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 $\delta^{13}C$ values and 4.7 (‰) for $\delta^{18}O$ values respectively. Diluting CO2 standard gases with synthetic air resulted in a standard deviation of 6.44(‰) and 6.818(‰) for $\delta^{13}C$ and $\delta^{18}O$ 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 $\delta^{13}C$ and 0.04(‰) for $\delta^{18}O$ (see Fig.2a-b (residual distribution), Fig.3a-d (Corrected $\delta^{13}C$ and $\delta^{18}O$ 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

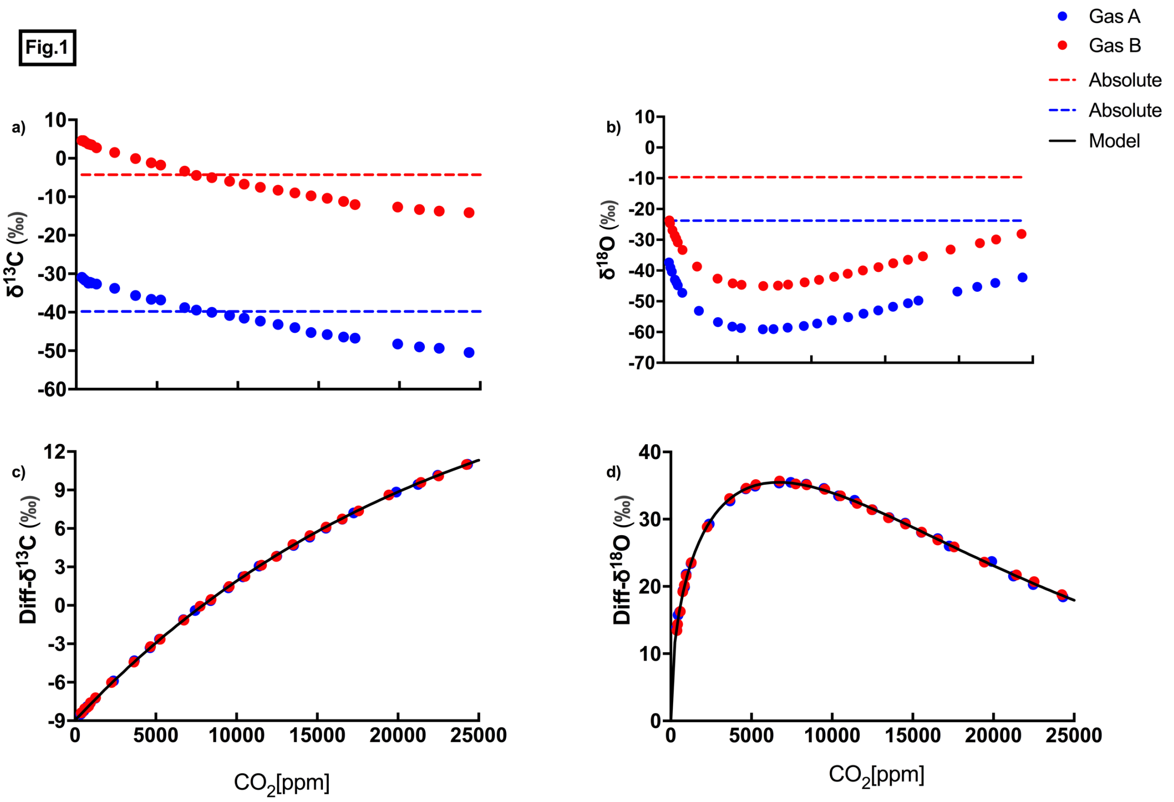


Figure.1: Deviation of measured $\delta^{13}C$ and $\delta^{18}O$ of CO2 (a,b) when diluted using synthetic air. (c-d) shows diff- $\delta^{13}C$, diff-$\delta^{18}O$ values across a concentration gradient. Red and Blue dots shows measured $\delta^{13}C$ & $\delta^{18}O$ values of two different gases 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

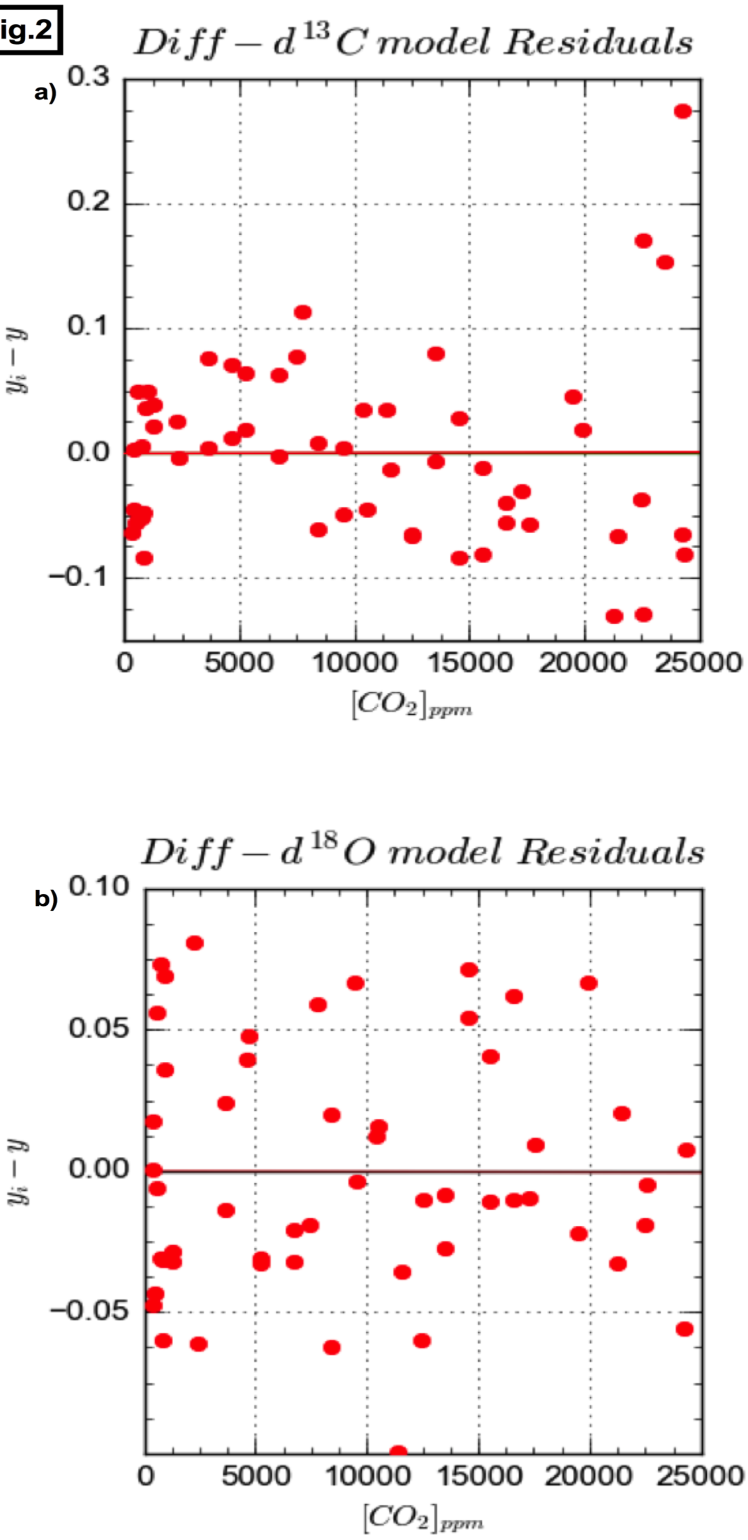


Figure 2: Residual distribution of modeled data for diff- δ^{13}C, diff-δ^{18}O values across changing CO2 concentration (300 – 25000 ppm).

Fig.3

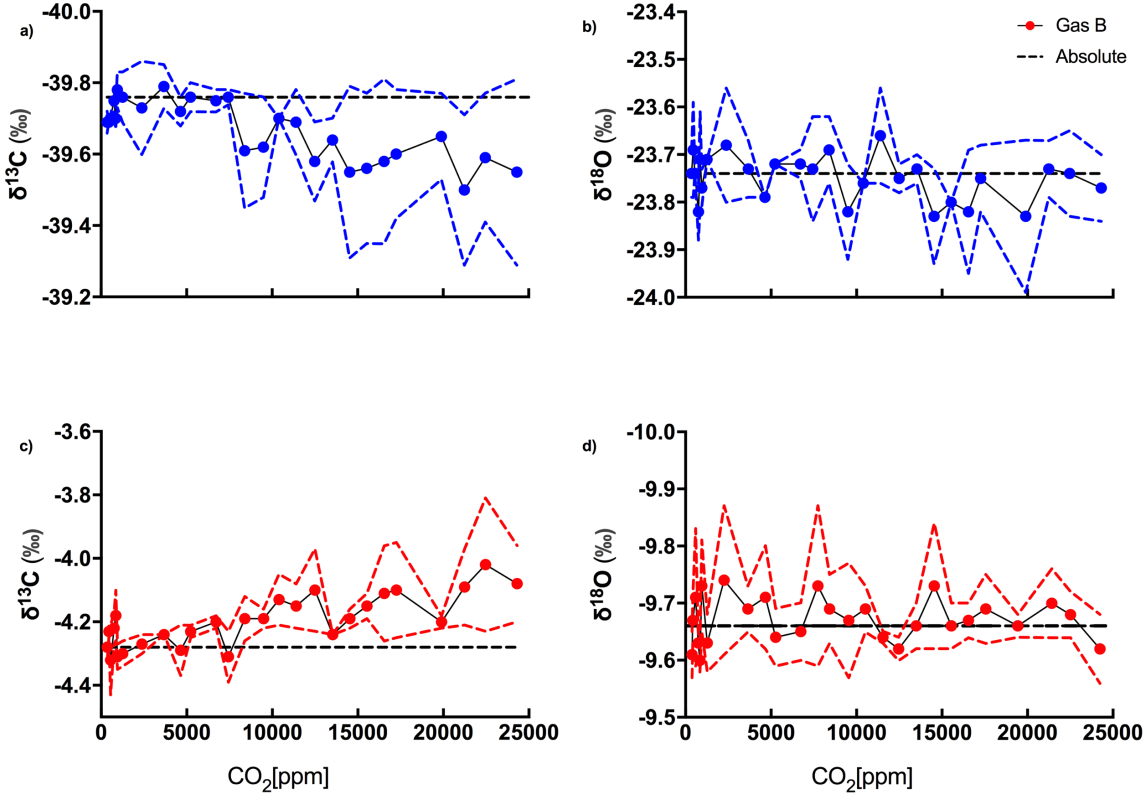


Figure 3: Corrected $\delta^{13}C$ & $\delta^{18}O$ values of two different standard gases measured after correcting for concentration dependent drift.

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

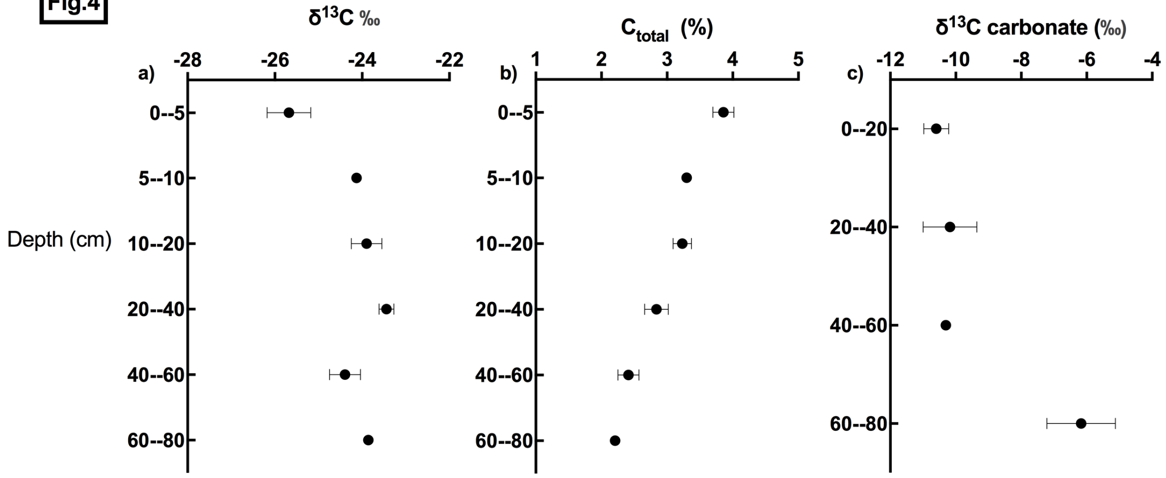


Figure.4: Bulk $\delta^{13}C$ (a), bicarbonate $\delta^{13}C$ (c) and % of carbon (b) in soil across a depth profile of (0-80 cm).

Fig.5

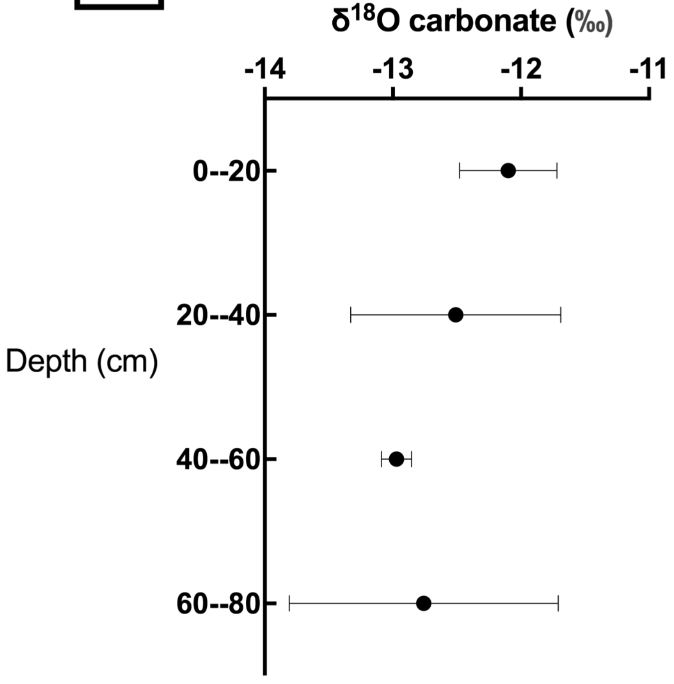


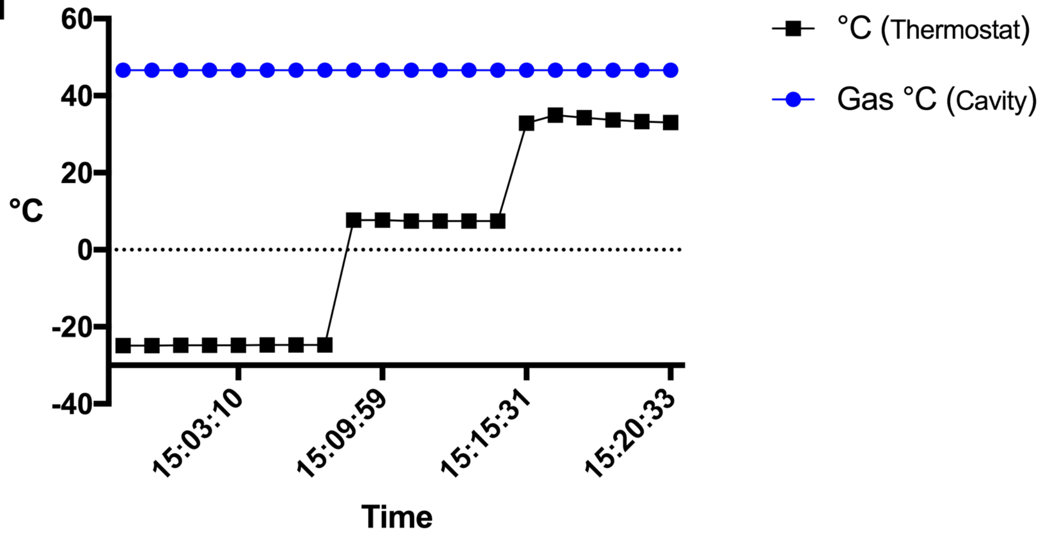
Figure.5: bicarbonate $\delta^{18}O$ in soil across a depth profile of (0-80 cm).

Response: We have measured soil samples for bulk $\delta^{13}C$, bicarbonate $\delta^{13}C$ & $\delta^{18}O$ values and also % of 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 $\delta^{13}C$ enriched CO2 in deeper soil layers.. Our aim is to establish a method which enables continuous online measurement of soil gas $\delta^{13}C$ & $\delta^{18}O$ 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.

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, never the less, 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



3. 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 will also let a native speaker do the final editing of the manuscript.

4. 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.

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

Response: Yes, water vapor was removed using drierite desiccant cartridges. We will 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		