Paper title: Monitoring Pedogenic Inorganic Carbon Accumulation Due to Weathering of Amended Silicate Minerals in Agricultural Soils

Journal: Journal of Visualized Experiments (JoVE)

Responses Guidelines:

1. **Green** font represents new additions/modifications throughout the revised version of the submitted manuscript. ***Italic*** texts are directly quoted from the submitted manuscript. Responses are indicated in **Blue** font.

2. In order to reply to each comment separately, comments have been numbered sequentially.

3. Comments from each reviewer start on a new page.

Editorial comments:

Comment 1.

Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues. Please define all abbreviations at first use.

Authors' response:

The manuscript was reviewed by the authors and all spelling/grammar issues were corrected. We also defined all abbreviations at first use.

Comment 2.

Please revise the title to "Monitoring Pedogenic Inorganic Carbon Accumulation Due to Weathering of Amended Silicate Minerals in Agricultural Soils".

Authors' response:

The comment was addressed in the revised manuscript accordingly.

Comment 3.

For in-text formatting, corresponding reference numbers should appear as numbered superscripts after the appropriate statement(s), but before punctuation.

Authors' response:

All the in-text references were checked and revised based on the mentioned comment.

Comment 4.

Unfortunately, there are sections of the manuscript that show overlap with previously published work. Please revise the following lines: 129 (soil fractions…)-132 (…programs).

Authors' response:

The mentioned sentences were paraphrased.

Comment 5.

> **The Protocol should contain only action items that direct the reader to do something. Please move the discussion about the protocol and background to the Discussion and Introduction, respectively.**

Authors' response:

The mentioned sections were relocated in the revised manuscript accordingly.

Comment 6.

> **Please ensure that all text in the protocol section is written in the imperative tense as if telling someone how to do the technique (e.g., "Do this," "Ensure that," etc.).**

Authors' response:

The comment was addressed in the manuscript by revising the protocol section as requested.

Comment 7.

> **Please note that your protocol will be used to generate the script for the video and must contain everything that you would like shown in the video. Please add more details to your protocol steps. Please ensure you answer the "how" question, i.e., how is the step performed?**

Authors' response:

The comment was addressed in the manuscript by revising the protocol section as requested.

Comment 8.

> **For ease of filming, please clarify how many plots you are considering—four (Figure 1)?**

Authors' response:

The comment was addressed in the manuscript by adding the following sentence in NOTE of protocol 1.1:

"These four plots were considered in order to facilitate field filming campaign."

Comment 9.

> **As you have highlighted the text for recording moisture content, please provide more details to facilitate filming.**

Authors' response:

The comment was addressed in the manuscript by revising protocol 1.5 as follow:

"Transport the soil samples into buckets, one for each sampled depth at each plot. Hand-blend the soils in each bucket thoroughly. Place the portable moisture tester into the mixed soil sample. Wait until the moisture content reach a stable value on the gauge of device. Press the holder button and record the value as the real time moisture content of blended soils."

Comment 10.

> **Please mention the formula you use in protocol 3.4**

Authors' response:

The comment was addressed in the manuscript by adding the following to protocol 3.4:

NOTE: The $CaCO_{3(eqv)}$ content of the sample is calculated from the below formula:

$$w\left(CaCO_{3(eqv)}\right) = 1000 \times \frac{m_2(V_1-V_3)}{m_1(V_2-V_3)} \times \frac{100+w(H_2O)}{100} \qquad (4)$$

Where:

$w\left(CaCO_{3(eqv)}\right)$ = the carbonate content of the oven-dried soil
m_1 = the mass of the test portion
m_2 = the mean mass of the calcium carbonate standards
V_1 = the volume of carbon dioxide produced by the reaction of the test portion
V_2 = the mean volume of carbon dioxide produced by the calcium carbonate standards
V_3 = the volume change in the blank determinations
$w(H_2O)$ = the water content of the dried sample

Comment 11.

Even if you do not film these methods, please mention in the protocol the various methods you have described in the Representative Results section, stating that these can be used to determine the stated parameters.

Authors' response:

Along with comment 7 and 11, all the procedures carried out for calculating inorganic carbon content in soil were explicitly described in Protocol section. We also referred our methodology to a standard procedure.

Comment 12.

Please include a scale bar for all images taken with a microscope to provide context to the magnification used. Define the scale in the appropriate Figure Legend.

Authors' response:

Scale bars are present in Figures 4 and 5 for each SEM image, so this comment is not clear. To provide more information, we have defined the magnification and the scale size in the figure legend for both Fig 4 and Fig 5, as follows:

"Figure 4: (a) SEM image of an wollastonite-amended soil at 250x and 400 µm scale; (b) closer view of the weathered wollastonite grains present in the soil at 2000x and 50 µm scale; (c-f) EDS elemental mapping of Si, C, Ca, and Mg present in the area visualized in Figure 4b at 2000x and 50 µm scale; (g) EDS spectrum and semi-quantitative elemental composition of the area visualized in Figure 4b. SEM-EDS data was collected using FEI Inspect S50, which was equipped with energy dispersive spectroscopy (Oxford X-Max20 SSD) used for elemental composition analysis of selected areas and spots with an excitation volume at 20 kV. Prior to SEM-EDS analysis, the samples were mounted on carbon tape and sputter-coated with gold.

Figure 5: SEM image of encircled particulate shown in Figure 4b at 40000x and 2.5 µm scale, and EDS spectrum and semi-quantitative elemental composition of two spots marked on the SEM image."

Comment 13.

As we are a methods journal, please revise the Discussion to explicitly cover the following in detail in 3-6 paragraphs with citations

Authors' response:

Major revisions were done in discussion section. Accordingly, two paragraphs were removed/relocated in introduction section. Also, the following paragraph was added to address "significance of used method comparing to existing methods":

"Thermogravimetric analysis (TGA) can also be used to determine the calcium carbonate content in the soil by measuring the mass loss in the temperature range of 500–800 °C, which is the decomposition temperature range for $CaCO_3$ (Huijgen et al., 2006). Automated carbon analyzer or TGA methods are suitable for analyzing soil samples from smaller confined areas such as a pot experiment because these methods require only 20 miligrams of soil sample for analyses, therefore for agricultural studies, the calcimetry results are deemed more precise and accurate given the larger mass of sample analyzed (10 g versus 20 miligrams), the triplicate readings obtained, and the use of pure calcium carbonate as the method standard. More details and examples can be found in Haque et al. (2019, b)."

Other sections of the discussion were revised as well.

Comment 14.

> **Please ensure that the references appear as the following: [Lastname, F.I., LastName, F.I., LastName, F.I. Article Title. Source. Volume (Issue), FirstPage–LastPage (YEAR).] For more than 6 authors, list only the first author then et al. Please include volume and issue numbers for all references.**

Authors' response:

The comment was addressed in the reference section of manuscript.

Comment 15.

> **Please sort the Materials Table alphabetically by the name of the material.**

Authors' response:

The Material Table was re-sorted as requested. Also, the details of soil moisture meter were added to the table.

<u>Reviewers' comments:</u>
Reviewer #1:

Manuscript Summary:
This manuscript addresses a really important issue, by setting out a protocol to determine, quantitatively, the amount of carbon sequestered in a soil as a consequence of application of a calcium silicate mineral, wollastonite. The research is topical, as recent papers in Nature and elsewhere have investigated the phenomenon. The contribution that this manuscript makes is by defining a protocol to measure the amount of inorganic carbon that is in a soil.

Comment 1.

> **I'm not sure who the audience is, but the detail given appears to assume a non-specialist, a reader who has not used mineralogical techniques before. That is fine. It does mean, however, that some of the method is described beyond what would be expected. For example, the use of the calcimeter is absolutely standard, and a single reference to a standard protocol, published elsewhere, would be appropriate.**

Authors' response:

The protocol of a JoVE article is written according to what is expected to be filmed. So if the procedure for the calcimeter is entirely replaced by a reference to a standard procedure, it will not appear in filming. The calcimetry result is the "visual proof" of CO_2 sequestration in soils, or at least of the presence of carbonates, so having it be a meaningful part of the video is important. As such, this section was revised rather than removed. In order to address this comment, the standard procedure was cited in the revised manuscript and the following was added to references:

Eijlelkamp Soil & Water. Calcimeter Manual. https://www.eijkelkamp.com/download.php?file=M0853e_Calcimeter_b21b.pdf. Retrieved on November 22, 2020.

Furthermore, we added the details of the formula used for calculating the $CaCO_3$-equivalent content of the sample (to address the comment 10 of the editor).

Comment 2.

> **It is not clear how the data for different depths are used; an example would be useful. In fact, no data are shown at all that allow comparison between the different sample depths (or indeed, the different sieved fractions - that would justify choosing the pan over other fractions). In**

the absence of data, it is hard to accept that the method proposed here is robust and has been robustly tested by the authors.

Authors' response:

We added new set of data to demonstrate the evolution of $CaCO_3$ in different depths of the soil. Based on the new results a new table (Table 2) was added to manuscript. Also, the following paragraph was added to representative results section to address this variation of $CaCO_3$ in different depths of the soil:

"The $CaCO_{3(eqv)}$ content (calcimeter results) over a vertical profile (0-15, 15-30, and 30-60 cm) are demonstrated in Table 2. Accordingly, the highest content (of unsieved samples) was 10.13 $g,CaCO_{3(eqv)}\cdot(kg,soil)^{-1}$, detected in 0-15 cm depth zone. The 15-30 cm samples showed the lowest amount of $CaCO_{3(eqv)}$. The $CaCO_{3(eqv)}$ content increased again in 30-60 cm zone. The results from two deep profile samples also suggest the much higher content of carbonates in deeper layers (60-100 cm profile). Based on the results of shallow and deep samples, it is implied that there are two zones enriched in carbonates. The first is the depth zone of 0-15 cm, representative of weathering products due to wollastonite application, given the significant increase versus the control. The other zone includes deeper samples, starting at around 30 cm and increasing to great extent down to 1 m. Since deeper samples are generally indicative of soil's parent materials, it can be inferred that carbonates at this zone are of geological origin. Table 2 also depicts a comparison of the unsieved and sieved sample (pan fraction) to investigate the impact of fractionation on the carbonate content. Based on this, the carbonate content is slightly higher in the pan fraction, implying possibility of accumulation of carbonates in smaller particles, in agreement with our previous study[28]."

We also edited last paragraph of representative results (calculation of Areal SIC) as follow:

"Using the calcimeter reading of the soil amended with wollastonite, the pedogenic inorganic carbon sequestration rate can be calculated using the steps outlined in Section 3 of the Protocol. To this end, the difference of calcimeter reading for analyzed sample and its corresponding control should be calculated for the depth of interest. Then a factor of 0.44 is applied to the difference to convert $CaCO_{3(eqv)}$ to CO_2. In case of the 0-15 cm depth samples presented in table 2, deducting the calcimeter reading of the control (2.51 $g,CaCO_{3(eqv)}\cdot(kg,soil)^{-1}$) from of 0-15 cm depth reading (10.13 $g,CaCO_{3(eqv)}\cdot(kg,soil)^{-1}$), results in a net amount of 3.35 $kg,CO_2\cdot(tonne,soil)^{-1}$ sequestered at this depth. Considering only the soil thickness of 0.15 m, and a disturbed BD of 1.01 $tonne\cdot(m^{-3})$ measured in the laboratory, the Areal SIC is then can be calculated using Eq. 5. Based on the ratio between undisturbed and disturbed BD and the uncertainty existing in reporting BD due to climatic and land use factors, we have estimated the undisturbed BD of our 0-15 cm layer to be 1.386 ± 0.23 $tonne\cdot(m^{-3})$. Considering the uncertainty present in calcimeter reading, we determined the cumulative uncertainty of calculated SIC_{Areal}, using a Gaussian equation for normally-distributed errors method, to be ± 39%. Accordingly, we estimate the sequestrated CO_2 in the 0-15 cm layer of our field to be 6.96 ± 2.71 tonne,CO_2·(hectare)$^{-1}$. Using similar procedure, the Areal SIC of 15-30 cm and 30-60 cm layers can also be calculated (for this field, control values were not available to demonstrate this calculation). Summing up the values for the full 0-60 cm profile, the estimated sequestrated carbon could be determined in the study area."

On the soil fractionation we have added the following:

Table 2 also depicts a comparison of the unsieved and sieved sample (pan fraction) to investigate the impact of fractionation on the carbonate content. Based on this, the carbonate content is slightly higher in the pan fraction, implying possibility of accumulation of carbonates in smaller particles, in agreement with our previous study[28].

The fractionation procedure was found to be beneficial in our prior work[28] for concentrating the pedogenic carbonates and residual silicates in a single soil fraction (pan fraction), allowing more precise chemical, mineral, and morphological analyses to be done. Other studies have indicated that accumulated organic carbon is more likely to be stored in fine particle aggregation of agricultural soils[37, 38], similar to the particle analyzed in Figure 5. Furthermore, the higher surface area of fine silicate rock particles leads to a higher weathering rate comparing to the coarser fraction[15]. However, fractionation could vary from soil to soil and even may not be required for soil containing large amounts of carbonates and silicates. Also, its need or benefit should be further verified for different soil types and various silicate amendments than the ones used in our prior work.

Comment 3.

> **Much is made of the use of SEM analysis, with some nice images. But discussion of the Ca:Si ratio is fraught with problems. For a start, the size of the excitation volume at 20kV (I deduce this from Figure 4; it is not stated anywhere) is likely to be around 10 μm in diameter and depth. So the spectrum reports anything within this volume, which exceeds the size of the particles in Figure 5. I don't think the discussion of the Ca:Si ratio has any validity. At the very least, the Ca:Si ratio for the wollastonite used in the study should be given.**

Authors' response:

The authors checked the available literature to understand the range of specimen excitation volume at 20 kV and we found that it ranges between 1-5 μm, depending not only on the beam energy, but also on the angle of incidence of the electron beam, as well as the atomic number of the material being examined [1-3]. The EDS spectrum shown in Figure 5, at 40000x and 2.5 µm scale, is on a sample size greater than 1 µm, thus indicating that the EDS analysis is being conducted on a spot on the particle which is within the 1-5 µm excitation volume range. Thus, the SEM-EDS analyses reported in this study are valid. An excitation volume of 10 µm at 20 kV seems to be a very high value, and the authors did not come across this number in literature. If the Reviewer can provide us with a reference for this, then we can inspect further into it. Nevertheless, based on the available literature, the typical excitation volume at 20 kV is 1-5 µm, and hence, the EDS spectrum reported in this study is valid.

[1] Lyman, C. E., Newbury, D. E., Goldstein, J., Williams, D. B., Romig Jr, A. D., Armstrong, J., ... & Peters, K. R. (2012). Scanning electron microscopy, X-ray microanalysis, and analytical electron microscopy: a laboratory workbook. Springer Science & Business Media.

[2] Scrivener, K. L. (2004). Backscattered electron imaging of cementitious microstructures: understanding and quantification. Cement and concrete Composites, 26(8), 935-945.

[3] Wong, H. S., & Buenfeld, N. R. (2006). Monte Carlo simulation of electron-solid interactions in cement-based materials. Cement and Concrete Research, 36(6), 1076-1082.

The authors agree with the Reviewer's concern regarding the Ca:Si ratio. We have omitted the detailed discussion on Ca:Si ratio from the manuscript, and made the following changes:

~~The overall EDS spectrum of the mapped area is shown in Figure 4g. The Ca-to-Si ratio is relatively high, at 0.97:1, close to such a ratio in wollastonite (1.42:1). The lower ratio of the wollastonite found in soil is suggestive that some calcium has leached from these particles due to weathering.~~ *Figure 5 shows spot EDS analysis on the smaller fragments, at 40000x, scattered in the soil sample and shown in Figure 4b (at 2000x) marked within the yellow circle. Spot EDS analysis at two different points indicates that this fragment is rich in C and O, suggesting that it is made up primarily of organic matter, which matches the amorphous particle morphology*~~. Its Ca:Si ratio is much lower than that of wollastonite (Ca:Si 0.7) or weathered wollastonite, at 0.59:1, but much higher than that of the overall soil as determined by WDXRF, of 0.08:1. This suggests that the organic-rich particles contain calcium that was leached from wollastonite and likely re-precipitated as small carbonate grains that become trapped in organic matter. A more detailed study is needed to accurately determine the fate of carbonates in the soil if they do, in fact, reside in these organic-rich amorphous particulates.~~ *Additionally, by looking at the Ca:Si ratio SEM-EDS analysis can be potentially used to identify signs of wollastonite weathering in soil, such as leaching of Ca from the wollastonite or the fate of the formed carbonates in the soil.*

Also, the composition of the wollastonite has been added, instead of the Ca:Si ratio (as its not needed since the discussion of Ca:Si ratio from the SEM-EDS analyses has been omitted).

"WDXRF and XRD can also help in the characterization of the wollastonite used. The nominal elemental composition of the wollastonite used in this study, sourced from Canadian Wollastonite's Ontario mine, includes 26% silicon (55% SiO_2), 18% calcium (26% CaO), 4.0% magnesium (9% MgO), 1.8% sulfur, 0.11% nitrogen, 0.10% P_2O_5, 0.10% K_2O, 11 ppm copper, and 1.1 ppm zinc. The main mineral phases present in this wollastonite, as determined by XRD analysis, includes wollastonite ($CaSiO_3$), diopside ($CaMgSi_2O_6$), and quartz (SiO_2)."

Comment 4.

> **The choice of samples down to 2 m depth is described in the context of providing background TIC contents (around line 200). How can this be so? No data are presented to support this claim. It is far from safe to assume that a soil is homogenous to that depth**

Authors' response:

Although we mentioned the 2.5 m as the deepest sample in the protocol, we reached muddy sediments (water table) after one meter in our field sampling campaign. We modified this as "up to 2.5 meter" in the protocol. Therefore, the depth of choice may vary for other locations/land uses, and should be justified with researchers based on setting of the field of choice.

Based on our findings of deep profiles collected with auger sampler (and added to manuscript (Table 3)), the higher contents of $CaCO_3$ in deeper samples were detected. The high difference of carbonate contents through the vertical profile (30-100 cm), is representative of high level of heterogeneity in soil's characterization (This was also obvious based on the appearance of samples collected from different depths). We imply these "deep" carbonates are of naturally occurring pedogenic carbonates pool in the soil. Since relatively short time has passed from the application of wollastonite, we can only see the short-term impact of application over the vertical profile of the soil. However, we expect the downward migration of newly formed pedogenic carbonates (already observed in depth of 0-15 of the soil, Table 2), resulting in mixture of carbonates from weathering process and parent rock. Accordingly, we expect to see a mixture of naturally occurring and newly formed (as the weathering products) carbonates in the deeper layers. Hence, choice of a conservative depth for seeing carbonates of parent soil origin (in short term) and the mixture of sources (in long term) is of importance in such analysis.

Comment 5.

> **The choice of the pan fraction is valid for the reasons stated, but it is very common to see pedogenic carbonates as a coating on pebbles in a**

soil. These would be excluded from the analysis, as they would be removed by the sieving. So one could argue that the protocol reported here gives an underestimate of the SIC.

Authors' response:

This comment addresses a good challenge, which we also detected in our previous work[28]. Based on that results, the second-highest content of SIC was observed in the largest size fraction (course fraction) just after pan fraction. This large fraction simply may be indicative of "coating on pebbles". Therefore, we agree with argument mentioned with the reviewer and believe other fractions of soil should be also analyzed.

In the protocol 2, we said that sieving can be applied to further confirm presence of carbonates in the analyzed soils. Therefore, it would be optional. It is encouraged that other fractions of the soils to be analyzed as well and see if they contain significant content of carbonates or not.

In our case, we use the sieving as a "indirect confirmation" of carbonates accumulation due to mineral weathering. To further verify the occurrence of carbonates due to weathering process in the soil, more robust methods (such as carbon isotopes) may be required. As we illustrated in the comment 9, analyzing C and O isotopes may not be required in our case, as we detected two distinct zones of carbonates in shallow and deep horizon of soil, indicative of weathering products and naturally occurring origins, respectively.

Comment 6.

The composition of the wollastonite should be given, as it underpins the discussion of the accumulation of C and Ca. I think I am happy with the way this is argued, but I can't test what is said without knowing more about the wollastonite.

Authors' response:

The composition of the wollastonite is added:

"The nominal elemental composition of the wollastonite used in this study, sourced from Canadian Wollastonite's Ontario mine, includes 26% silicon (55% SiO_2), 18% calcium (26% CaO), 4.0% magnesium (9% MgO), 1.8% sulfur, 0.11% nitrogen, 0.10% P_2O_5, 0.10% K_2O, 11 ppm copper, and 1.1 ppm zinc. The main mineral phases present in this wollastonite, as determined by XRD analysis, includes wollastonite ($CaSiO_3$), diopside ($CaMgSi_2O_6$), and quartz (SiO_2)."

Comment 7.

It is not clear how the SIC content varies with depth, and to what depth the wollastonite was mixed with the soil. It appears that the 0-15 cm sample shows the effects of adding wollastonite. Was anything similar observed for deeper samples?

Authors' response:

As explained for the Comment 2, we added new set of data to demonstrate the evolution of $CaCO_3$ in different depths of the soil. Based on the new results a new table (Table 2) was added to manuscript. Also, the following sections were added to representative results section:

"The $CaCO_{3(eqv)}$ content (calcimeter results) over a vertical profile (0-15, 15-30, and 30-60 cm) are demonstrated in Table 2. Accordingly, the highest content (of unsieved samples) was 10.13 $g,CaCO_{3(eqv)}\cdot(kg,soil)^{-1}$, detected in 0-15 cm depth zone. The 15-30 cm samples showed the lowest amount of $CaCO_{3(eqv)}$. The $CaCO_{3(eqv)}$ content increased again in 30-60 cm zone. The results from two deep profile samples also suggest the much higher content of carbonates in deeper layers (60-100 cm profile). Based on the results of shallow and deep samples, it is implied that there are two zones enriched in carbonates. The first is the depth zone of 0-15 cm, representative of weathering products due to wollastonite application, given the significant increase versus the control. The other zone includes deeper samples, starting at around 30 cm and increasing to great extent down to 1 m. Since deeper samples are generally indicative of soil's parent materials, it can be inferred that carbonates at this zone are of geological origin. Table 2 also depicts a comparison of the unsieved and sieved sample (pan fraction) to investigate the impact of fractionation on the carbonate content. Based on this, the carbonate content is slightly higher in the pan fraction, implying possibility of accumulation of carbonates in smaller particles, in agreement with our previous study[28]."

Comment 8.

It is stated that other rocks, such as dolerite, can be used as alternatives to wollastonite. There is no discussion of this, or of how analysis of such rocks might compare with what is reported here.

Authors' response:

Methods illustrated in the current manuscript are among usual tools used for analyzing SIC in the soil due to mineral weathering of wollastonite. However, the other minerals such as those containing magnesium may have different impact on the soil once they are applied. To address this comment, the following section was added to the discussion of the manuscript:

"We have demonstrated methods for detecting and analyzing SIC due to application of wollastonite to agricultural soils in the current study. Although these techniques could be utilized for investigating SIC in the soils amended with other enhanced weathering candidates, such as basalt and olivine, the mineral of choice may have different effects on the soil, which should be considered during the analysis. For example, the weathering process may take longer for some minerals compared to others. This is can be due to dissimilar dissolution rate of several minerals, concluding in different mineralization rate in short and long terms. The other issue is concerned with occurrence of precipitated carbonates over vertical profile of the soil, which could vary based on the silicate mineral properties and resulting geochemical condtions of the soil, inductive or not to immediate carbonate precipitation in the shallow soil. Accordingly, amendment of soils with some types of silicates could yield significant pedogenic carbonate formation in deeper layers, in contrast to the shallow accumulation of carbonates due to weathering of wollastonite detected in our studied fields."

Comment 9.

> **One key issue that is omitted concerns how newly formed pedogenic carbonates can be distinguished from geological carbonates. There is a lot in the literature about this, referring to the use of C and O stable isotopes to 'fingerprint' the carbonate.**

Authors' response:

We understand that carbon and oxygen isotopes are a robust tool for discriminating sources of carbonates in the soil (and other porous medias). However, based on our new results (Table 2 and Table 3) of vertical distribution of $CaCO_3$ content, it can be inferred that two separate zones in soil are enriched in the soil. One includes the 0-15 cm (and to some extent 15-30 cm) layer(s). The other comprises the deeper horizons start to appear at 30-60 cm and continue to extremely increase up to 100 cm (The deepest section we could collect sample in our field). We hypothesize that the latter belongs to naturally occurring C pool of the soil, usually formed over long-term intervals. In contrast, the former seems to belongs to newly pedogenic carbonates formed due to application of wollastonite to the soil. This is more corroborated as the upper most layer is the younger horizon of the soil and likely shouldn't contain significant content of carbonates of rock origin.

It is also worth mentioning that we applied the wollastonite around 1 year earlier of time of sampling to the soil, and we expect weathering process occur mainly in the shallow horizons. We predict downward migration of weathering products in the soil over longer time periods (e.g. after a few years). In this case, we can argue the co-occurrence of carbonates induced by weathering and geological origin. However, we don't anticipate this in the case of analysis depicted in current paper. Therefore, we believe the methods we utilized (e.g. calcimetry, SEM, XRD, XRF and ICP-MS) should be sufficient for corroborating occurrence of pedogenic carbonates in agricultural soils due to weathering of wollastonite and claiming carbon credits. Such procedure should be performed with a reasonable cost that make the verification process “affordable”. Therefore, involving the costly analysis such as isotope analysis may not be required in all cases.

The following section was added to discussion of the manuscript to emphasize the importance of such analysis in the cases it needs to be done:

“Due to co-occurrence geological- and atmospheric-deriven pedogenic carbonates in soil, distinguishing between different sources is of significance. Stable isotopic ($\delta^{13}C$) and radiogenic (^{14}C) carbon signatures as well as isotopic oxygen signatures are regarded as the robust tools employed for identifying source of carbon in soil (Bughio et al., 2016; Carmi et al., 2019; Manning et al., 2013; Washbourne et al., 2015). Such analysis can further verify sequestration of atmospheric CO_2 in studies aiming to monitor fate of pedogenic carbonate in soils.”

Comment 10.

Around Line 257 - does the bulk density use the ground sample, or an undisturbed sample? The results could be very different.

Authors’ response:

The method originally was explained for measuring BD in protocol was based on calculating “disturbed bulk density”. However, after reviewing this comment, the authors thought it makes more sense to use “undisturbed bulk density” which usually is measured at site using some standard method such as Cylindrical Core Method widely applied by researchers.

However, since we received this comment after our sampling was concluded, we tried to make a correlation between our measurements (as disturbed bulk density) and “undisturbed bulk density” values reported in the literature for similar soil texture (e.g. sandy loam). Accordingly, we found a ratio of undisturbed/disturbed bulk density (1.51/1.1) of 1.373 gr/cm^3 for similar soil structure in the literature [1]. Given that we measured a disturbed bulk density of 1.01 for the 0-15 cm depth sample in the lab and use the obtained ratio, we hypothesized the undisturbed bulk density would be around 1.386 gr/cm^3.

Based on the findings of a study focused on determining soil bulk density for carbon stock calculation [2], we concluded that the bulk density is a function of several climatic parameters and practice settings and may vary both spatially and temporally. Therefore, we inferred that it makes sense to report bulk density and (accordingly sequestrated CO_2), with a range to cover the inherent variability of bulk density. Given that the average of undisturbed bulk density in [2] is around 1.63 gr/cm^3, we calculated difference between our calculated undisturbed bulk density and 1.63 gr/cm^3 to be 17%. So, we consider this as the range of uncertainty to report BD.

We also have some uncertainty with our calcimetry measurements, which means we have to consider two uncertainty for calculating Areal SIC (Eq 5). Therefore we have to "propagate to the uncertainty of SIC. Based on the below formulations:

$$Q = a.b.c.d \ldots..$$

$$\frac{\delta Q}{Q} = \sqrt{(\frac{\delta a}{a})^2 + (\frac{\delta b}{b})^2 + (\frac{\delta c}{c})^2 + (\frac{\delta d}{d})^2 + \cdots}.$$

Where a, b, c and d are the parameters of the formulation with uncertainties of $\delta a, \delta b, \delta c, and\ \delta d$

Based on the uncertainties of each parameters, we can determine the uncertainty of Q.

Accordingly, for Eq. 5 we have:

$$SIC_{areal}\left(\frac{\text{tonne CO}_2}{hectare}\right) = SIC_{measured}\left(\frac{\text{kgCO}_2}{tonne\ soil}\right) X\ BD\left(\frac{tonne\ soil}{m^3}\right) X\ DT\ (m) \times \frac{1}{1000}\left(\frac{tonne}{Kg}\right) \times 10000\left(\frac{m2}{hectare}\right)$$

Since SIC measurement and BD are the parameters with uncertainties, we will have:

$$\frac{\delta SIC_{areal}}{SIC_{areal}} = \sqrt{(\frac{\delta SIC_{measured}}{SIC_{measured}})^2 + (\frac{\delta BD}{BD})^2}.$$

For 0-15 depth sample,

$SIC_{measured}$ = 3.35 ± 1.19 kg, CO2·(tonne,soil)$^{-1}$ (Note we calculated the uncertainty for "sample reading – control value" separately).

BD = 1.386 ± 0.23 t/m^3

$$\frac{\delta SIC_{areal}}{SIC_{areal}} = \sqrt{(\frac{1.19}{3.35})^2 + (\frac{0.23}{1.386})^2} = 0.39$$

Therefore, we will consider a range of SIC_{Areal} ± 39% = 6.96 ± 2.71 tonne, CO_2·(hectare)$^{-1}$

1. Shaykewich, C.F. Hydraulic properties of disturbed and undisturbed soils. Canadian Journal of Soil Science. 50 (3), 431–437 (1970).

2. Walter, K., Don, A., Tiemeyer, B., Freibauer, A. Determining soil bulk density for carbon stock calculations: a systematic method comparison. Soil Science Society of America Journal. 80 (3), 579–591 (2016)

We also add the following discussion to the manuscript on methods measuring undisturbed bulk density of soil:

"Core samplers have been widely used for extracting undisturbed samples and measuring undisturbed BD in the field[39]. Since BD is a function of several climatic parameters and practice settings, and it may vary both spatially and temporally, a reasonable number of replicates is required to yield an acceptable range of BD uncertainty in the study area[39]. This is particularly necessary for addressing big uncertainties in SIC_{areal} calculated estimates, such as the value determined in the current study (i.e. ± 39%)."

Reviewer #2:

Manuscript Summary:

This paper provides a clear and detailed protocol for sampling soil inorganic carbon in agricultural fields, and the response of soils to the addition of wollastonite. This work clearly describes how to sample soils in the field, and analytical methods of determining carbonate storage (calcimetry) and minerology (various methods).

It was with great interest that I read this submission; I am very interested in soil inorganic carbon quantification and the possibility of sequestering carbon as carbonates. I am hoping that you are also submitting another paper that is focused on the implications of this study.

Comment 1.

> **I am confused about whether there is supposed to be a video accompanying this paper? I did not see one if so. Following on the comment above, if this is strictly a methods paper, you might be better served to also submit your implications to a journal (e.g. Catena) where a different range of researchers will see your work.**

Authors' response:

Yes, a video comes along with this manuscript to visualize some key steps described in the Protocol for calculating soil inorganic carbon. The filming campaign includes two sections. First part comprises steps performed for collecting samples in the agricultural field site (this part has been completed). The other part involves measurements carried out in laboratory for calculating SIC using calcimetry method. This hasn't been completed yet as laboratory access is restricted due to COVID pandemic.

Comment 2.

> **The organization of the paper is a bit awkward at the end of the introduction. You have framed your thesis statement in terms of carbon credits (and the need for quantifying carbon to obtain carbon credits). This is true, but does not follow smoothly on the rest of your intro. Why not just stick with the need for a well-developed and consistent protocol for measuring inorganic carbon? This is needed for many reasons (estimations of SIC storage, changes, etc).**
> **Extra 'as' on line 86In the discussion,**

Authors' response:

Authors agree that the final paragraph of the introduction did not address importance of a consistent protocol. To do so, the sections were revised as follow:

"At the field scale, an important limitation is the use of low application rates of silicate soil amendments. As there is limited knowledge on the effect of many silicates (such as wollastonite and olivine) on soil and plant health, commercial producers avoid testing higher application rates that could result in significant carbon sequestration. As a result of such low application rates, as well as the large area of crop fields, a research challenge commonly faced is to determine changes in SIC when values are relatively low, and to recover and isolate the silicate grains and weathering products from the soil to study morphological and mineralogical changes. In our past work, we reported on how physical fractionation of the wollastonite-amended soil, by using sieving, enabled better understanding of the weathering process, especially the formation and accumulation of pedogenic carbonates (Dudhaiya et al., 2019). Accordingly, the higher contents of wollastonite and weathering products were detected in finer fraction of soil, which provided reasonably high values during analyses, ensuring more precise and reliable results. The findings highlight the importance of using physical fractionation, through sieving or other segregation means, for reliable estimation of the sequestrated carbon accumulation in silicate-amended soils. ~~This is particularly crucial for verification of carbon sequestration in carbon credit programs. It is thus recommendable that soils amended with silicates, especially under low application rates, are analyzed following fractionation using sieving.~~ However, the degree of fractionation could vary from soil to soil and from silicate to silicate, so should be further researched.

Accurate measurement of SIC is critical for establishing a standard and well-developed procedure that can be adopted by various researchers interested in analyzing the evolution of SIC (and also organic carbon) over time and depth of the agricultural soil. Such methodology also enables farmers to claim carbon credit as a result of SIC formation in their field soils. ~~In order to enable farmers to claim carbon credit as a result of SIC formation in their field soils, there must exist a scientific protocol for statistically significant soil sampling method, followed by soil preparation and characterization to precisely verify and quantify the changes in SIC over time.~~ The following protocol describes, in detail: (i) a soil sampling method to be used following soil silicate amendment, which accounts for the statistical significance of the analyzed soil data; (ii) a soil fractionation method that improves the accuracy of quantifying changes in pedogenic inorganic carbonate pool as a result of enhanced silicate weathering, and (iii) the calculation steps used to determine the SIC sequestration rate as a result of soil silicate amendment. For the purpose of this demonstration, wollastonite, sourced from Canadian Wollastonite, is assumed to be the silicate mineral applied to agricultural soils, and the agricultural soils are considered to be similar to those found in Southern Ontario's farmlands."

The extra 'as' was removed from the manuscript.