Comment on soil-2021-115

The responses are in blue and the track-changes in green.

Anonymous Referee #2
Referee comment on "Dynamics of carbon loss from an arenosol by a forest/vineyard land use change on a centennial scale" by Solène Quéro et al., SOIL Discuss., https://doi.org/10.5194/soil-2021-115-RC2, 2022

The manuscript "Dynamics of carbon loss from an arenosol by a forest/vineyard land use change on a centennial scale" presents, as tittle says, the results of a research about long term variations in soil organic carbon (SOC) stocks and their dynamics in a 80 cm deep Mediterranean Arenosol that had undergone a land use change from forest to vineyard over more than 100 years. According to their results a stock of 50 GtC ha-1 in the 0-30 cm forest soil horizon was reduced to 3 GtC ha-1 after long-term grape cultivation. Analyses of 14C showed that deep ploughing (50 cm) in vineyard plot redistributed the remaining carbon both vertically and horizontally. Authors concluded that this soil would have a high carbon storage potential if agricultural practices, such as grassing or organic amendment applications, were to be implemented within the framework of the 4 per 1000 Initiative.

The text denotes a considerable amount of field and laboratory work. In general, manuscript is well written (English grammar and spelling are correct). It is a very interesting research dealing with SOC stocks in soil profiles under different land uses. The natural radiocarbon (14C) abundance analyses present a significant contribution to the discussion. References are updated and they support properly introduction and discussion sections. Tables and Figures are of good quality and all necessary. However, I consider manuscript needs a MODERATE revision before being accepted for publication. It needs to consider the following remarks.
=>We thank Referee #2 for this positive evaluation and for all of the suggestions proposed to improve the paper.

General comments:
There are not statistical analyses supporting the data discussion. Authors are comparing values and treatments and this should be done by means of statistics.
=>We have taken this important remark of Referee#2 into account and have called upon the expertise of a statistician (Joel Chadoeuf) with whom we had worked on the Balesdent et al. (2018) paper published in Nature. We detail the different statistical approaches we used in the specific remarks below.

Specific remarks:

L76-82. Even if understandable, this paragraph is a mix of Material and Methods with objectives. I suggest authors to re-write it focusing on clear objectives. Research hypotheses are also much appreciated.
=>We agree with the reviewer and ~~will reword~~reworded the paragraph as follows:

"~~This~~The present study was therefore carried out to highlight the impact of the long-term conversion (>100 yr) of a forest to a vineyard ~~conversion~~ on the C dynamics at the profile scale, while focusing ~~on the establishment and management of a vineyard~~ on an arenosol under a Mediterranean climate. We hypothesized that the combination of arenosol, vineyard, and conventional practices ~~has~~would, overall, have a major impact on C stocks and the dynamics of C remaining ~~C dynamics~~ in ~~both~~the topsoil and subsoil. To ~~quantify~~test our hypothesis, we ~~chose to work~~worked on paired soils, measuring carbon contents and stocks, vertical and intra-horizon heterogeneity of carbon, as measured by ^{14}C, and correlating the C:N ratio and

radiocarbon (F^{14}C). These parameters enabled us to: (1) determine how ~~vine~~vineyard cultivation and deep ploughing impact carbon stocks and dynamics in a Mediterranean arenosol, at soil layer and entire soil profile scales, and (2) use this case study to estimate, according to different calculation hypotheses, the time required for the vineyard soil to recover a C stock equivalent to that prevailing pre-cultivation."

L81-82. It is not clear why authors applied a rate of carbon incorporation in their cultivated arenosol according to the proportions and rate put forward in the remediation study of Kazlauskaite-Jadzevice et al. (2019).
=>We acknowledge that, when presented in this way at the end of the introduction, our approach was confusing. ~~So we will just mention in the introduction that we tested different computational assumptions, without citing, at this step, the work of Kazlauskaite-Jadzevice et al. (2019) upon which we relied. We~~In the revised version, we explain the different assumptions and detail them in the last section of the discussion.

Köppen-Geigerclassification can be interesting to be used. Particularly because authors refer to it several times through the manuscript.
=>This classification was indeed used in selecting the papers underpinning the discussion: only the papers listed under "Mediterranean climate" (BSk, BWh, Cfa, Csa, Csb and Csc, see Appendix) were retained. We will add this information in the Materials and Methods section and in the SI.

It should be explained in sampling whether rocks were eliminated (as well from calculations?). What happened with vegetation fragments (from roots to branches)? This should be clearly explained particularly in SOC stock studies. Is this related to the presence of less solid fragments (rocks, vegetation, etc.)?
=>Coarse material (rocks and organic matter > 2 mm) was removed with a 2 mm sieve. The remaining root tips were removed by hand. SOC stocks were calculated on the fine soil stock (STF), i.e. by removing the coarse elements from the bulk density:

$$STF = \frac{M_{samp} - (M_{samp} * EG)}{V_{samp}}$$

With STF in g.cm^{-3}, M$_{samp}$ in g, EG in Mass % and V$_{samp}$ in cm^3.

$$SOC\ stock = STF * TOC * e/10$$

With SOC stock in t.ha^{-1}, TOC in g.kg^{-1} and e in cm.
This is now added in the "material and methods" part.

Are these results?
=>The amount of soil to be analysed with respect to ^{14}C was defined according to the carbon content. The target was 1,000 µg of carbon for the solid source and 100 µg for the gas source, with the limitation of cumulating a maximum of 2 capsules for the solid source and 1 capsule for the gas source. One capsule can contain a maximum of 40 mg of soil. Unfortunately it was not possible to reach the 100 µg target for the deepest samples. The carbon masses used are now detailed (M&M and data table in ~~the results section.~~SI).

Refer to "Total Organic Content (TOC)".
=> We disagree with Reviewer #2, the carbon concentration is clearly expressed in total organic carbon: TOC. This confusion may come from line 119 where we were talking about carbon content. We ~~will change~~changed this to total organic carbon, here at line 119 and all over the article.

Is this 0 or 5-6 to 60?
=>This was a mistake. 5 was missing. The correct depth is 50-60 cm, not 0-60 cm. This is now corrected.

L141-151. Authors should present similar depths in both treatments in order to compare them. And use p values to make sound conclusions.

=>We used a Student's t-test to compare, depth by depth, the TOC between vineyard and forest soils. This test is applicable if the variances are in the same order of magnitude. We therefore performed the test on ~~Log~~log$_{10}$(TOC) to have similar orders of magnitude of the variances between vineyard and forest soils. The p-value results are:

Depth [cm]	t-test p-value
0-5	0.00059
5-10	0.00015
10-15	0.00024
15-20	0.00028
20-30	0.00104
30-40	0.00118
40-50	0.00928
50-60	0.00100
60-70	0.07454

The p-values showed a significant difference (<0.05) of TOC between forest and vineyard soils to 60 cm depth. This ~~will be~~is added in the ~~article~~revised version (methodology and results).

It might be good to explain why authors chose to use composite sample at these two depths (5-10 and 40-50 cm) and not others.

=> In order to minimize the 14C analysis cost (€300/sample), we opted to use composite samples for all depths: we thus obtained a mean ^{14}C value (mean of profiles A, B and C). However, the composite samples did not enable us to determine the variability in ^{14}C at the scale of the same layer. We estimated this variability by testing it on 3 layers: a C-rich topsoil layer (5-10 cm), a C-poor subsoil layer from the vineyard ploughing horizon (40-50 cm), and a layer below the ploughing horizon for which only the soil in the vineyard was measured (50-60 cm) (in view of the 5-10 and 40-50 cm results in the forest, we did not expect that there would be any variability in the forest 50-60 cm 14C).

Section 4.3. Please include statistical analyses results that help to explain this variability.

=>Given the limited number of data, we applied a permutation test on the ratio $\frac{\sout{RMS_{forest}}\ RMS_{vineyard}}{\sout{RMSvineyard}\ RMSforest}$ (the residual ~~sum of~~mean squares), calculated on F^{14}C data. The RMS ratio allowed us to compare the ~~degrees of~~variance between forest and vines. The permutation test allowed us to test whether the ratio result was significant or not (Manly, 2006).

At 5-10 cm depth, the observed ratio was ~~1.46~~9.16 (≠1). We repeated 1,000 times a permutation test of the RMS ratios between forest and vines (simulation), which we then compared to the observed ratio value (Figure 1). The observed value was outside the simulated critical values with a p~~-value = ~~=0~~ (<~~<.02 (<0.05). This showed that the variance under vines was significantly different from the variance under forest.
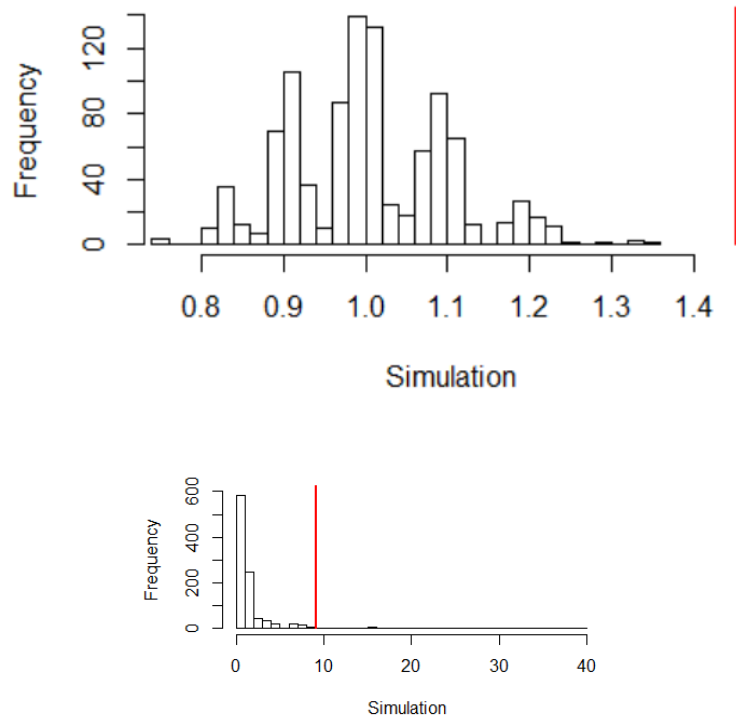
Figure 1÷: RMS's simulation ratios in relation to the observed ratio (red) at 5-10 cm depth

At 40-50 cm depth, the observed ratio is ~~0.98 (close to~~ 27.53 (≠1). Similarly, we repeated a permutation test 1,000 times. The observed value was within the simulated critical values (Figure 2), with a p ~~value = =~~0.~~67 (>>~~01 (<< 0.05). This showed that the ~~variances~~variance under vines ~~and under forest~~ was ~~not~~ significantly different~~.~~ from the variance under forest
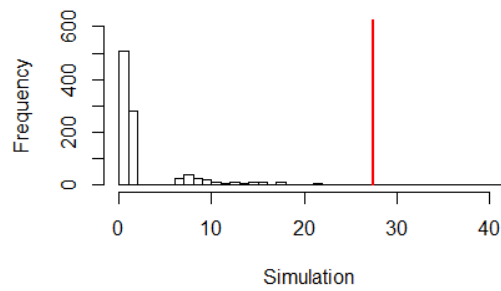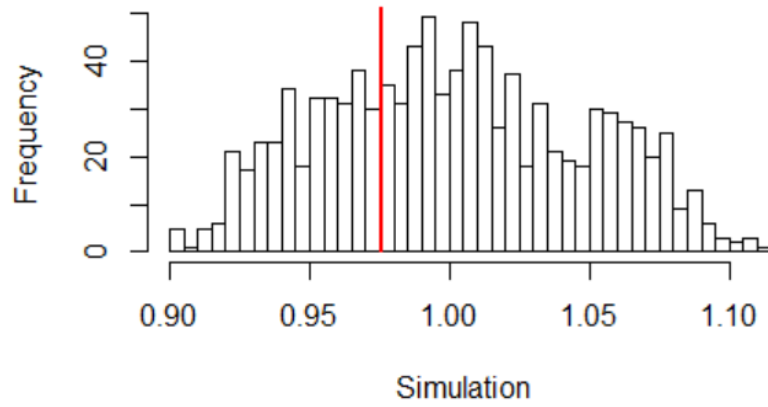
Figure 2 : RMS's simulation ratios in relation to the observed ratio (red) at 40-50 cm depth

In Fig.4. Why don't present both soils in one depth? Legend can be moved
The reviewer is right, this way of presenting the data is better.
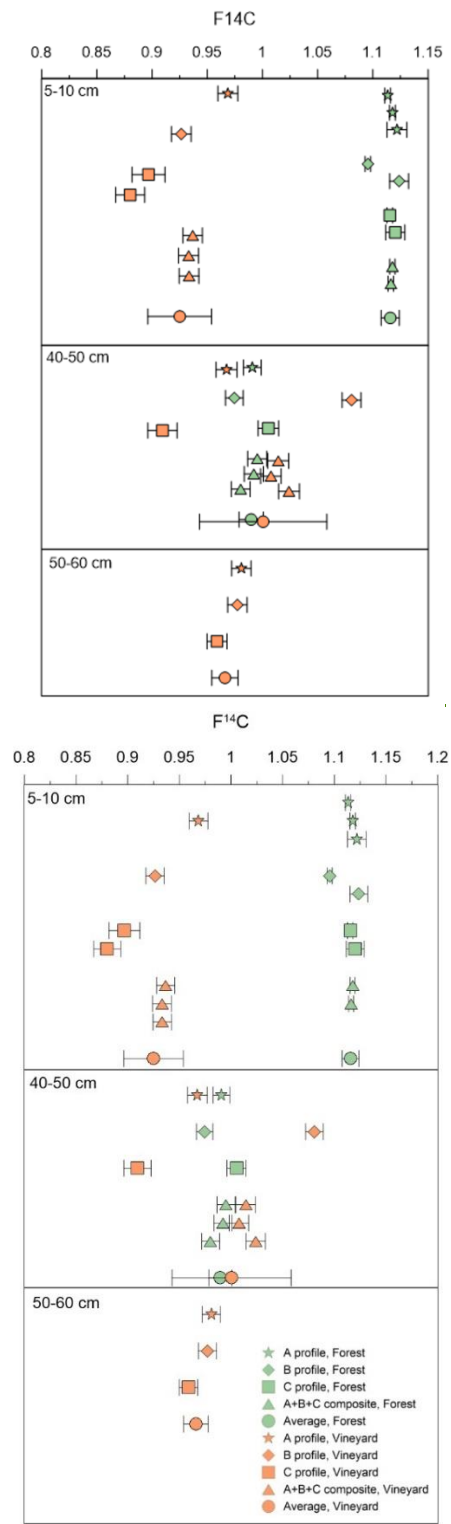=>Here is the new graph Figure 3 that is now in the article:

Figure 3 : Comparison of intra-layer F^{14}C heterogeneity at three depths (5-10, 40-50 and 50-60 cm) in forest and vineyard soils. F^{14}C data were obtained for profiles A (star), B (diamond), C (square), composites A+B+C (triangle) and the average of these data (round), in forest (green) and vineyard (orange) soils. Error bars represent the analytical error for ~~the~~ profiles A, B and C and the standard deviation ~~of~~for the mean.

5. Very interesting comparison.
=>Thank you.

Section 6 should be probably renamed as "Possible origin of OM". In this section there is a comparison of C:N ratios that is related to a probable origin of the OM. Authors based their discussion in Cotrufo et al., 2019. According to theses researchers, OM of plant origin shows C:N = 9.8 -17.8 and the OM of microbial origin associated with minerals C:N= 7.9-17.3. There are not great differences in these thresholds particularly when one compares results of this research with soil under forest (13 < C:N <16) and under vines (7<C:N<12). It could be in any of the two origins, don't you think?

=>The reviewer is right our approach was a bit speculative. However, we also applied a statistical approach (Student's t--test) to compare the C:N ratios between vine and forest soils. Up to 50 cm depth, the p-values were under 0.05 except for the 15-20 cm and 30-40 cm horizons, where they were less than 0.1. This result ~~shows~~showed that there was a significant difference in C:N, with lower values in the vineyard than in the forest soils. This result tended to confirm that, at equivalent depth, the C pool remaining in the vineyard had a more marked microbial signature than the C pool in the forest soil. We ~~will rewrite~~rewrote this section by changing the title as proposed by Referee #2, using the statistical results explained above, and by qualifying our statement.

Depth [cm]	t-test p-value
0-5	0.0255
5-10	0.0143
10-15	0.0122
15-20	0.0990
20-30	0.0098
30-40	0.0778
40-50	0.0310
50-60	0.4627
60-70	0.7696

6. Nothing is mentioned about Normality of data. Are these correlations made by Pearson or Spearman?

=>In our initial manuscript, we applied a simple linear regression (R^2=0.79). There was no normality of data (p~~-value~~=0.002), which is why the ~~Spearman~~Spearman's test should be preferred to the ~~Pearson~~Pearson's test. The Spearman correlation coefficient was r=0.78, showing that $F^{14}C$ and C:N were strongly linked by a linear relationship, which supported the regression results.

In Fig. 6. Authors should change symbols to see composite vs single samples as well. Are there any differences? I'm not sure about the independency of these data to perform correlations?

=>For a given depth, the composite samples had their own $F^{14}C$ measurement, so they were independent of the single samples (with regard to the $F^{14}C$) and were subjected to the same errors due to the analysis. With regard to the C:N, the composite samples had a single measurement per $F^{14}C$ (C:N mean of the A, B and C sides), making their independence questionable. However, the results showed that the composite samples were ~~included~~spread in the ~~scatter plots~~point cloud without showing any ~~preferential areas~~aberrant behaviour (Figure 4). So they were included in the regression. Hereafter is the new graph showing the sample types (single or composite) (Figure 4):
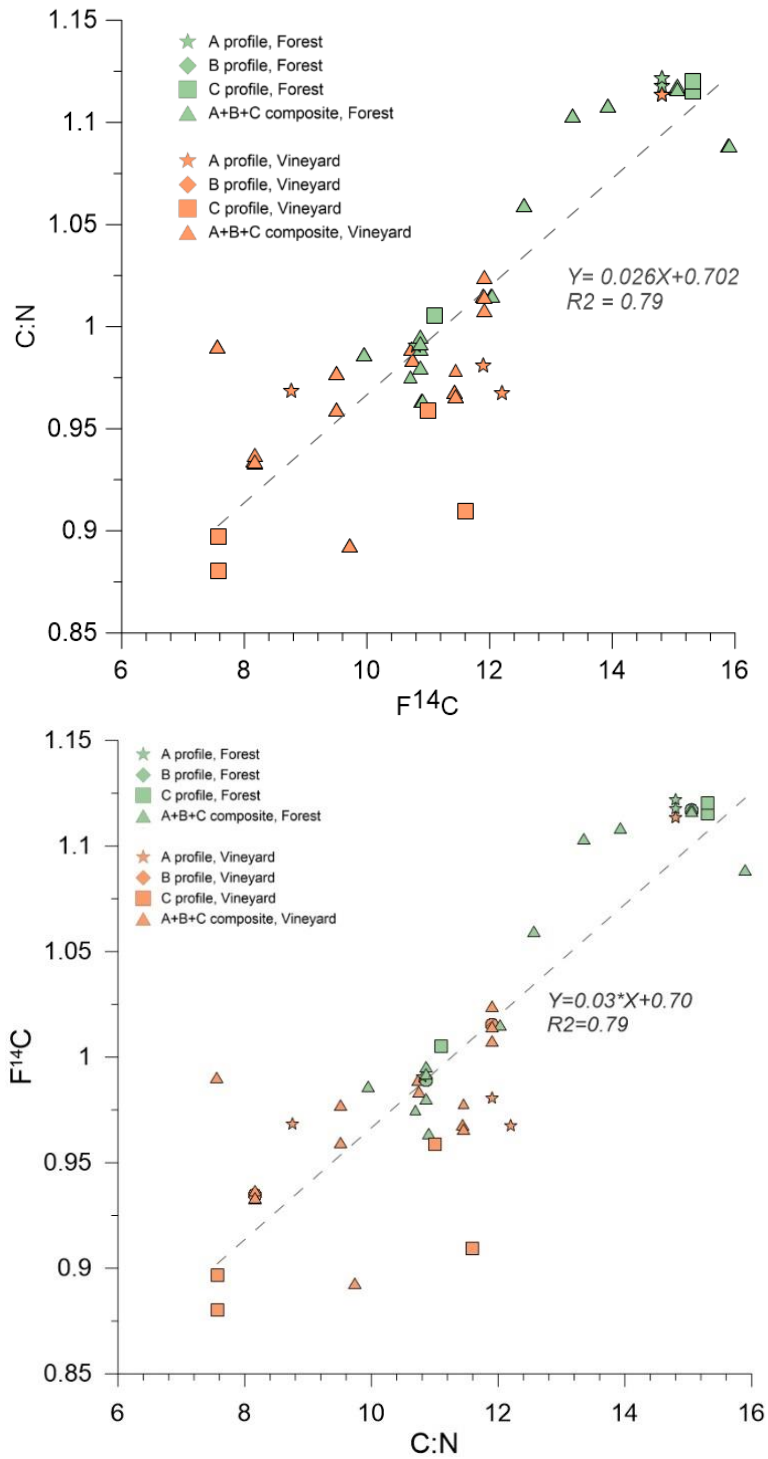
Figure 4 : Correlation between the F14C and C:N ratio. The correlation was calculated on composite samples (F14C) and the average for the 3 profiles A, B and C (C:N), as well as on simple samples of profiles A, B and C, from the forest (green) and vineyard (orange) soils

In addition, there was no difference in the behavior of the samples according to the depth at which they were sampled (Figure 5).
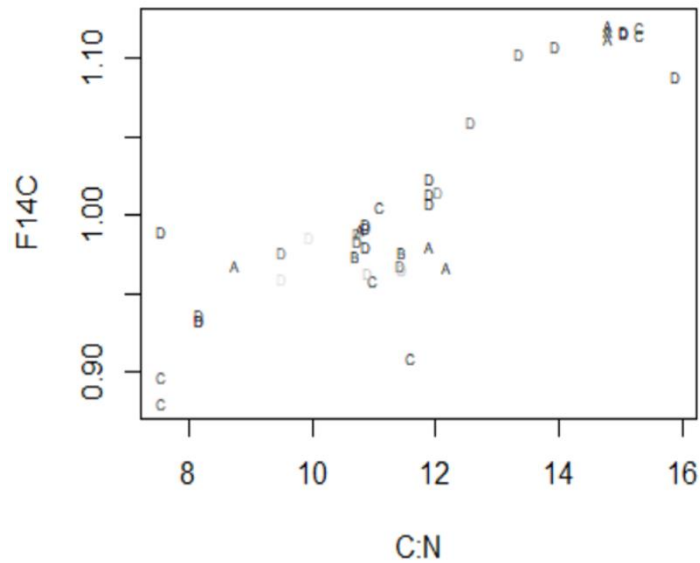
Figure 5 ~:~ F14C as a function of C:N. The letters correspond to the faces of the pit, their colour to their depth (the lighter the colour, the deeper the sample).

Therefore, we used the whole dataset to apply a linear regression and a Spearman correlation.

Are the experiments economically viable? Is the owner of vineyard willing to make this change?
=>According to a study by Pellerin et al. (2019), at the scale of France, very few of the stocking practices generate income for farmers (only 2 of the 9 studied). The economic viability of these practices will therefore depend on those chosen by the farmer, as well as on potential state aid.

Payen et al. (2022) showed that the decision to adopt stocking practices by farmers was dependent on many socioeconomic and behavioral factors (farm size, number of hired workers, attitude towards stocking practices), and on specific wine production aspects (e.g. being an independent winegrower).

We continue to work on these plots and are in contact with the wine growers of the agricultural cooperative. We hope that our work will help boost their awareness of the importance of changing agricultural practices to preserve the soil.

These issues ~~will be~~is addressed ~~following~~at the ~~discussion on "4.7 Are arénosol a good target for~~end of the ~~4:1000 Initiative?".~~conclusion.

Include depths (0-5 cm) to make it more accurate
=> OK.

L311-313. Even if the assumption of relating older age, i.e. F14C (old and stabilized carbon), to decreased C:N ratio is true, it is based on a "discussion" not completely clear (Section 6). I invite authors to re-think this part and present sound conclusions.
=>The Referee is right and, as we mentioned above, our approach was a bit speculative. However, the new statistical approaches (Spearman correlation, r = 0.78) confirmed a strong linear relationship between $F^{14}C$ and C:N, thereby confirming the hypothesis that an advanced age of C is related to a decrease in C:N. We ~~will rewrite~~rewrote this section.

Is equation A.3. correct?

And the statistical analysis to confirm this?

=>The correct equation is:

$$As = \frac{^{14}C \; sample \; atoms}{^{12}C \; sample \; atoms}$$

In Table C1 caption, refer to Total Organic Carbon (TOC).

=>We ~~will change~~changed it to total organic carbon.

In Table C2, C values are significantly different between A, B and C?

=>As mentioned above, amount of soil to be 14C analysed was defined according to their carbon content. The target was ~~1000µg~~1000 µg of carbon for the solid source and ~~100µg~~100 µg of carbon for the gas source with the limitation of cumulating a maximum of 2 capsules for the solid source and the limitation of 1 capsule for the gas source. One capsule can contain a maximum of ~~40mg~~40 mg of soil. Unfortunately it has not yet been possible to reach the ~~100µg~~100 µg target for the deepest samples.

References:

Manly, B. F. J.: Randomization, Bootstrap and Monte Carlo Methods in Biology, Third Edition, CRC Press, 488 pp., 2006.

Payen, F. T., Moran, D., Cahurel, J.-Y., Aitkenhead, M., Alexander, P., and MacLeod, M.: Factors influencing winegrowers' adoption of soil organic carbon sequestration practices in France, Environmental Science & Policy, 128, 45–55, https://doi.org/10.1016/j.envsci.2021.11.011, 2022.

Sylvain Pellerin et Laure Bamière (pilotes scientifiques), Camille Launay, Raphaël Martin, Michele Schiavo, Denis Angers, Laurent Augusto, Jérôme Balesdent, Isabelle Basile-Doelsch, Valentin Bellassen, Rémi Cardinael, Lauric Cécillon, Eric Ceschia, Claire Chenu, Julie Constantin, Joël Darroussin, Philippe Delacote, Nathalie Delame, François Gastal, Daniel Gilbert, Anne-Isabelle Graux, Bertrand Guenet, Sabine Houot, Katja Klumpp, Elodie Letort, Isabelle Litrico, Manuel Martin, Safya Menasseri, Delphine Mézière, Thierry Morvan, Claire Mosnier, Jean Roger-Estrade, Laurent Saint-André, Jorge Sierra, Olivier Thérond, Valérie Viaud, Régis Grateau, Sophie Le Perchec, Isabelle Savini, Olivier Réchauchère, 2019. Stocker du carbone dans les sols français, Quel potentiel au regard de l'objectif 4 pour 1000 et à quel coût ? Synthèse du rapport d'étude, INRA (France), 114 p.