|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| |  | | --- | | **Nam Nguyen <em@editorialmanager.com>** | | AttachmentsJun 3 (2 days ago)  https://mail.google.com/mail/u/0/images/cleardot.gif |  | **https://mail.google.com/mail/u/0/images/cleardot.gif**  **https://mail.google.com/mail/u/0/images/cleardot.gif** |
| |  | | --- | | to me  https://mail.google.com/mail/u/0/images/cleardot.gif | | | |

Dear Dr. Lawrence,  
  
Your manuscript JoVE54815R1 "Methods of soil resampling to monitor changes in the chemical concentrations of forest soils" has been peer-reviewed and the following comments need to be addressed. Please keep JoVE's formatting requirements and the editorial comments from previous revisions in mind as you revise the manuscript to address peer review comments. Please maintain these overall manuscript changes, e.g., if formatting or other changes were made, commercial language was removed, etc.

Please track the changes in your word processor (e.g., Microsoft Word) or change the text color to identify all of the manuscript edits. When you have revised your submission, please also upload a separate document listing all of changes that address each of the editorial and peer review comments individually with the revised manuscript. Please provide either (1) a description of how the comment was addressed within the manuscript or (2) a rebuttal describing why the comment was not addressed if you feel it was incorrect or out of the scope of this work for publication in JoVE.

Your revision is due by **Jun 17, 2016.** Please note that due to the high volume of JoVE submissions, failure to meet this deadline will result in publication delays. To submit a revision, go to the [JoVE Submission Site](http://www.editorialmanager.com/jove" \t "_blank) and log in as an author. You will find your submission under the heading 'Submission Needing Revision'.

**Editorial comments:**

The manuscript has been modified by the Science Editor to comply with the JoVE formatting standard. Please maintain the current formatting throughout the manuscript. The updated manuscript (54815\_R1\_042716.docx) is located in your Editorial Manager account. In the revised PDF submission, there is a hyperlink for downloading the .docx file. Please download the .docx file and use this updated version for any future revisions.  
  
Changes to be made by the Author(s):  
  
1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues. The JoVE editor will not copy-edit your manuscript and any errors in the submitted revision may be present in the published version.  
  
2. Please upload the Tables as xls or xlsx files for proper integration when published.  
  
3. Please clearly state in both the short and long abstract the goal of the protocol.  
  
4. Formatting: Please format all author names identically, either First Last or Last, First.  
  
5. Grammar:  
-Please remove all instances of “you” or “your”.  
-Section 6 – Please use appropriate punctuation (commas) when formatting a list.

NOTE: Line numbers listed in the responses below are approximate because they shifted as further changes were made. All changes are tracked.

6. Additional detail is required:  
-1.1 – Approximately what size should a unit be?

*We can’t approximate the study unit size because it will depend on the considerations discussed in the Introduction. There isn’t a right or wrong size. The study unit needs to be sized to properly meet your monitoring objectives. To make this clear we have added the following sentence that starts on Line 168.*

-2.2 – How is it removed?

*Information added on line 326.*

-2.4 – Is recording done by hand?

*Information added on line 335.*

-3.1 – Are there any criteria for the horizons/depths to be sampled? Please list in a note.

*Information added on line 369-375.*

-3.2 – What is the necessary mass?

*Information added starting on line 378.*

-3.3 – Please clarify “to the extent possible”.

*Information added starting on line 386.*

7. Discussion: Please discuss the significance with respect to alternative methods, the limitations, and the future applications of the protocol.

*Information added starting on line 711*

**Reviewers' comments:**

**Reviewer #1:**  
*Manuscript Summary:*  
The manuscript explains an appropriate method of soil sampling.  
  
*Major Concerns:*  
N/A  
  
*Minor Concerns:*  
Such information is at best conveyed in practical soil classes and practical work including guidance by experts may be the only valid way to get an understanding of the dos and donots of soil sampling. However, when a community asks for a narrative on soil sampling the text will be useful. The pictures certainly give a good account of well-organized soil sampling and sample handling. The attached spreadsheet with the list of required items may be too much, as people engaging in field work will find out their needs on themselves.  
  
*Additional Comments to Authors:*  
N/A  
  
*Since the spreadsheet of needed equipment is required by the journal, we have not made any changes in response to this review.*

**Reviewer #2:**  
*Manuscript Summary:*  
This paper is a well-written and straightforward guide for repeated sampling of soils over time, with special attention to avoiding bias from spatial variability and analytical methods. I believe it merits publication and visualization in JoVE.  
  
The one main point in the paper that I feel was not sufficiently addressed is whether changes occur in dried soils in storage, and how such changes may be differentiated from analytical bias.

*For most of the methods commonly used in forest soil analyses (based on citations given in Section 5), information currently in the literature supports the air-dried storage of soil samples without detectable change for up to 30 years, as discussed and cited in the paragraph starting on line 262. However, the reviewer is correct in pointing out that more work needs to be done in this area. We feel that delving further into this area in this particular paper, exceeds the defined scope, and doesn’t warrant a visual presentation. This same group of authors is currently working a separate paper that tackles this question directly.*  
  
Following are additional minor comments, by line number:  
228-231. This sentence could be clarified. How does deep sampling help reduce uncertainty for detecting changes near the surface?

*An example was added starting on line 233.*

248. No changes in dried soil? Needs ref.

*This was used as an introductory statement. The discussion of changes in air-dried soils, with citations is presented in the following paragraph.*

310. Section 1.6 is a little vague, I am not sure what is meant by "land surfaces" here. Also it may help to give an example or two for the second sentence.

*Information added with specific examples starting on line 319.*

364. You touched on it earlier, but it may be helpful here to reiterate why to sample one way or another (by depth or horizon). Or refer reader to the Discussion section, where it is discussed more in depth.

Per editor’s suggestion, specifics on this were added in the procedure starting at line 375  
375. If sampling by horizon, still record depth?

*Yes, as is done in the NRCS protocol. Knowing the depth of the horizon is important in maintaining sampling consistency.*

399. Specify "oven" drying here.

*I couldn’t find this on line 399 or anywhere in the section to which I think they was referring*.

419. Not clear if "those" refers to forest soils or analytical methods.

*Replaced “those” with soil samples.*

455. Person name also?

*We left this out because it seemed that over 5-10 years or longer the individual overseeing the archived could change.*

502-508. How can you differentiate analytical bias from storage effects?

*If there is no difference between the original data and the results of the reanalysis of the same samples years later you can rule out storage effects. The citations provided showed this for all of the common measurements except pH, as mentioned in the text.*

615. "P<0.10" for consistency.

*Correction made*

Table 1. Need delineation to set off the three blocks from one another. "measurement" misspelled.

*Corrections made.*

I found typos on these line numbers: 218, 301 (comma not needed), 398, 425 ("inter" not stand-alone), 496, 655, 665.

*Corrections made.*  
  
*Major Concerns:*  
N/A  
  
*Minor Concerns:*  
N/A  
  
*Additional Comments to Authors:*  
N/A  
  
  
**Reviewer #3:**  
*Manuscript Summary:*  
This manuscript provides a thorough description of a repeated soil sampling method to monitor changes of chemical characteristics in forest soil. It discusses the background information and protocols of soil sampling site design, sample collection, sample processing and analysis, sample archive and consistency verification. Examples from multiple studies are used to emphasize the critical steps in resampling. Error in soil sampling methods is the major source of uncertainty in soil change studies. This paper could be a guideline for future study, especially for large scale monitoring networks. The introduction of this method is very valuable and timely. The topic of this paper is suitable for JoVE. I'm looking forward to see the video.  
  
*Major Concerns:*  
-This manuscript described the soil sampling method in idea condition. However, forest soils could be rocky, or saturated etc. In most of cases, it will be difficult to dig a soil pit with a clear and smooth profile. This manuscript did not provide any back up plan about how to deal with a non-ideal situation. If the soil is rocky, the content of coarse fragment could be very different within a small distance, and coarse fragments are highly correlated with chemical concentration. But coarse fragment was not even measured or estimated in the method. For this reason, the practicality of this method is questionable.

*Specifics on where not to attempt pit excavation has been added to Section 1.6. However, coarse fragments within the soil profile will not be apparent until excavation is underway. This method has been used extensively in soils with high and variable amounts of coarse fragments without serious complications. Clearing a pit face for description and sampling in these types of soils is not generally overchallenging, although in some cases the pit might need to be expanded. Mention of this has been added to Section 2.3. The comment regarding the measurement of coarse fragments is not applicable. The method described in this paper is designed to identify changes in soil chemical concentration, which does not require measurements of coarse fragments. The measurement of total mass of chemical elements (which does require measurement of coarse fragments), is a different method that provides different information. To incorporate this additional component to the approach presented here would substantially increase the size of the paper well beyond journal limits and therefore could be addressed more readily in a separate paper. With regard to the comment that “coarse fragments are highly correlated with chemical concentration, we respectively disagree with the reviewer and feel that the cited papers which have used this method in soils with large amounts of rocks successfully support our view. Much of the soil resampling work has been done in glaciated soils with various silicate mineralogy, where rocky soils are the rule rather than the exception. It’s the chemical composition of coarse fragments (mineralogy) that strongly effect soil chemistry, not the volume or mass of the coarse fragments. The soil parent material within a study unit will more often than not be a variable mixture of differing mineralogy, but the replication of pits is used to address this form of variability, as well as the other soil-controlling factors that also vary. The practicality of the method has been demonstrated in the peer-reviewed literature that appears in the bibliography.*

-A very important element of the soil sampling design is the number and size of study unit. But this manuscript did not discuss much about how to determine the number and size of the study unit. Forest soils are heterogeneous. To detect the change of a certain chemical concentration over time, the spatial variation of the targeted characteristics across and within the study unit should be considered. In addition, the resampling interval that needs to detect the change also depends on the sampling density (how many soil pits over a certain area). The role of spatial and temporal variation in determining study unit and interval should be adequately explained in the method.

*Text has been added to the paragraph that begins on line 161 to better explain how to select a study unit. Text has also been added at the end of the Section “Designing the Soil Monitoring Study” on the frequency of sampling.*

-Figure 8b is a very bad example of adjusting the analytical bias. The model was driven by an outlier and not statically reliable.

*Yes, one point falls much further down the axis, but in this case it actually alters the best fit line very little. This is shown by the R2 value of 0.99. The model is reliable. As an example, if you use the model that includes this point and plug in an original analysis value of 1.0, the model yields a reanalysis value of 0.81; and if you remove the point the model yields a reanalysis value of 0.73, a difference of about 10%, within the tolerance of normal analytical variability.*   
  
*Minor Concerns:*  
Line 165-167 The resampling interval is very important in sampling design. Please discuss more about how the interval should be determined in different study. In my opinion, the interval should be determined based on the variation of the targeted variables such as chemical concentrations, not the driver of the changes.

*Actually both the variation of measurements and the driver of changes are important factors. As indicated above, a paragraph has been added* *at the end of the Section “Designing the Soil Monitoring Study” on the frequency of sampling.*

Line 181-185 In fact, disturbance could be a very important driver of soil changes in forest. Soil resampling is a useful way to detect the effect of disturbance on soil chemical concentrations. A well design resampling method should consider the possibility of disturbance, not avoid them.

*Note that this sentence and paragraph refers to UNDESIRED changes. I think the wording of this paragraph makes it clear in its current form.*

Line 190-191 How large was the area of interest? How large was the study unit? Why five study units was selected, not four or six? Which factors determined the number of study units within a certain area?

*As indicated in the caption for Figure 1, this is a generalized example. The additional information on study units should clarify this for the reader.*

Line 224-225 How to verify the organic-mineral interface with laboratory analysis? To be specific, what are the criteria for the organic or mineral horizons? Are multiple samplings around the possible interface necessary for this verification?

*The defining criteria for an organic horizon is a concentration of organic carbon > 20%. This information has been added with citation.*

Line 241-244 Is the air-dry process good enough to stabilize total C in the soil sample? Is there any evidence to prove that C was not consumed by microbial activity.

*There may be some consumption of organic matter during the drying period, but once air-dried, the organic carbon contcentration is stable. This is shown by Figure 10 and the discussion that begins on line 565.*

Line 263-264 The unused samples were archived after air-dry or after sieving? Since sieving can dramatically change the mass of the samples, this will also affect the mass of soil that should be collect in the field. It is also need to be notice that changes in sieving protocol, or even personal error in the sieving process, can be important sources of uncertainty in soil resampling studies.

*See section 6.1, archiving occurs after chemical analysis (section 5) (which happens after sieving (Section 4). Little loss of soil mass occurs with most soils (although a few soils have peds that are very difficult to break up). The sieving is for removal of stones and roots. Correct sieving is important, particularly the sieve size, which is why its included in the archiving information (section 6.2).*

Line 292-297 What if the highest or lowest point in the study unit is not on the edges?

*“Aspect” has been added to the list of landscape features that should be the same or similar within a study unit to reduce variability (Line . Our approach provides a consistent way to provide a general slope measurement for the study unit.*

Line 305-308 The tree layer is much more important and complicated than the understory. More explanation is need to describe how to identify the forest type and dominate tree species. Photo should be taken for trees as while.

*Wording to define dominant tree species (those that reach the top of the canopy) has been added to section 1.5. Effectively photographing the canopy requires special camera equipment and techniques. Not necessary for soil monitoring.*

Line 310 How did the sampling location selected within the study unit? They were randomly located or located in a certain point of the gird?

*The text (Line 174) specifies that either approach is suitable.*

Line 310-313 Avoiding certain land surfaces may induce bias to soil sampling. Forest soils are commonly rocky. Soils close to outcropping or trees could have very different chemical concentrations. It seems impractical to avoid the effect of location selection in resampling in rocky area.

*This is covered in Section 1.6, which has had some additional wording added.*

Line 339 Figure 3 didn't show a 'white piece of paper with small amounts of soil on it to assist horizon identification'.

*Yes it does.*

Line 345 No 'pins or similar objects' in figure 4 and 5.

*Figure number changed to “Figure 2, which does show the objects. Also, placement of Figures 4 and 5 was changed.*

Line 372 If the soil could be stony, why the present of rocks are not be evaluated in profile description.

*This has been added to section 2.11.*

Line 406 The content of coarse fragment and roots should also be measured.

*This has been added to section 2.11.*

Line 447 What temperature is recommended? Can different labs keep samples archived at different temperature?

*The temperature itself does not change the soil, but fluctuations in temperature can cause condensation in the bags.*

Line 475-478 Did the archived samples from the first measurement reanalyzed with the new samples? The results of reanalyzed should also be compared with the original data to prove a significant changes over time without analytical bias.

*This is covered in the second paragraphs 2-6 in the Representative Results section.*

Line 452-455 Is this listed information archived in a single file or separated spreadsheet? It would be helpful if an example could be given to show the table head of the database.

*We are leaving the database structure/organization up to the user because they need to incorporate the archive information into their own system.*

Line 459 Not clear. Twelve samples for each horizon, or twelve samples from different horizons?

*!2 samples from each horizon. The section on “Soil Sample Collection – Background Information” discusses the importance of treating samples from each horizon discreetly.*

Line 464-467 What if the significant difference between previous analysis and current reanalysis is a result of different sample processing methods or changes of concentration during storage? Rerun all the archived samples cannot prevent these problems.

*That is correct and why archived samples are important to help identify if these potential problems occurred so that differences resulting from artifacts are not assumed to be changes in soils caused by some type of environmental driver.*

Line 486-517 Why only Ca and Al were analyzed in this study? Is there any intrinsic characteristic of these elements that will affect the result? It is likely that Ca is stable during storage, while Al are not. Therefore the Al loss in storage is combined with bias from changing analysis method. What about other elements (Fe, N, etc.)? Are they suitable for this reanalysis?

*Ca and Al were used as examples because there has been a focus on these measurements in the literature as a result of acid rain effects on soils. This was provided so that the user can set up their own similar system for checking their own measurements and procedures. The journal does not allow room for comparisons of a long list of other elements but information on a number of elements is available in the literature. , I am not aware of why Al should not be stable over time in an air-dried sample. There is some information in the literature that indicates that Al is stable.*

Line 494-496 I can't see a cause-effect relationship between" analytical bias was negligible between analyses" and "changes in exchangeable Ca concentrations during the 14-16 years of storage were also be assumed to be minimal". The propose of this sentence is not clear.

*Sentence has been rewritten.*

Line 505-508 The linear relationship was mostly driven by outlier. Even though the R2 was high, this model should not be used to adjust the original values. If the sample with largest Ca has 5% increase (within the range of possible error) in the reanalysis, the model will have a very different slope, which means the regression model itself is not reliable. If more samples were reanalyzed (e.g. all the 40 samples are reanalyzed), it is likely that other outliers will show up and significantly change the regression model. Then figure 8b will looks no better than figure 9b.

*As pointed out above, running the regression with or without the “outlier” doesn’t make much different in the result. The data distribution does create some uncertainty (ie what would additional points look like?), but whether to use the point or not is a judgement call because the point does not have a strong biasing effect on the relationship. Anyway, the point of this example is to demonstrate the method to be used, not to provide an actual model that other users should apply in their own studies.*

Line 542-544 This sentence is too long. Break into two sentences.

*Just two phrases, it seems to read clearly.*

Line 555-620 Captions of figures and tables should be put at the end of the manuscript, or at least after the discussion and acknowledgments sections.

*The layout of the manuscript follows the journal rules.*

Line 556 The location of the site should be briefly described here.

*It’s a generalized diagram, the location is irrelevant.*

Line 580-596 The sources of the data (such as Buck creek and Turkey Lake) should be indicated in the figure captions. Otherwise the readers will fail to notice that figure 10 came from a different study from figure 8 & 9.

*This information comes from published articles (in the reference list), that provides all the site details.*

Line 615-616 Change to "Analyses with P < 0.1 are shown in yellow to indicate significant differences."

*Change made.*

Line 632-636 Horizons with unclear boundaries are less desirable for sampling with horizons. Then what? Is the depth increments method should be used and accurate enough in this situation?

*Yes, as discussed in the Introductory material.*

Line 640 Figure 11 --> Figure 12

*Correction made.*

Line 642 "Sampling the upper 10 cm of the B horizon" Is this a method combining the horizon and depth increment methods? If yes, this would be a third method that should be discussed in detail. In what situation this combing method should be recommended?

*This is discussed starting on line 243: “Sampling by both horizon and depth within the same soil profile can be effective in addressing variations in the distinctness of horizons within that profile.”*

Line 644 Photo 5 -> Figure 5?

*Figure 12 is correct here.*

Line 658-670 This paragraph is trying to provide a way to evaluate the bias from sampling inconsistencies. However, the example showing here is a very special case that can barely be used in other soils. In addition, the comparisons of measurements among horizons seems only work qualitatively. Is there any method that can assess the sampling consistencies quantitatively?

Again, the second sentence in this paragraph starts with “For example”. This TYPE of comparison can be used in many different soils even though the actual horizons might differ.

Figure 1 A scale bar should be added to the map.

*Again, this is just a generalized schematic.*

Figure 4 and 5 What is the white thing on the ground? Snow? Did it have any effect on the sampling process?

*Yes, there was a dusting of snow on the ground surface. This small amount of snow does not affect sampling of this particular soil. The point of the photo was to show examples of horizination.*

Figure 8 The data point at upper right is an obvious outlier, which should not be used to establish a adjusting model. Why this sample had such a high Ca concentration? How many other samples had similar problem in the original analysis?

*Why has the reviewer brought this up 3 times?*

Table 1 Should show the gridlines, especially separate the measurements from n=12, 8 and 4 into three clearly framed sections.

Correction made.

Table 2 Reverse columns for PH and Al concentration to put similar variables together. Why some sites were labeled with forest type (CF, HW and MF) , but others were not?

*Switch pH and Al columns to put similar variables together? Don’t follow. Table 2 was modified from Lawrence et al. 2015. Some sites had two different study units based on forest type. This information has been added to the Table caption.*

*Additional Comments to Authors:*  
N/A  
  
  
**Reviewer #4:**  
*Manuscript Summary:*  
Lawrence et al. describes a standard practice of resampling soils to monitors changes in the chemical concentrations of forest soils. The paper is clearly written and the procedures are well defined. I think it will be helpful to have some discussion on the limitation of this method and the comparison to other soil sampling procedures in the field of soil ecology.  
  
*Major Concerns:*  
My major comments are as follow:  
1. Designing the soil monitoring study.  
-It would be better to have a table to summarize a list of factors needed to be considered in the study design. For the current version, it is not easy to maneuver thorough this dense paragraph to find key information. I would suggest to follow the order of: how to define/select a study unit, the area of the study unit, sampling size and sampling interval.

*We have made a number of additions to the section on Study Design in response to the various review comments. Most of the decisions regarding how the study is designed are dependent on multiple considerations, as described in the text. We feel that this information would be difficult to organize in a table.*

-Another thing that is also relevant to the study design: If the study is to monitor the consequence of environmental change (e.g., acid deposition) on soil properties, then additional study sites where the environmental change is minimal may be necessary to serve as control to avoid confounding changes from soil development. Or study sites that are under clear successional changes need to be avoided.

*What the reviewer is describing is more along the lines of an experiment, rather than monitoring change over time, as water and air quality have been monitored for decades. The approach that we describe here focuses on monitoring for effects of large-scale environmental drivers, but could be applied to long-term experiments. Some text on this has been added to the beginning of the second paragraph of the Introduction, and to the final paragraph of the Conclusion.*

2. Soil sample collection  
-In addition to where to sample, I think it is also important to consider the time/season for soil sampling. For example, avoid soil sampling right after heavy rain events. Make sure consistent sampling season (or when plants are in similar phenology) among sampling over time.

*Additional text on when to sample has been added at the beginning of the Section “Soil Sample Collection-Background Information”.*

-Pit excavation is very destructive to the study sites. It need to be cautious whether the sampling itself would bring significant disturbance that may change the under story plant community which may have further consequence on soil properties. Having a buffer zone around plots or it may be necessary to evaluate and determine the influencing zone of an excavated pit on neighboring intact plots.  
Therefore, I think it is necessary to have in the discussion the pros/cons of pit excavation compared to other soil sampling methods such as using soil cores that has fewer disturbances.

*The minimum area needed for pit excavation is discussed starting on line 203. We do not recommend the use of corers for in this procedure because (1) they are very ineffective at sampling in rocky soils, which is typical in most forested settings, and (2) there is not sufficient information obtained on the profile to help verify that the sampling is done consistently between samplings. Furthermore, compression of the soil collected often occurs in the corer so the depth over which the core was collected is uncertain.*  
  
*Minor Concerns:*  
Protocol  
2.2). Any protocol if running into a big bed rock that compromise digging?

*Text has been added on this to section 2.3.*

3.2) Is there a minimum amount of soil mass for a full set for general soil chemical analyses?

*This will depend on which analyses you are performing and which chemical analysis methods are being used. Guidelines for archiving soil mass are provided starting on line 300.*

4.1) A subset of fresh soils need to be weighed out if the determination of soil water content is desired.

*Section 4 only deals with moisture measurements to determine whether samples can be considered air dried. I think this is explicit in Section 4.2. and 4.3. Converting chemical concentration data to oven-dried soil mass requires oven drying of subsamples known to be already air-dry. This would be covered by the chemical analysis SOP.*

4.2) Can also mention other drying methods, such as freeze-dry, if quantification of certain chemicals/DNA is needed.

*Beyond the scope of this procedure.*

7.2) line 464-467: Contradict to result in lines 504-506. If significant correlation is found, regression model can be used to adjust the values from the remaining samples.

*Correction has been made in Section 7.2.*

Table 2, line 612: T tests?. Also there is one \* in the table was not explained.

Information added to Table 2 caption. Asterisk has been removed.

Figure 1. Two lines for "B. Study units within the area of interest"

Written as intended to be explicit in explaining that the study units occur within the area of interest.  
  
*Additional Comments to Authors:*  
N/A