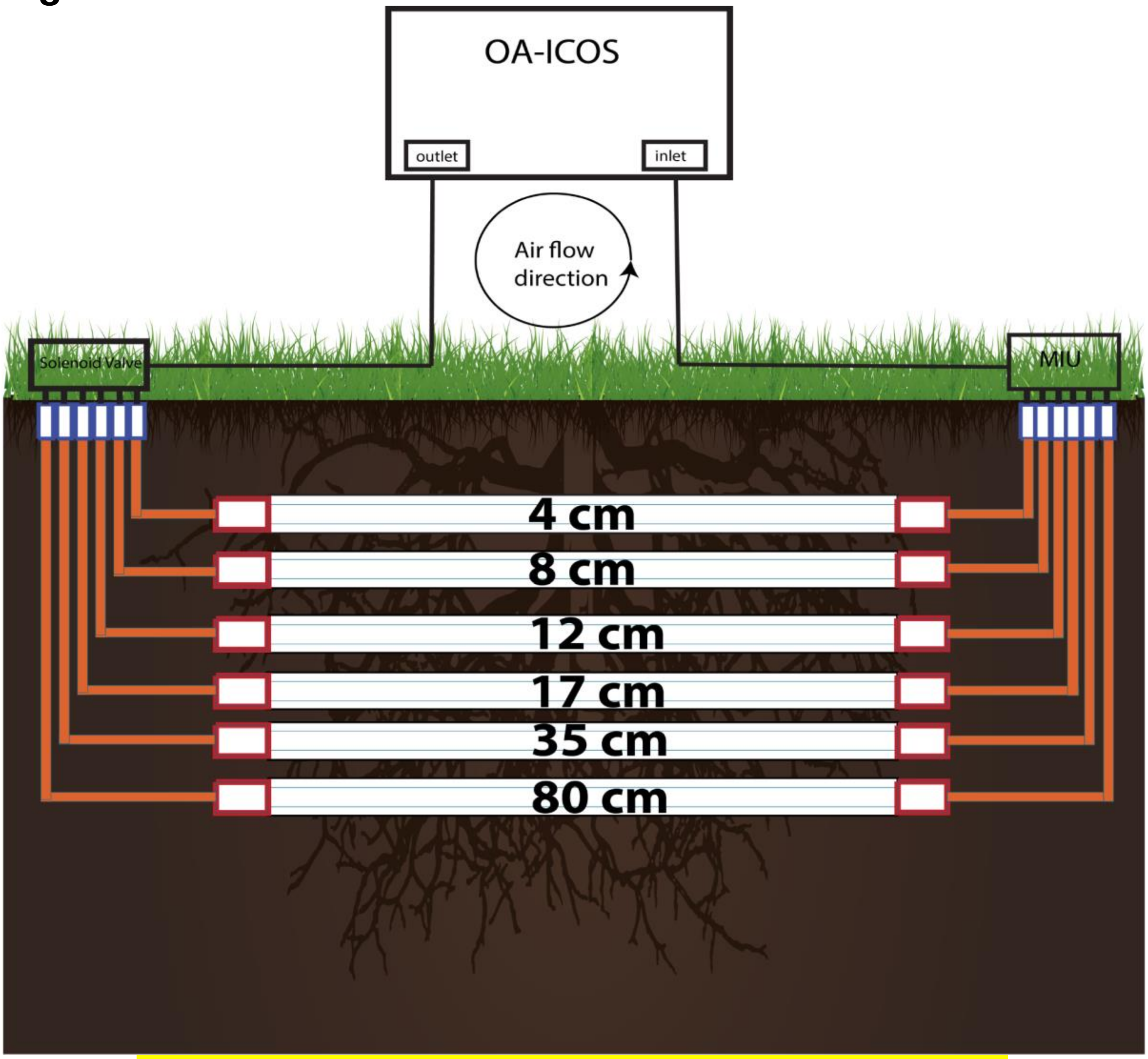

Figure 2: Installation made for soil air CO_2 [ppm], $\delta^{13}C-CO_2$ and $\delta^{18}O-CO_2$ measurements using an Off-Axis integrated cavity output spectrometer (OA-ICOS). Hydrophobic membrane tubing were installed horizontally in soil at different depths. MIU: multi-port inlet unit

Figure 3

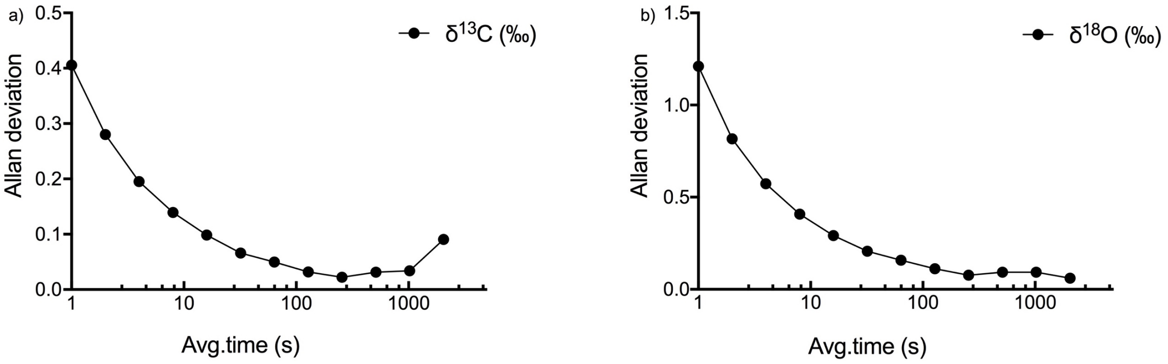

Figure 3: Allan deviation curve for $\delta^{13}C$ (a) and $\delta^{18}O$(b) measurements by OA-ICOS CO_2 Carbon isotope analyzer (LGR CCIA-36d).

Figure 4

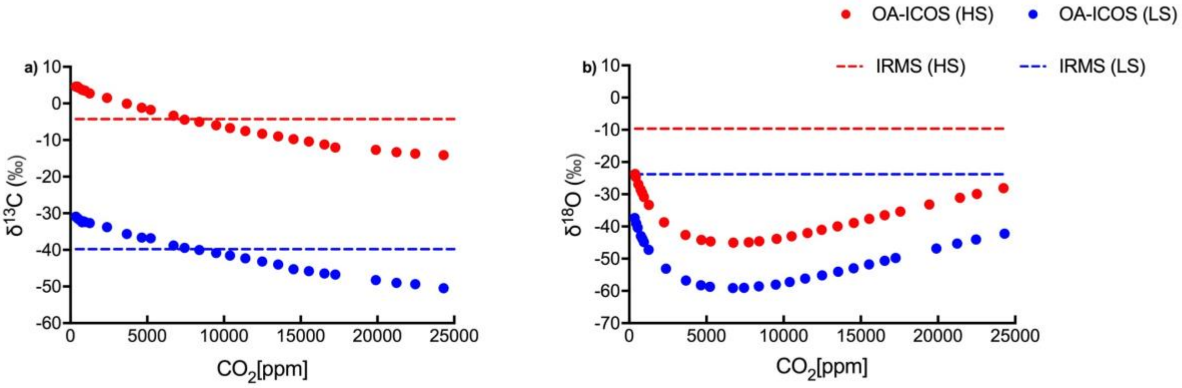

Figure 4: Variability observed in (a) $\delta^{13}C$ and (b) $\delta^{18}O$ measurements using OA-ICOS before calibration. $\delta^{13}C$ and $\delta^{18}O$ measured using OA-ICOS for Heavy Standard and Light Standard are shown as red and blue circles respectively. Actual $\delta^{13}C$ and $\delta^{18}O$ values reported after measuring by IRMS for heavy standard and light standard are shown as red and blue dashed lines respectively.

Figure 5

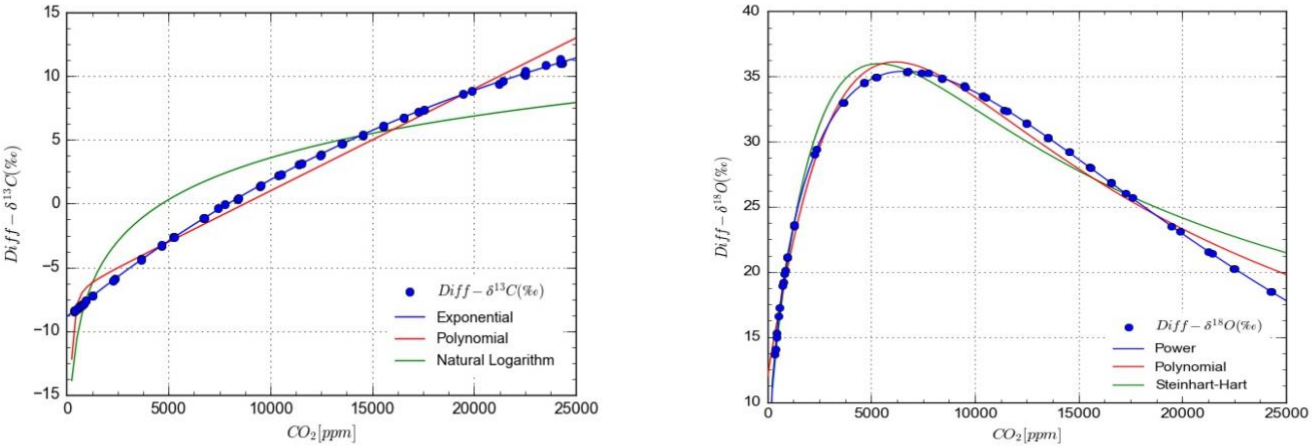

Figure 5: Mathematical models for concentration dependent drift in OA-ICOS measurements of stable isotopes of Carbon (a) and Oxygen (b) in CO_2 from IRMS measurements. Blue circles show Diff-δ^{13}C (a) and Diff-δ^{18}O (b) data points and lines represents different mathematical models fitted on the measured data.

Figure 6

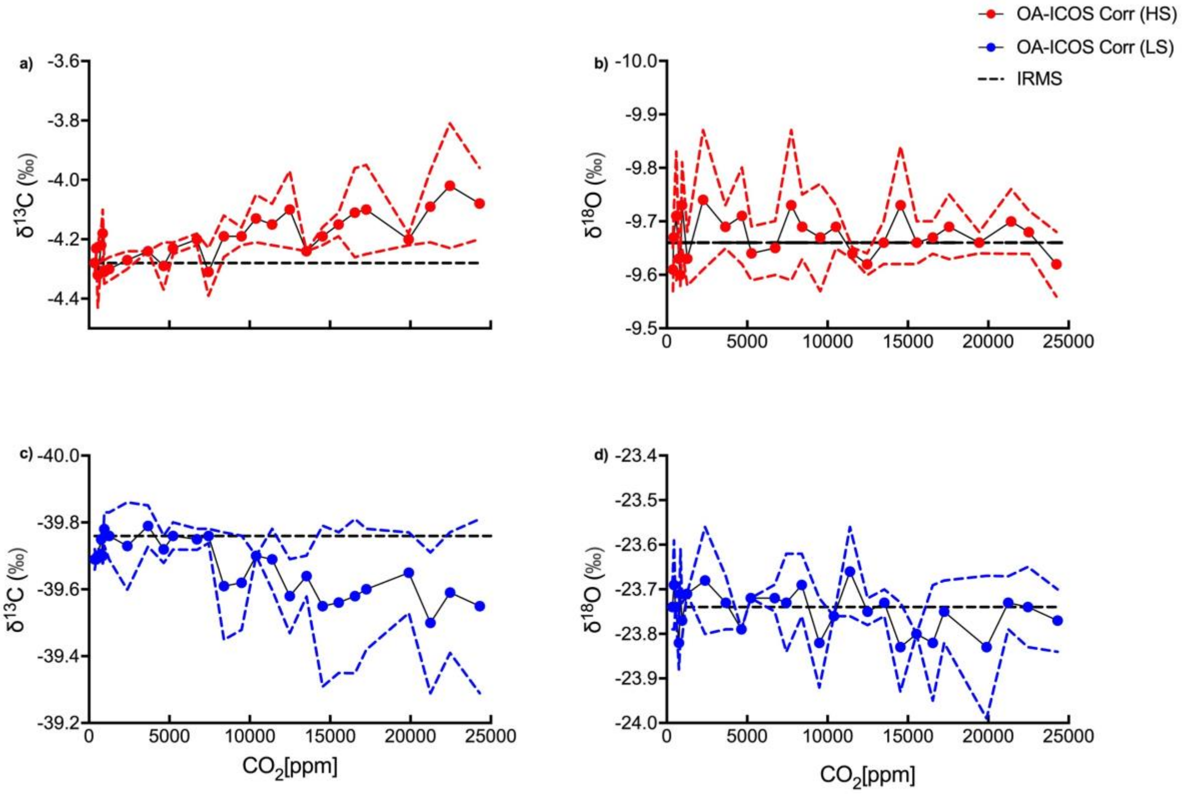

Figure 6: Corrected (a,c) $\delta^{13}C$ and (b,d) $\delta^{18}O$ measurements by OA-ICOS CO_2 Carbon isotope analyzer. $\delta^{13}C$ and $\delta^{18}O$ measured for Heavy Standard and Light Standard are shown as red and blue circles respectively. Actual $\delta^{13}C$ and $\delta^{18}O$ values reported after measuring by IRMS are shown as black dashed lines respectively.

Figure 7

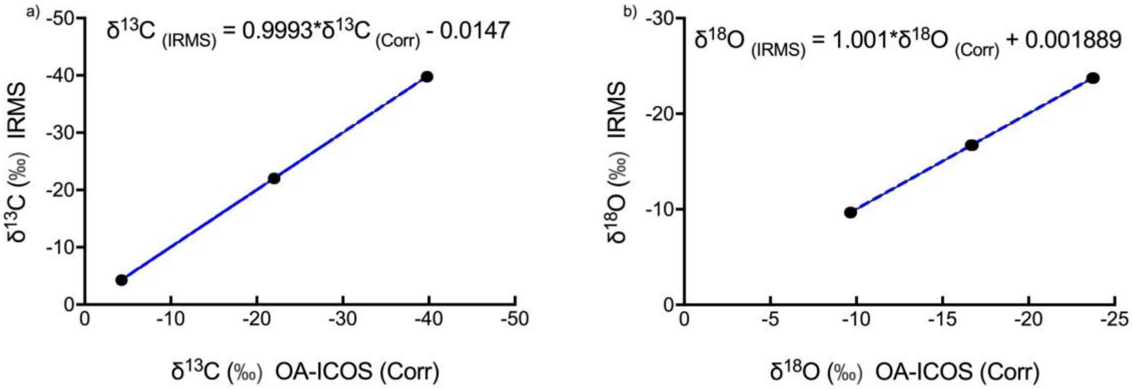

Figure 7: Three point Calibration lines for (a) $\delta^{13}C$ and (b) $\delta^{18}O$ measurements using OA-ICOS with 95% confidence interval.

Figure 8

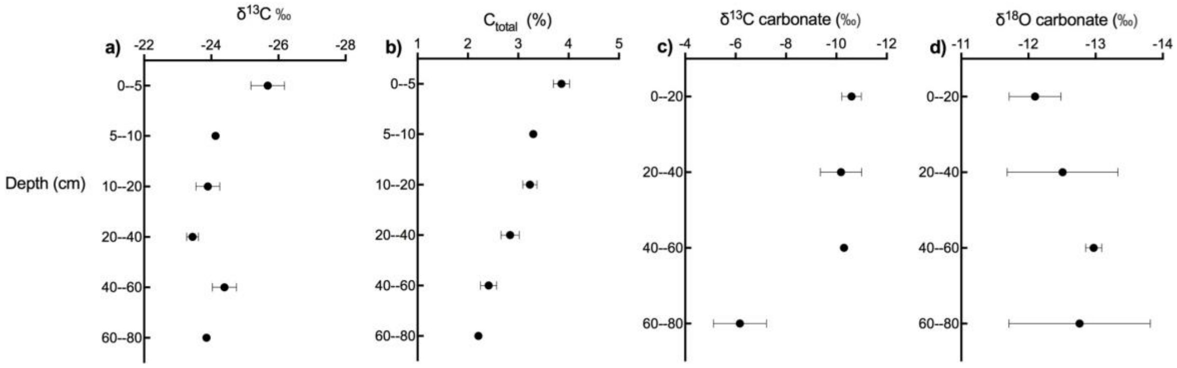

Figure 8: Depth profile of (a) δ^{13}C, (b) Carbon content, (c) δ^{13}C of soil carbonate and (d) δ^{18}O of soil carbonate in calcareous soil.

Figure 9

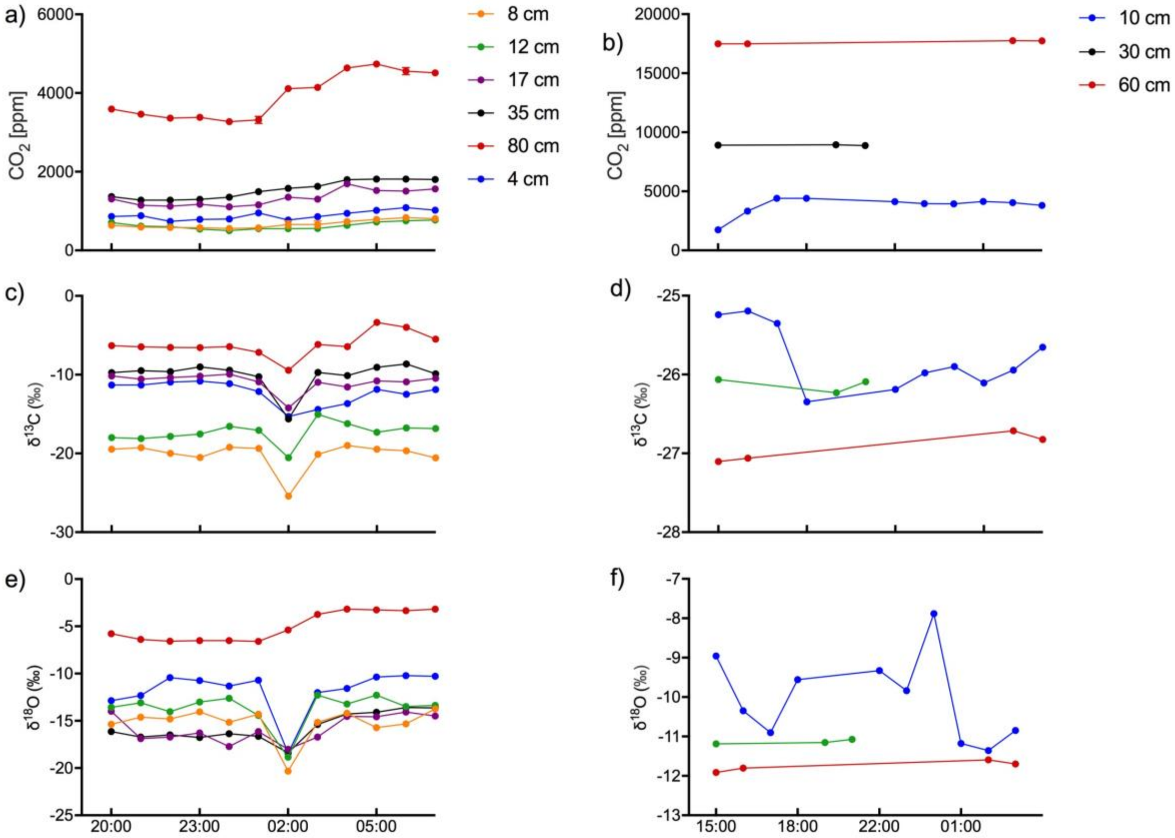

Figure 9: Time course of the evolution of soil gas CO_2 [ppm], $\delta^{13}C$ and $\delta^{18}O$ in calcareous (a,c,e) and acidic (b,d,f) soils. Data collected continuously over a 12 hour time frame for the calcareous soil and a 14 hour time window with intermittent data collection for the acidic soil.

Figure 10

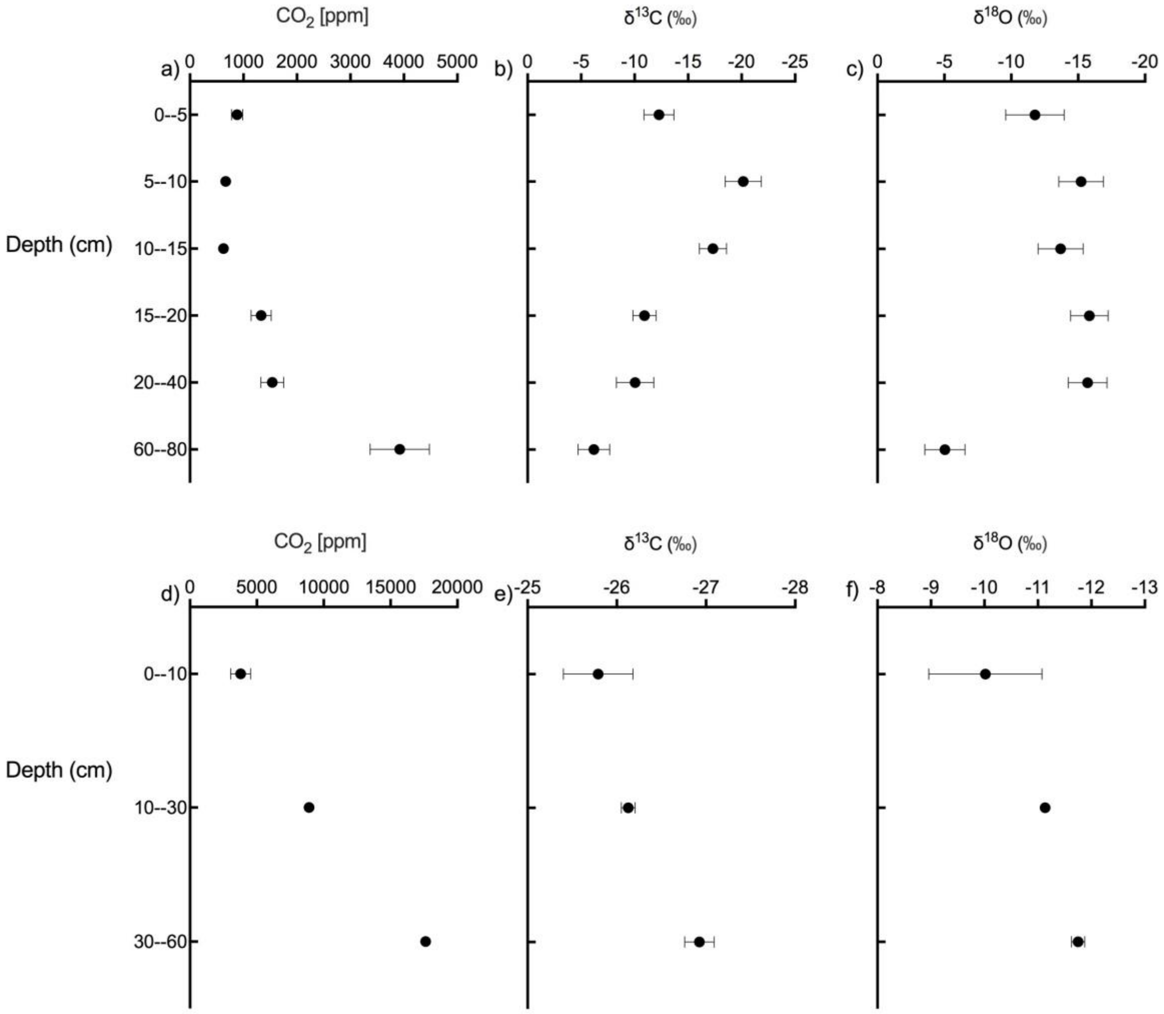

Figure 10: Daily average data of soil CO$_2$ [ppm], δ^{13}C and δ^{18}O in calcareous (a,b,c) and acidic (d,e,f) soils across soil depth profiles.

Table 1. Correction factor models are fitted for Diff-δ^{13}C, DF (Degrees of Freedom), AIC$_C$ (Akaike information criterion) and [CO$_2$] CO$_2$ concentration in ppm

Model Fit	**Equation**	**R^2**	**AIC$_C$**	**DF**
Exponential	$\boldsymbol{Diff-\delta 13C = \mathrm{a}*(\mathrm{b}-\exp(-\mathrm{c}*[CO2]))}$	**0.99**	**-294.6**	**54**
Polynomial	$\boldsymbol{Diff-\delta 13C\ =\ a\ +\ b*[CO2]+\ c/[CO2]\text{^}2)}$	**0.98**	**-27.56**	**54**
Logarithmic	$\boldsymbol{Diff-\delta 13C\ =\ a\ +\ b*ln([CO2])}$	**0.89**	**91.68**	**55**
Lowess	**------**	**0.99**	**-170.24**	**54**

Table 2. Correction factor models are fitted for Diff-δ^{18}O, DF (Degrees of Freedom), AIC$_C$ (Akaike information criterion) and [CO$_2$] CO$_2$ concentration in ppm.

Model Fit	**Equation**	**R^2**	**AIC$_C$**	**DF**
Power	$Diff-\delta 18O = a*(b^{[CO2]})*([CO2]^{c})$	**0.99**	**-337.04**	**51**
Polynomial	$Diff-\delta 18O = (a+b*x)/(1 + c*[CO2] + d*[CO2]\,\hat{}2)$	**0.98**	**-19.34**	**50**
Stein-Hart	$Diff-\delta 18O = 1/a+(b*\ln[CO2])+(c*(\ln[CO2])^{3})$	**0.96**	**29.77**	**51**
Lowess	-------	**0.78**	**128.66**	**51**

Table 3. Parameter values for correction factor model fit for Diff-δ^{13}C & Diff-δ^{18}O.

Parameter	**Value**	**Std Error**	**95% Confidence**
a^{13}C	**31.007**	**0.2149**	**30.57 - 31.43**
b^{13}C	**0.713**	**0.002376**	**0.708995 - 0.718522**
c^{13}C	**0.000043**	**0.000000**	**0.000042 - 0.000043**
a^{18}O	**0.85**	**0.003**	**0.8455 – 0.8576**
b^{18}O	**0.99**	**0.00**	**0.999928 – 0.9999283**
c^{18}O	**0.477**	**0.0047**	**0.476871 – 0.478767**

Figure S1

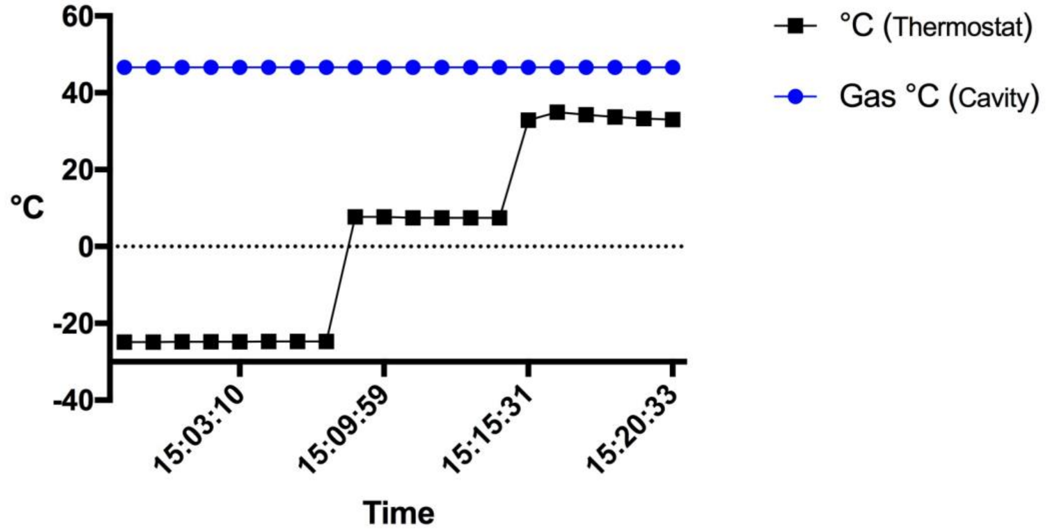

Figure S1: Temperature sensitivity of OA-ICOS. Influence of temperature fluctuations in thermostat unit (black square) on gas temperature inside the optical cavity (Blue circles).

Figure S2

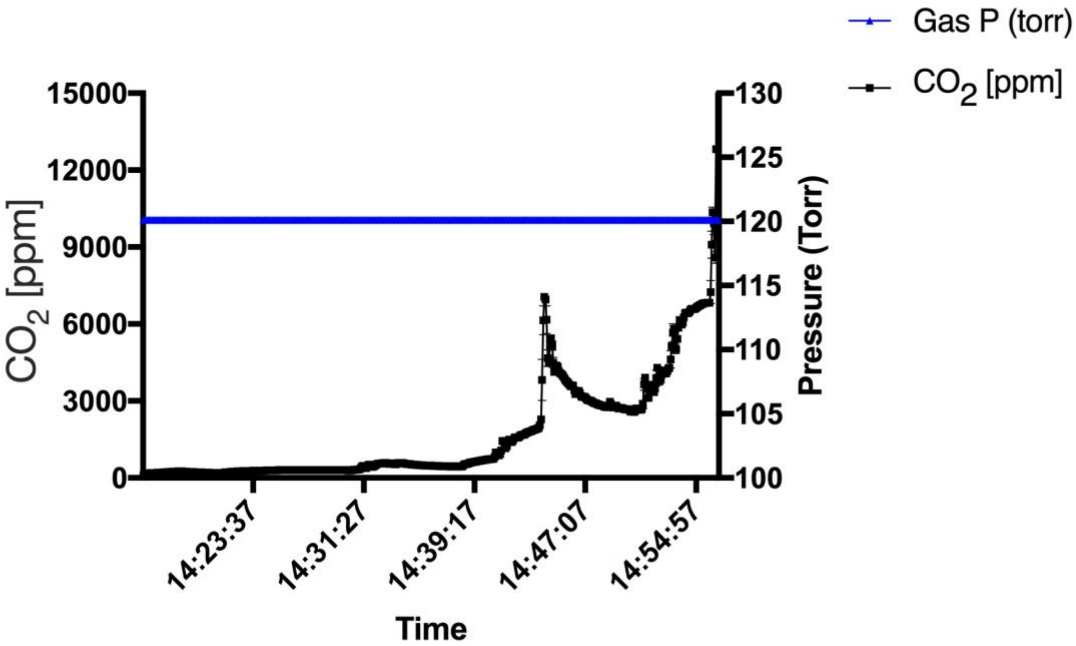

Figure S2: Optical cavity pressure data (blue line) of an OA-ICOS maintained in a closed loop of gas supply.

Figure S3

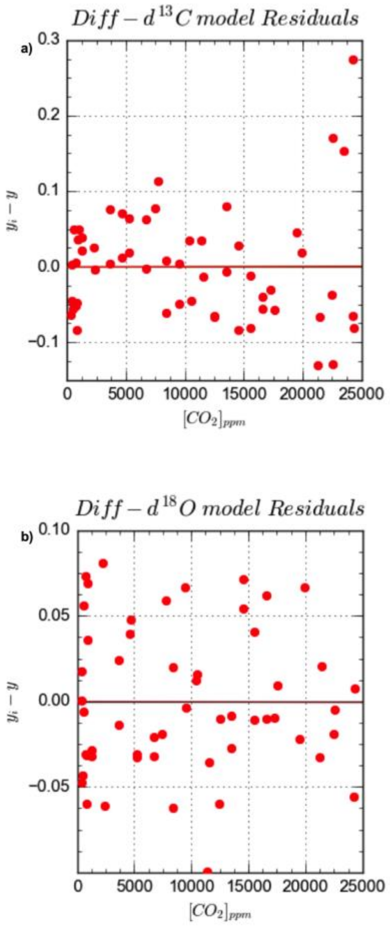

Figure S3: Residual distribution of modelled data for (a) Diff-$\delta^{13}C$ and (b) Diff-$\delta^{18}O$ values across changing CO_2 concentration (300 – 25000 ppm).

Figure S4

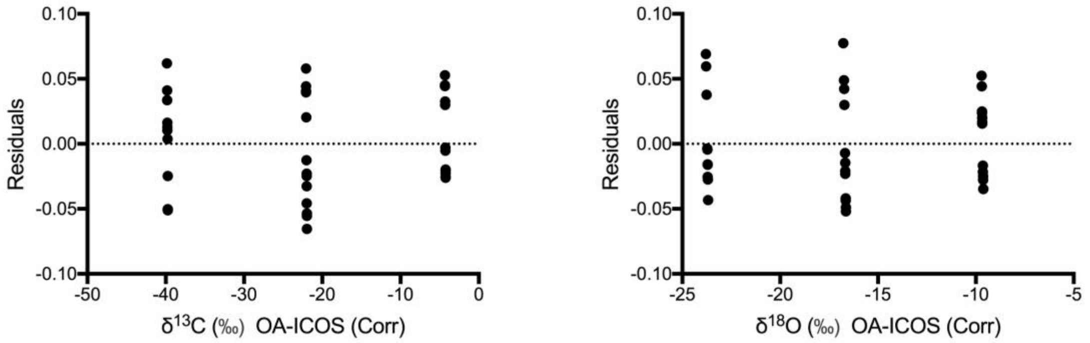

Figure S4: Residuals: Linear regression of Three-point calibration of (a) $\delta^{13}C$ (‰) OA-ICOS (Corr) and (b) $\delta^{18}O$ (‰) OA-ICOS (Corr).

Supplementary Table.1 The $\delta^{13}C$ and $\delta^{18}O$ values of the calibration standards used measured against VPDB.

CO_2 standard	$\delta^{13}C$	$\delta^{18}O$
Heavy standard	-4.28 ± 0.03‰	-9.66 ± 0.06‰
Validation standard	-22.02 ± 0.04‰	-16.63 ± 0.035‰
Light standard	-39.76 ± 0.04‰	-23.74 ± 0.035‰