Author comments, Anonymous referee #1

I thank referee 1 for perceptive and constructive comments and make the following responses:

Major comment:

The author recommends using the "direct calibration by isotopologues amounts" due to its apparent advantages (no pressure dependence, the need of only one reference gas in different "dilutions") as opposed to the calibration via different reference gases with different δ-value ("Calibration by delta values"). I think this recommendation can only be done if the targeted precision in δ-value is not exceeding the precision of the absolute concentrations of the reference gas and its "dilutions". For example, the precision of CO_2 absolute concentration in reference gases from international reference labs is around 0.07 ppm[1]. The same type of labs can provide the $δ_{13}C(CO_2)$ in such gases using IRMS with a precision in the 0.02‰ range or better, while this δ-value is relative to a material with a specific isotopic composition (reference material) and therefore independent of the absolute concentration of CO_2 in that gas. The 0.02‰ in $δ_{13}C(CO_2)$ corresponds to a precision in absolute concentration of about 0.008 ppm (for a 400 ppm concentration gas), so about one order of magnitude better than the absolute concentration actually can be known (in absolute, SI-traceable fashion).

As far I understand the recommended calibration approach, it ultimately depends on the precision of the absolute CO_2 concentration of the used gases. Since the current limit of these values is around 0.07 ppm, the corresponding precision in $δ_{13}C(CO_2)$ cannot be better than about 0.2‰ (exceptions exist if the measured value is very close to the used reference gas in the calibration). If the user dilutes its reference gas in a self-made experiment, it's very likely that the dilution process will introduce additional uncertainty making the in $δ_{13}C(CO_2)$ precision (and obviously also the precision of the absolute CO_2 concentration) worse.

This disadvantage of the "direct calibration by isotopologues amounts" has to be addressed in the manuscript and the suggestion has to be adapted accordingly. A 0.2‰ in $δ_{13}C(CO_2)$ is one order of magnitude above the GAW recommendations for which reason the proposed calibration method seems not applicable for instruments applied in the context of GAW. Some of the references used in the manuscript as an example of the alternative "Calibration by delta values" are used in the GAW context with corresponding precision targets. So the context of these references has to be reconsidered.

Response:

This is a valid comment, also raised by referee 2, but highlights some misunderstandings which clearly should be clarified.

Firstly there is the minor misconception that the reference standards for absolute isotopologue calibration are obtained by dilution of a high-level standard. This is not usually the case, the reference gases are normally obtained as whole air mixtures individually calibrated by a certified GAW laboratory (such as NOAA, ICOS or Gaslab) with a range of CO2 mole fractions but in a matrix of other gases (N2, O2, Ar, CH4,, CO, N2O) which are typical of atmospheric composition. Thus there is no loss of accuracy due to an in-lab dilution process. If standards are prepared in the reference gas supply lab over a range of mole fractions using the same CO2 source gas the delta values will be the same for all standards over the range of mole fractions – this was the case in Flores et al. Such standards are easier to prepare but suitable only for isotopologue-based calibration.

The referee points out that for reference standards with absolute mole fractions known to 0.07 ppm (0.18‰), the best possible absolute accuracy for $\delta^{13}C$ would be ~0.2‰. However this is only true if the individual isotopologue mole fractions are each individually known to 0.18‰ with independent errors(the uncertainty in $\delta^{13}C$ would actually be sqrt(2) greater, or 0.25‰ because there are two measurements in the calculation of delta). In practice the calibration lab provides a total CO2 amount fraction (accuracy say 0.18‰) and $\delta^{13}C$ value (accuracy say 0.02‰). Following the calculations in this paper, the 636/626 ratio is as accurately known as the reference $\delta^{13}C$ value provided (not the total CO_2 accuracy), and the individual isotopologue values as accurately as the total CO2 accuracy. The accuracy of the isotope ratio provided by the calibration lab is preserved through the isotopic calculations and the calibration process.

Now consider the optical measurement, whether FTIR or laser-based. Using the 1-min Allan deviation figures from Griffith et al (2012) as an example, we take a repeatability for CO2 isotopologues of 0.02 ppm (0.05‰) and for $\delta^{13}C$ we take 0.07‰ (which is approximately 0.05*sqrt(2) ‰). For laser instruments the figures are similar. If errors in retrieving the individual isotopologue amounts from the spectra were to some degree correlated, the $\delta^{13}C$ repeatability would be better. (This may be more the case for laser instruments analysing single lines than for FTIR analysing whole spectral bands, as pointed out in a specific comment by this referee). Thus the *repeatability* of the spectral $\delta^{13}C$ measurement in this example is of the order of 0.07‰, but its *accuracy* is determined by the calibration against reference standards, which is governed by the reference $\delta^{13}C$ values and may be better (ie the measurement of delta may be more accurate than it is precise). The ultimately reported accuracy of the sample delta value will be limited by the largest source of error, the isotopologue measurement repeatabilities, and thus of the order of 0.07‰. It is not determined by the absolute accuracy of the total CO2 amounts in the reference gases. This accuracy is still larger than the GAW-GGMT requirement, but is the current state of the art for optical measurements.

Finally, it is important to recognise that both the absolute and the ratio calibration methods take a ratio of 636/626 to determine ^{13}r and $\delta^{13}C$ and thus both take advantage of the accuracy of the isotope ratio calibration of the reference gases. There is no equivalent in optical measurements to IRMS, where the instrumentation primarily measures a ratio of ion counts, but not individual amounts. In this sense both methods are equally precise.The difference between the absolute and ratio calibration of optical measurements lies in whether the calibrated or uncalibrated 636/626 ratio is used. In the former case (ratio of calibrated 636 and 626) the calibrated $\delta^{13}C$ is calculated directly from the calibrated isotopologue amounts, but in the latter (ratio calibration) a range of $\delta^{13}C$ in reference gases is required, and there is an unavoidable concentration dependence (Eq. 15) which must be characterised. The form of eq 15 suggests a 3-parameter fit to characterise this concentration dependence, but the coefficients β and γ are not actually constants so the parameterisation is only approximate, and the values of β and γ are related to the instrument calibration and should be in principle re-determined with every calibration. In my FTIR experience, the residuals in fitting data to Eq 15 may result in residuals as large as 0.5‰. Other instruments may take a different approach to determining this concentration dependence, but it is likely to add error to the determined delta values, and can never reduce the error.

To address these comments I have extended the discussion of ratio calibration in section 4.2 to include a figure illustrating the concentration dependence through application of Eq. 15 and the potential errors using real FTIR data, and I have added a new section 4.3 which is a condensed form of the response given above.

Added to 4.2

describe the concentration dependence. The linear term becomes relatively more important than the inverse term at high CO_2 mole fractions where the inverse CO_2 term becomes small and any quadratic contribution to the calibration equation leading to the linear term becomes large.

Figure 1 illustrates this concentration dependence with a typical $\delta^{13}C$ vs CO_2 dependence for an FTIR analyser similar to that used in the example of section **Error! Reference source not found.** below. The dependence was determined by continuous flow measurements of a single CO_2-spiked air tank while the CO_2 content was gradually reduced by passing a fraction of the flow through Ascarite. The measured $\delta^{13}C$ vs CO_2 data are fitted to Eq **Error! Reference source not found.** with fitted parameters β = - 1227 ‰ ppm and γ = 0.0054 ‰ ppm^{-1}, corresponding to CO_2 - dependent corrections of up to 5‰ over the CO_2 range 400-1000 ppm. The residuals of the fit illustrate potential errors from the modelled behaviour of up to ± 0.3‰. Uncertainties in calibrating the CO_2 concentration dependence can lead to significant errors in Keeling-type analyses over a wide range of total CO_2 amounts even if the isotopologue calibration non-linearity is very small (Pang et al., 2016; Wen et al., 2013).
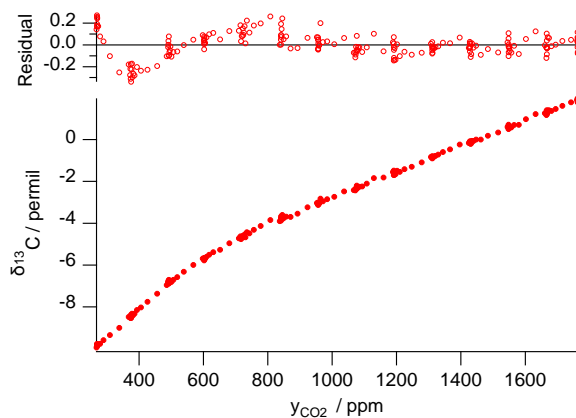

Figure 1. Example of $\delta^{13}C$ dependence on CO_2 mole fraction for a Spectronus FTIR analyser. The measured data are fitted with a function of form of Eq. Error! Reference source not found. **with fitted parameters β = - 1227 ‰ ppm and γ = 0.0054 ‰ ppm^{-1}.**

Added 4.3

Comments on the accuracy of optical isotopologue and ratio calibration

As an example assume a calibration laboratory provides calibrated reference gases with an absolute accuracy of 0.05 ppm for total CO_2 amount (0.12‰ in 400 ppm CO_2) and 0.02‰ for $\delta^{13}C$ measured by IRMS. The isotope ratio is thus more accurately determined than the total amount fraction for the reference gases. Now take as a practical measurement repeatability for optical analysers 0.02 ppm

(0.05‰) for total CO_2 amount and 0.07‰ for $\delta^{13}C$ (e.g. Griffith et al. (2012), laser instruments similar). The absolute accuracy for the calibrated optical measurement of total CO_2 is limited by the reference gas amount fraction, but the more accurately known reference ^{13}r or 626/636 ratio is carried through the calibration calculations and this accuracy is preserved when measured isotopologue amounts are ratioed. The accuracy of measured ^{13}r or $\delta^{13}C$ is thus limited by the optical measurement (0.07‰) which is less precise than the IRMS-provided reference accuracy (0.02‰). This does not currently meet GAW requirements for clean background air measurements (WMO-GAW, 2016), but is nevertheless useful for continuous analysis of air in non-baseline scenarios such as urban air or agricultural flux measurements.

This reasoning applies to both isotopologue and ratio calibration schemes, which both benefit from the higher accuracy in the isotopologue ratios than in absolute isotopologue amounts. The principle differences between the isotopologue and ratio calibration schemes are twofold:

- The isotopologue scheme does not require calibration gases spanning a range of delta values, it is sufficient to span the range of total amount fractions of interest. This simplifies the preparation of reference gases for calibration laboratories.
- The ratio scheme has an unavoidable CO_2 concentration dependence which must be characterised and leads potentially to a loss of accuracy, as shown in section **Error! Reference source not found.**. This complicates the calibration procedure for optical analysers.

Errors are discussed further in section 6.

Specific comments

Clumped isotopes are mentioned a few times in the manuscript, but there is no example of it. It would be nice to have an example for the uneducated reader. In general, one could consider to extend table 2 with all 18 (or 12 distinct) possible isotopologues as an overview and list its abundances (as much as known) as an overview.

There is a confusion (from both referees) here between multiply-substituted isotopologues (isotopologues with more than one minor isotope) and clumped isotopes (referring to multiply-substituted isotopologues where the relative amounts of the isotopes are not statistical, eg where two isotopes are both enriched together above their bulk statistical abundances). To introduce clumped isotopes more clearly, as requested, I have amended the text at first usage in section 2:

Using CO_2 as an example, considering the stable isotopes ^{12}C, ^{13}C, ^{16}O, ^{17}O and ^{18}O, there are eighteen possible isotopologues (2 x 3 x 3 isotopic possibilities). ^{14}C is a negligible proportion of total carbon for these purposes and is neglected. Only twelve of these eighteen possibilities are distinct due to symmetry. Assuming the substitution of each isotope at each position in the molecule follows its bulk statistical abundance (i.e. no clumping, see section **Error! Reference source not found.**), only four independent quantities are required to fully define the total amount and full isotopic composition of CO_2. These quantities may equivalently be the total CO_2 amount and three isotopic ratios ^{13}r, ^{17}r and ^{18}r (or delta values $\delta^{13}C$, $\delta^{17}O$ and $\delta^{18}O$), or the amounts of four individual isotopologues with each isotope substituted, most conveniently $^{16}O^{12}C^{16}O$, $^{16}O^{13}C^{16}O$, $^{16}O^{12}C^{17}O$ and $^{16}O^{12}C^{18}O$. Once these are known, the abundances of all multiply-substituted isotopologues can be calculated.

I have also expanded and clarified this description of "clumping" in section 6:

The relative amounts of multiply-substituted minor isotopologues are assumed to be in statistical relative abundance, i.e. there is no isotope clumping. Clumping refers to the case where the enrichment (or depletion) of two or more isotopes in a multiply-substituted isotopologue are correlated rather than each following their statistical amounts independently. Clumping effects are normally much less than 1‰, and according to **Error! Reference source not found.** therefore insignificant.

-Page 4, line 2 and following formulas: you shorten from n($_{12}$C) to $_{12}$C. I recommend to not do this because it complicates the comparison with the previous formulas and the shortening is only used in the following formulas (as much I can see).

Done:

$$
^{12}x = \frac{n(^{12}C)}{n(^{12}C) + n(^{13}C)} = \frac{1}{(1 + {}^{13}r)}
$$

$$
^{13}x = \frac{n(^{13}C)}{n(^{12}C) + n(^{13}C)} = \frac{{}^{13}r}{(1 + {}^{13}r)}
$$

$$
^{16}x = \frac{n(^{16}O)}{n(^{16}O) + n(^{17}O) + n(^{18}O)} = \frac{1}{(1 + {}^{17}r + {}^{18}r)}
$$

$$
^{17}x = \frac{n(^{17}O)}{n(^{16}O) + n(^{17}O) + n(^{18}O)} = \frac{{}^{17}r}{(1 + {}^{17}r + {}^{18}r)}
$$

$$
^{18}x = \frac{n(^{18}O)}{n(^{16}O) + n(^{17}O) + n(^{18}O)} = \frac{{}^{18}r}{(1 + {}^{17}r + {}^{18}r)}
$$

-Page 4, line 11-16: This section is not very clear to me. You only take 4 distinct isotopologues into consideration, but R_{sum} seems to be the sum of all 18 (according to the current text). Or how do I have to understand this? R_{sum} seems to be a rather abstract parameter, or does it have any easy- understandable meaning?

R_{sum} is analogous to a partition sum over all possible energy levels of a molecule and provides a convenient normalising factor when calculating relative isotopologue abundances (cf relative populations in the case of energy levels). In practice it is most easily thought of as the ratio of the total amount of CO_2 in a sample to the that of the major isotopologue. For example if Rsum=1.01, 1/Rsum = 0.99 and 99% of the sample is 626. I have expanded the wording in this paragraph as follows to try and make this clearer:

The labels 626, 636, 628, 627 are the common isotopic shorthand used in spectroscopy and the Hitran database. The sum of all isotopologue abundances x over all 18 isotopologues is equal to unity. R_{sum} is a sum of 18 products of isotope ratios, one corresponding to each of the 18 possible isotopologues of CO_2. R_{sum} conveniently accounts for all possible isotopologues in calculations of abundances, providing a normalising factor somewhat analogous to a partition sum over all energy levels of a molecule. From Eq. **Error! Reference source not found.**, $x_{626} = 1 / R_{sum}$ i.e. $1 / R_{sum}$ is the fractional abundance of the major isotopologue and $R_{sum} - 1 \approx 1 - x_{626}$ is that fraction of the sample that is made up of all the minor isotopologues. Equivalently from Eq.
Error! Reference source not found. and the following paragraph it can be seen that R_{sum} is the ratio of the total amount of CO_2 to that of the major isotopologue in a sample.

Abundances of the major and three singly-substituted isotopologues and R_{sum} values for standard reference scales are given in **Error! Reference source not found.**. Abundances of the multiply-substituted isotopologues can be calculated following the examples of Eq.
Error! Reference source not found.. They are also listed for Hitran isotope ratios on the Hitran website https://www.cfa.harvard.edu/hitran/molecules.html.

-Page 7, line 35 and following: IRMSs can also be used to measure absolute abundances of the single isotopologues, but the measurement is in general noisy and very difficult to calibrate. This "absolute" approach has been used in the beginning of mass spectrometry before it has been realized that a relative measurement with IRMS is much more precise than an absolute measurement3. This is also the case for isotopologue-specific optical systems. The noise in a line ratio is much smaller than the noise of the individual lines because there is a lot of correlated/technical noise on two simultaneously measured lines which cancels out in a ratio (given that the instrument can measure the targeted lines simultaneously in real-time as possible e.g. with a direct absorption spectrometer). It's not due to the nature of IRMS that the community has converged to go for relative δ-scales, but rather due to the more fundamental analytical advantage of measuring relative values with respect to a reference material as opposed to absolute measurements.

This point has been addressed in the response to this referee's major comment on the absolute vs ratio calibration. Some sources of noise are common to the retrieval of amounts from two isotopologue absorption lines or bands, and these may ratio out, but fundamentally the spectrum is analysed for each isotopologue independently and then the results are ratioed. Technically this is also true of the ion counts in IRMS, but as the referee points out the absolute ion counts are very noisy, more so in IRMS than in retrievals from spectra.