

The authors would like to thank the Anonymous Referee #2 for his/her attentive reading and his/her numerous, useful, and constructive comments. We agree on all of them, see our detailed response below. Please note that the reviewer comments are in bold and that the line numbers correspond to the ones of the Ms with track change. The sentences or the quotations in italics are the modified parts of the Ms.

The study by Hazera et al. is a methodological manuscript dealing with the quantification of total carbon (TC), soil organic carbon (SOC), and soil inorganic carbon (SIC) in single analysis with the Rock-Eval6 method (RE). The proposed method is compared against the standard method using an elemental analyzer (EA) combined with acid fumigation or heating. The main conclusion by the authors is a necessary adjustment of the standard protocol to ensure complete combustion of SIC by increasing the last isotherm from 4 to 7 minutes.

The scientific significance itself is excellent, as RE offers the advantage to derive additional properties of SOC. Advancing the method, so that TC, SOC and some of its properties, as well as SIC could be measured in a single run would be an excellent methodological step forward.

However, as methodological work requires a very high standard, I have some reservations regarding the scientific quality of the description or application of the methods. As it is, I judge the scientific quality as “Fair”, but I hope the authors can amend their manuscript further. Please find some comments and questions in the paragraphs below.

Overall, I guess that addressing the major comments below would need additional work and analysis. This will take some time. If the authors and editors agree, I suggest the following major change to the manuscript: In my opinion, there are several topics which are addressed more in a “work-in-progress” way. The main conclusion, however, is the necessary prolongation of the last isotherm. By significantly cutting the word count it would be an alternative to focus on the topic of the last isotherm and change the “Research Article” into a “Technical Note”. This would mean to focus on the second panel of samples, for which I can see convincing results.

Considering the number of elements we added, we kept the Research Article format, and we hope that our arguments are now convincing.

P.S.: After writing this review, I found the poster of Sebag et al. (2022, DOI: 10.13140/RG.2.2.27606.52800). Using the presented datasets or getting samples and re-analyzing them would render several of my comments unnecessary. Please justify why this large dataset was not used here.

Most of the soil samples of this large dataset were non-calcareous and/or no more available. However, all the calcareous soils of the dataset presented in Sebag et al. (2022) were included in the data set of this article (first panel).

General comments:

Number and range of samples

For a methodological study the number of samples (n = 24) is relatively low. Moreover, the samples are all (1) from agricultural soils, and (2) topsoil samples. Therefore, all results are strictly only valid for this limited sample set. This is discussed in the conclusion (see also “conversion factors” below) but should also be mentioned clearly in the Abstract and Discussion.

We added 6 soils to the first panel. To identify the domain of validity of the RE method, we specified clearly the number and the soil use of the soil samples of this study in the *Abstract* section: “30 agricultural topsoils” (l.28), in the *1 Introduction* section: “30 agricultural topsoils” (l.104) and in the *3.1 Comparison between the estimations of SOC and SIC contents of the 30 soils (first panel) measured by RE and EA* section: “Thus, for these 30 agricultural topsoils, ...” (l.373-376) and “Thus, for these 30 agricultural topsoils, ...” (l.410-403).

There is also no description of soil sample properties. At least, the range of TC (measured by EA) and a corresponding pH value should be given.

We added the Table 1 (section *2.1 Material*, l.130) with the Land use, Soil type, Depth, Texture, pH, TC, SOC and SIC contents for each soil.

It is also not clear if all soils contain carbonates. From Results & Discussion I suspect that all samples contained carbonate. It is highly preferable to mix some samples without carbonates into the set to show that these carbonate-free samples do not show any (or only the stated 9.2%) signal in the “MinC” range. It might even be a good idea to treat these carbonate-free samples exactly as the carbonaceous samples (muffle oven, acid pre-treatment) to show caveats of these methods.

You are right, we did not explain how much non-calcareous soils we had and how they were analysed. Now the Table 1 gives the information. Also, we added the missing information about the non-calcareous soil samples in the *2.1 Material* section: “Among these 30 soils, four were considered non-calcareous [...]. These four non-calcareous soils have been analysed by EA and Rock-Eval® (*Supplementary Materials, SM 1*) just as the other soils.” (l.111-115). We added an example of the 5 thermograms obtained with a non-calcareous soil in SM 1 where the signals in the MinC range are visible. We added explanations on the calculation and the corrections of the TOC and MinC parameters for non-calcareous soils in the *2.2.2 Corrections of the standard parameters* section: “When the MinC parameter is lower than 2.0 gC.kg⁻¹soil, the sample is considered non-calcareous. The signals associated to the MinC parameter (*SM 1*) are then ...” (l.249-251), “For non-calcareous soils, the MinC is added to the TOC parameter and set to 0” (l.258) and “For non-calcareous soils, the corrected TOC and MinC parameters

are calculated as described by Eq. (5) et (6) ..." (l.266-268). We also added the average of the uncorrected MinC parameter for non-calcareous soils in the 3. *Results & discussion* section: "For the non-calcareous soils, the uncorrected MinC parameter averages $1.07 \pm 0.26 \text{ gC.kg}^{-1}\text{soil.}$ " (l.392-393).

There was only one sample of "natural calcite". The description must be expanded. Was it taken from carbonaceous rock? If yes, which kind of rock? Calcite is also available as certified standard material and for methodological work, this should be preferred over a natural sample with unknown properties (e.g. purity, presence of other carbonates such as dolomite or magnesite, presence of traces of organic matter).

We analysed this calcite sample by X-ray Diffraction (XRD, SM 2), EA (SM 3), and RE (SM 4). The results show a purity sufficient for this study, but we cannot affirm that this calcite sample is a certified one... We added the results of these analyses in SM and their conclusions in the 2.1 *Material* section: "As the most common carbonate mineral in soils is calcite, a sample of calcite was analysed by X-Ray Diffraction (XRD, SM 2), EA (SM 3), and RE (SM 4) to check its purity..." (l.117-124).

Precision and accuracy

In methodological studies two measures are essential, that is "precision" and "accuracy" (sometimes termed "bias"). EA, for example, is widely accepted as a standard method because it is both accurate (i.e. measures the true value) and precise (i.e. repeated measurements are very close to each other). The manuscript presents some indications for accuracy, but none for precision. A quantification of precision and accuracy often starts by using reproducible standard materials, which should be mixed together, and measured in replication. Bisutti et al. (2007) provide an excellent example for this. Vuong et al. (2013), for example, offer simple methods to compare precision and accuracy with well-known *F*- and *t*-tests.

We made four EA and RE analyses on three natural geostandards materials with reference values for total C content and the calcite sample. Unfortunately, we did not find any geostandards with both SOC and SIC certified values. We used soil samples or geostandard materials rather than mixes of pure standard materials because we think that the associations of organic and inorganic C in natural samples generate different thermal behaviors of the organic and inorganic C forms than in mixes of pure standard materials. The three geostandard materials are presented in the 2.1 *Material* section: "[...] two soil standards (ERMCC690 from the European Commission – Joint Research Centre and ISE850 from the WEPAL International Soil-analytical Exchange Program) and one Norwegian Geochemical Standard of rock (SR1)." (l.125-128). The results are shown in SM 3 and interpreted in the 3.3 *Adaptation of the RE standard cycle of analysis* section: "[...] On these samples, the precision of the RE method is comparable or better for inorganic content, than the precision of the EA method. [...] Thus, the

RE method gives accurate estimation of total C content and similar values and precision for SOC and SIC contents than EA_{HCl} and EA_{550°C}, respectively, without any soil sample pretreatment.” (l.495-501).

Accuracy in the manuscript is quantified by linear regression. It is a very good start that not only R² is given, and there is more emphasis on the slope. However, I wonder (1) why no intercept is presented (I do hope the regression was not forced through zero, please confirm), and (2) why there is no measure of dispersion (e.g. standard error, confidence interval) for slope and intercept. The dispersion of the regression parameters is important to know.

We did not show the linear regressions with intercept because the intercepts were not significant at 99%. We added the details of this choice in the 2.4 *Data analysis* section: “*If the intercept was not significantly different from 0 with a confidence interval of 99%, the regressions were performed without intercept.*” (l.331-333) and in the figure captions: “*For both regressions, the slopes were significantly different from 0 (p-value (P) < 0.001) but not the intercepts (P > 0.01).*” We did not add the intercept values to avoid overloading the captions. The intercepts were: 0.46 ± 0.33 with the uncorrected TOC (Fig. 4); 0.76 ± 0.38 with the corrected TOC; 1.15 ± 0.49 with the uncorrected MinC and 0.02 ± 0.56 with the corrected MinC (Fig 5).

We added the standard errors of the regression slopes in the figures (4 and 5) and in the text (l.370-374 and l.404-406). For instance: “*Moreover, the slope of the regression line between the SOC contents estimated by EA_{HCl} and by the uncorrected TOC parameter is significantly different from 1 (0.84 ± 0.01, Figure 4, Student test: P < 0.05) while the slope of the regression line corrected TOC vs EA_{HCl} does not significantly differ from 1 (0.98 ± 0.01, Figure 4, Student test: P > 0.05).*” (l.370-374).

Moreover, the authors present the p-value for the regression, which is correctly interpreted as the probability that the slope differs from 0. In this case this is not of interest. We have to know if the slope is different from 1 to judge if methods correspond with each other. This is addressed in the text but should also be integrated into the figures.

We added statistical tests to assess the significance of the difference between the regression slopes and 1. We specified it in the 2.4 *Data analysis* section: “*The significance of difference of the regression slope from 1 was tested with a Student test (H0: μ_{SLOPE} = 1).*” (l.335-336) and we replaced the previous p-value with the Student one in the figures (4 and 5) and specified it in the figure captions, for instance: “**The slope of the regression was significantly different from 1 (P < 0.05).*” (l.368).

Furthermore, a large error of intercept or slope will indicate low precision. Do not only look on significances, but also on variability. With high variability, the difference to 0 or 1 will be insignificant, but the high error may render the method unsuitable for routine analysis.

Thank you, we agree with you, this data was definitively missing. As mentioned above, the intercepts were not significantly different from 0. However, we added the standard errors of the regression slopes in the text and figures. For all the regressions, the standard error of the slope was 0.01 (I.370-374 and I.404-406).

Use of certified standard materials and device calibration

The use of reproducible standard material is highly encouraged. The readers also must be informed about the calibration of all involved devices; including calibration range, precision, and accuracy. There is some information about the assumed relative error, but the authors need to show this data.

As mentioned above, we added four EA and RE analyses on three geostandard materials and one calcite sample. Relative errors of the method are given and compared to the relative error of EA in the *3.3 Adaptation of the RE standard cycle of analysis* section: *“For the three geostandard materials, the relative errors for the TOC, MinC and TOC+MinC parameters were comprised between 0.13% and 5.88%, 0.83% and 2.44% and, 0.35% and 1.92%, respectively. These relative errors are equivalent to the ones of EA: between 0.22% and 5.02% for organic C content, between 1.57% and 23.80% for the inorganic C content and between 1.12% and 2.44% for the total C content.”* (I.497-501).

We also added details on the RE device calibration in the *2.2.2 Rock-Eval® thermal analysis* section: *“The calibration of all the RE devices and the quality of the RE analyses [...] compared with the reference values of the 160 000 standard.”* (I.230-235).

Conversion factors

First of all, I wonder why the authors gave a constant conversion factor, whereas Disnar et al (2003) provided a regression function for correction [this information was taken from Saenger et al. 2013, as I have no access to the manuscript of Disnar et al. 2003, so please correct me if I am wrong].

The constant conversion factor used in this study is the slope of the regression function gave by Disnar et al., 2003. See this sentence directly from Disnar et al., 2003: *“After the correlation presented in Fig. 2, the experimental TOC values must be divided by 0.916 (or multiplied by 1.092).”*

It is especially important, that the conversion factor (SOTHIS) was stated to be valid for samples “enriched in poorly degraded organic compounds (...)”. In the used sample set the

conversion factors seem to work well. However, to really establish the RE method it will be important to ensure that the method works well on a continuum of organic matter states. That is, from the litter, fermented, and humified forest floor layers down the profile of mineral soil. Conditions change through the profile from largely undecomposed to largely decomposed and mineral associated organic matter, often accompanied by a gradient of increasing carbonate content. It seems likely, the “standard” conversion factors will not work for all these conditions. And it is open to question at which depth the “additional 6.2%” shall be applied. Therefore, this will be important future work to address these conversion factors, or possibly change isotherm length or temperature settings. SOTHIS as presented here seems to be a mixture between Disnar et al. (2003) and Sebag et al. (2022). The latter is a poster, a peer-reviewed source or the actual data would be preferable.

We fully agree with the reviewer. The correction factors are useful but should be applied and discussed in specific soils and likely in specific soil depths. It is why we focused the study on agricultural topsoils. In the revised Ms, we specified that the study clearly focused on agricultural topsoils as mentioned above.

In addition, in the conclusion, we added *“The TOC and MinC parameters still need to be statistically corrected (Disnar et al. (2003) and SOTHIS corrections, Figure 2) even with the adaptation of the oxidation phase. To be independent of statistical corrections, which could depend on the SOC and SIC forms in the analysed soil, further study should focus on the distinction between the signals from the pyrolytic cracking and oxidative combustion of SOC organic matter and the signals ones from the SIC pyrolytic and oxidative thermal breakdown.”* (I.529-533).

Use of two different devices and settings

LL 117 ff. The authors used devices from different labs, with slightly different settings. They state that *“The minor differences between the standard cycles [...] do not affect parameter calculations”*. This may be so, but it has to be demonstrated with some samples. Overall, it would be essential to develop the method in one lab, using the same device with the same calibration, and after that set up a ring trial with different labs (inter-lab reproducibility).

To avoid any confusion, the analyses of the first panel have been reanalysed on the RE6 device of *IFP Energies Nouvelles*. Thus, in the revised Ms, only one device is described and the results for the first panel have been changed (Figure 4, I.360; Figure 5, I.380; Table 3, I.415)

Additional literature

I suggest looking at some additional work in the field of measuring SOC, SIC and TC in a single run (Bisutti et al., 2007; Vuong et al., 2016, 2013). Those references did not use RE but offer some methodological examples. Bisutti et al. (2007) made excessive use of different

material mixtures, which can be reproduced. Vuong et al. (2013) gave an example how to quantify precision and bias on a wider range of soil samples and calcite standard (though it would have been better to add an organic matter standard and mix it with calcite). Vuong et al. (2016) advance the former work by using dolomite, not only calcite. Although on marine sediments, the work of Carrie et al. (2012) should be recognized, as they already divide RE peaks into OC and IC and discuss some methodological issues, which may be relevant for some special soil conditions (e.g., saline soils). Additionally, Saenger et al. (2013) conclude that SOC (soil samples of different land use) can be quantified well with help of the correction provided by Disnar et al. (2003). Soucémarianadin et al. (2018 a; 2018 b) provide some insight on the potential for SOC fractionation – which gives reasoning why RE should be advanced in regards of SIC quantification.

Bisutti, I., Hilke, I., Schumacher, J., Raessler, M., 2007. A novel single-run dual temperature combustion (SRDTC) method for the determination of organic, in-organic and total carbon in soil samples. *Talanta* 71, 521–528. <https://doi.org/10.1016/j.talanta.2006.04.022>

Carrie, J., Sanei, H., Stern, G., 2012. Standardisation of Rock–Eval pyrolysis for the analysis of recent sediments and soils. *Org. Geochem.* 46, 38–53. <https://doi.org/10.1016/j.orggeochem.2012.01.011>

Saenger, A., Cécillon, L., Sebag, D., Brun, J.-J., 2013. Soil organic carbon quantity, chemistry and thermal stability in a mountainous landscape: A Rock–Eval pyrolysis survey. *Org. Geochem.* 54, 101–114. <https://doi.org/10.1016/j.orggeochem.2012.10.008>

Soucémarianadin, L., Cécillon, L., Chenu, C., Baudin, F., Nicolas, M., Girardin, C., Barré, P., 2018 a. Is Rock-Eval 6 thermal analysis a good indicator of soil organic carbon lability? – A method-comparison study in forest soils. *Soil Biol. Biochem.* 117, 108–116. <https://doi.org/10.1016/j.soilbio.2017.10.025>

Soucémarianadin, L.N., Cécillon, L., Guenet, B., Chenu, C., Baudin, F., Nicolas, M., Girardin, C., Barré, P., 2018 b. Environmental factors controlling soil organic carbon stability in French forest soils. *Plant Soil* 426, 267–286. <https://doi.org/10.1007/s11104-018-3613-x>

Vuong, T.X., Heitkamp, F., Jungkunst, H.F., Reimer, A., Gerold, G., 2013. Simultaneous measurement of soil organic and inorganic carbon: evaluation of a thermal gradient analysis. *J. Soils Sediments* 13, 1133–1140. <https://doi.org/10.1007/s11368-013-0715-1>

Vuong, T.X., Prietzel, J., Heitkamp, F., 2016. Measurement of organic and inorganic carbon in dolomite-containing samples. *Soil Use Manag.* 32, 53–59. <https://doi.org/10.1111/sum.12233>

The authors would like to thank the Referee 2 for his/her bibliographic suggestions that we were not aware of or had forgotten to mention. We added them in the *1 Introduction* section:

“The ramped combustion was a promising method to measure SOC and SIC on a single aliquot (Bisutti et al., 2007; Vuong et al., 2016; Apesteguia et al., 2018), but remains poorly tested.” (l.78-80) and “The RE thermal analysis has been progressively developed and used in soil science mostly to quantify SOC with the TOC parameter (Disnar et al., 2003; Saenger et al., 2013), and to characterize SOC stability through several indexes directly calculated from the signals (Sebag et al., 2016; Soucémarianadin et al., 2018; Malou et al., 2020)” (l.89-92). We did not mention the Carrie et al., 2012 reference because they used pure biochemical compounds and biological standards and focused on organic matters. See Carrie et al., 2012: “An important caveat in this study is that the pyrolysis and oxidation of mineral carbon fractions automatically measured by Rock–Eval analysis (portion of S3, S3CO₂ and S5CO₂/S5CO) was converted into an organic carbon quantity since our samples contain no carbonates and are strictly OM based. For this purpose, we manually integrated the IR signals to determine the total amount of oxygen-bearing OM and TOC, as peaks were observed past the temperature cutoffs for mineral carbon (Fig. S1).”

Specific comments:

This is not exhaustive due to the suggestion to change the manuscript into a Technical Note. As a non-native speaker, I refrain from language editing.

Some of the figures need more precise explanations. As a guideline, figures should be interpretable without referring to the text.

We added more details in the figure captions, especially for the Figure 1.

Figures show very different dimensions. This could be streamlined. As a guideline, text should be approximately of the same size in all figures

The scales of the graph have been resized to be as much as possible similar from a graph to another, especially the Figure 2.

LI 96: At which temperature were the samples dried?

We added the information: *“All soil samples have been dried at 40°C, sieved at 2 mm, and milled at 200 μm mesh before analysis.” (l.115-116).*

LI 100 ff: Were samples corrected for residual moisture (105°C)? Overall, more methodological details are needed. Which acid was used? Molarity? General conditions, such vacuum etc. Even if Harris et al. (2001) was followed exactly, please provide some details. The same applies to heating.

Methodology have been more detailed in the revised Ms, especially the pretreatments before the EA in the 2.2.1 *Elemental analysis* section:

- decarbonation pretreatment: *“The Ag-foil capsules were filled with 30±5 mg of soil and 50 µL of demineralized water and placed in a vacuum desiccator with a 100 mL-beaker of concentrated HCl (37%) during 8h. The capsules were then dried at 60 °C for 48h before being closed and analysed (Harris et al., 2001; Cardinael et al., 2015).”* (l.138-141)
- decarbonation pretreatment: *“The capsules were filled with 30±5 mg of soil and placed in a muffle furnace at 550°C during 6h (Bertrand et al., 2007). The capsules were then closed and analysed.”* (l.142-144)

The samples were not corrected for residual moisture but dried at 40°C before analysis.

L 143: The temperature was shifted to be consistent with the decarbonation treatment. However, decarbonation was likely performed in ambient air, pyrolysis without oxygen. Therefore, conditions are not comparable. Please justify the shift from 400°C/local minimum to 550°C and estimate the consequences.

You are right, this is not the good justification. We changed it: *“Regarding the boundary between the S3CO₂ and S3’CO₂ curves, Lafargue et al. (1998) set the temperature at 400°C for rock studies because the siderite and magnesite thermal breakdown starts at 400°C. When the most common carbonate mineral is calcite, operators usually shift this boundary to the local minimum of the CO₂ pyrolysis thermogram sample by sample. In this study, the thermograms did not show any of the specific curves of siderite, magnesite, or dolomite. Moreover, the thermograms obtained with the calcite sample showed that the calcite pyrolytic thermal breakdown starts at 550°C (SM 4). Thus, in this study, the boundary between the S3CO₂ and S3’CO₂ curves for all the samples was shifted to 550°C for all the samples to be consistent with the one used for the decarbonation pretreatment (Figure 1, Table 2).”* (l.207-215)

L199: As treated samples were used, I would prefer an indication, e.g. EA_{decarb}

Thank you for this suggestion. We choose EA_{HCl} and EA_{550°C} to make the distinction between the decarbonation and the decarbonation pretreatment, respectively. See in the 2.2.1 Elemental analysis section: *“without any pretreatment for TC (noted: EA), after an HCl decarbonation to remove SIC from the sample for SOC measurement (noted: EA_{HCl}) and after a 550°C heating pretreatment to remove SOC from the sample for SIC measurement (noted: EA_{550°C}).”* (l.136-138).

L 247: “...are correlated (R² = 0.9935...” please use the term “related” as “correlated” is used for Spearman or Pearson correlation coefficient

We changed where necessary (l.366).

Figure 5 and text ll 263 ff: This is a good example, why reporting an intercept for the regression is useful. It also demonstrates the issue when correcting values with a fixed factor

instead of of a regression (see “Conversion factors” above). Please discuss these consequences.

As mentioned above, the intercepts were not shown because they were not significant. The correction factor used is a fixed factor and did improve the estimation of SIC contents $> 62.50 \text{ gC.kg}^{-1}\text{soil}$. These surprising results result come from an underestimation by the MinC parameter because of incomplete thermal breakdown of a too large amount of SIC in the RE crucible of samples with high SIC contents (l.397-427).

Table 2, column S3'CO2 MinC: 12+/-20 does not seem to be a reliable estimate. This should be addressed

The previous results presented in Table 3 (previously Table 2) have been obtained on the overall first panel (20 calcareous soils and 4 non-calcareous soils). The four non-calcareous soils brought a lot of variability, and it was not correct to include the non-calcareous soils here. Thank you for your comment which make us realized this. We changed the results by considering only the calcareous soils (26). The variability is still high for some curves (*e.g.*, S3'CO₂) because it depends on the C forms in the sample, especially the amount of thermoresistant organic matters and/or thermolabile inorganic C forms.

LI323 ff, Figure 8: This finding indicates, that including carbonate-free soil samples in the test may be a good idea.

As mentioned above, the first panel is composed by four non-calcareous soils and 26 calcareous soils. The non-calcareous soils have been analysed just as the calcareous soils.

LI 340 ff: Would it be possible to prolongate the last phase even further? What does this finding mean for more thermostable carbonates?

Yes, it is possible, but, as far as we know, the calcite is the more thermostable carbonate (Pillot et al., 2014).

LI 351: If the SIC content is known, what is the advantage of the RE method?

We removed this confusing sentence because it is only for specific cases where the SIC content have been assessed by another method.

LI 356: Please indicate that further work on a wider range of samples and with different forms of carbonates is needed.

We added this sentence: *“These results have been obtained on 26 calcareous and 4 non-calcareous agricultural topsoils. The 26 calcareous agricultural topsoils contained calcite as main carbonate mineral. Thus, these results need to be repeated with other calcareous soils*

and on other carbonate mineral type with different thermal breakdown behaviour as siderite, magnesite and, dolomite for instance.” (1.522-525).