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Methods of soil resampling to monitor changes in the chemical concentrations of forest soils

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Abstract:	Recent soils research has shown that important chemical soil characteristics can change in less than a decade, often the result of broad environmental changes. Repeated sampling to monitor these types of changes in forest soils is a relatively new practice that is not well documented in the literature and has only recently been broadly embraced by the scientific community. The objective of this protocol is therefore to synthesize the latest information on the methods of soil resampling in a format that can be used to design and implement a soil monitoring program. Successful monitoring of forest soils requires that a study unit be defined within an area of forested land that can be characterized with replicate sampling locations. A resampling interval of 5 years is recommended, but if monitoring is being done to evaluate a specific environmental driver, the interval should be based on the rate of change expected in that driver should be taken into consideration. Here, we show that the sampling of the profile can be done by horizon where boundaries can be clearly identified and horizons are sufficiently thick to remove soil without contamination from horizons above or below. Otherwise, sampling can be done by depth interval. Archiving of sample for future reanalysis is a key step in avoiding analytical bias and providing the opportunity for additional analyses as new questions arise.
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TITLE:
Methods of soil resampling to monitor changes in the chemical concentrations of forest soils

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SHORT ABSTRACT:
Repeated soil sampling has recently been shown to be an effective way to monitor forest soil change over years and decades. To support its use, a protocol is presented that synthesizes the latest information on soil resampling methods to aid in the design and implementation of successful soil monitoring programs.

LONG ABSTRACT:
Recent soils research has shown that important chemical soil characteristics can change in less than a decade, often the result of broad environmental changes. Repeated sampling to monitor these changes in forest soils is a relatively new practice that is not well documented in the literature and has only recently been broadly embraced by the scientific community. The objective of this protocol is therefore to synthesize the latest information on methods of soil resampling in a format that can be used to design and implement a soil monitoring program. Successful monitoring of forest soils requires that a study unit be defined within an area of forested land that can be characterized with replicate sampling locations. A resampling interval of 5 years is recommended, but if monitoring is done to evaluate a specific environmental driver, the rate of change expected in that driver should be taken into consideration. Here, we show that the sampling of the profile can be done by horizon where boundaries can be clearly identified and horizons are sufficiently thick to remove soil without contamination from horizons above or below. Otherwise, sampling can be done by depth interval. Archiving of sample for future reanalysis is a key step in avoiding analytical bias and providing the opportunity for additional analyses as new questions arise.

INTRODUCTION:
Soil development has traditionally been viewed in terms of processes that take place over

centennial to millennial time scales [1]. Monitoring of soils that had not been perturbed by intensive uses such as agriculture was not typically considered important for policy or management concerns on the time scale of years to decades. However, recent soils research has shown that important chemical soil characteristics can change in less than a decade, often the result of broad environmental changes driven by consequences of human activities such as air pollution and climate change [2]. In eastern North America, repeated soil sampling is providing valuable information on the effects of acidic deposition through records of soil change in forested settings. In an effort to support and coordinate this work, the Northeastern Soil Monitoring Cooperative (NESMC) was formed in 2007 [3]. This paper is part of the continuing effort of the NESMC to provide information that advances the use of repeated soil sampling of forest soils as a valuable tool for monitoring our changing environment.

Repeated sampling has been used to assess changes from experimental manipulations, but long-term monitoring of forest soils in response to environmental drivers is a relatively new practice that is not well documented in the literature and has only recently been broadly embraced by the scientific community. Past skepticism was due in large part to the view that the rate of soil change was too slow to detect in the presence of the high spatial variability (horizontal and vertical) typical of forest soils. Because the collection of soil is destructive, resampling can only be done near the original sampling location. Therefore, spatial variability within the 3-dimensional space from which samples are collected must be properly quantified to detect real changes and avoid results that are an artifact of the collection method. Furthermore, the process of soil sampling and chemical analysis creates potential sources of measurement instability that can mask changes or bias results [4]. Measurement instability cannot be completely removed, but can be sufficiently controlled with the proper protocols to produce results with minimal uncertainty.

Designing the Soil Monitoring Study

Soil monitoring requires that soil samples are collected repeatedly over a time interval defined by the investigator. Shorter time intervals decrease the length of time needed to statistically detect a change, but longer intervals provide more opportunity for soil changes to occur [4]. A resampling interval of 5 years is recommended to balance these two factors, but if monitoring is being done to evaluate a specific driver, the interval should be set based on the rate of change expected in that driver [2]. Successful monitoring of forest soils also requires that a study unit be defined within an area of forested land that has been selected for soil monitoring. Repeated sampling at multiple locations within the study unit is used to determine if the soil of that specific study unit has changed over time. Additional study units can be selected, but each is statistically analyzed separately to evaluate if soil changes have occurred. Statistical results of multiple study units can then be grouped for the purpose of regional analysis, as demonstrated in Lawrence et al.[5]. The type and size of the study unit will depend on the monitoring questions being asked and the following study design considerations. Soil sampling within the study unit can be done at random locations or on a grid to obtain replicate samples as long as the sampling is done at enough locations to characterize the areal variability of the study unit without bias [4]. A study unit located within a single landscape type with regard to features such as slope, hillslope position, aspect, vegetation, parent material and drainage will tend to have less areal variability than a study unit that spans more than one landscape type. Avoiding sampling bias in each collection is needed to enable the values from pits sampled in any one collection to be statistically compared to the values obtained in prior and future collections. As the size of the study unit increases, the areal variability within the study unit may also increase from factors such as vegetation or slope changes. If potential causes of variability such as these become encompassed within the study unit, additional sampling locations will be needed to characterize the possible variability in soils that may occur. Therefore, the size of the study unit needs to be determined by the investigator based on the variability of the area being considered and the project resources available for sampling and resampling efforts.

A key criterion to be considered in locating the study unit is the potential for future undesired site disturbances. There should be some level of assurance that site conditions will remain suitable for the defined monitoring objectives for several decades or more. For example, a study unit with the single objective of monitoring climate change effects should be located in an area where logging will not occur in the foreseeable future.

The methodology described herein covers the sampling of an individual study unit. Study units can be replicated within a landscape type or study units can be added to characterize additional landscape types depending on the objectives and scope of the study, including whether the study involves an experimental manipulation. An example of a soil monitoring design is illustrated in Figure 1. Within the area of interest (western Adirondack region), six study units have been located. In this case, each study unit is gridded into 25 equal-sized plots. Each plot needs to be large enough to provide a space suitable for pit excavation. In forested upland terrain of the northeastern U.S. and eastern Canada, a suitable space to excavate a pit to a depth of 1.2 m can generally be found within a 10 m by 10 m area. Therefore, in our example, the total area of the study unit equals 1.0 ha. Each time the study unit is sampled, a chosen number of plots are randomly selected for sampling. If five replicate plots are randomly selected for sampling on a five-year interval, the study unit could be monitored for 25 years. The area required to excavate and sample one pit will vary among landscapes and must be taken into consideration in the sampling design.

The degree of replication within a study unit and the frequency of repeated sampling will vary depending on the study unit characteristics, the questions being asked and the nature of disturbances that are anticipated. Based on soil resampling studies that have detected changes with measurements commonly used in forest soils, a resampling interval of 5 years and a minimum of 5 replicate sampling locations within each study unit are recommended. Decreasing the frequency of resampling and increasing sampling replication will strengthen the ability to detect changes.

[Place Figure 1 here].

Soil Sample Collection – Background Information
The collection of soil samples should be done during the season when soils tend to be dry, which most often occurs in the latter part of the growing season. By resampling at this time, consistency is also achieved with regard to plant phenology, a possible influence on soil chemical conditions. Sampling should be avoided during or immediately after heavy rains or when the soils are exceedingly wet. At least one location within the study unit should be described and documented following the USDA Natural Resource Conservation Service (NRCS) Field Book for Describing Soils [6], or other appropriate protocols if following a soil classification system used outside the U.S. The field protocol provided herein follows the U.S. classification system and requires a copy of the NRCS Field Book for Describing Soils in the field. The sampler should have training and

experience describing and sampling the soil type being monitored before implementing the soil monitoring protocols.

Soil collection can be done in a variety of ways, but the use of a repeatable technique is crucial to monitoring soil change. The field methodology should be recorded in a standard operating procedure (SOP). Changes in collection procedures between samplings should be avoided, but when this is not possible, all details must be documented.

Tests should also be done to evaluate the potential for bias caused by procedural changes. Sampling can be done by horizon where (1) boundaries can be clearly identified in the field and (2) horizons are sufficiently thick to remove soil without contamination from horizons above or below. Where these criterion are not met, sampling by depth interval can be done. In any sampling, particular care must be taken to avoid mixing soil from the surface organic-rich horizon (usually O or A) with the uppermost mineral horizon (usually B or E). In some soils, changes in texture and color are readily visible across the organic-mineral interface, whereas in other soils color changes can be minimal so textural changes that reflect differences in organic carbon (C) concentration must be relied on to identify the location of the interface. Determining this interface from textural changes can be difficult, even for experienced soil scientists. Verification of the organic-mineral interface can be done with laboratory analysis of the carbon concentration (organic horizon is defined by organic carbon concentration > 20%[7]). In some soils, the O horizon can be less than 1 cm thick and may be too thin to sample. Sampling by both horizon and depth within the same soil profile can be effective in addressing variations in the distinctness of thicknesses of horizons within that profile. The horizons or depths to be sampled will also be dependent on the objectives of the monitoring program. Soil changes in layers closer to the surface have been more commonly identified than in deeper layers, but including deeper horizons or depth intervals can provide information that is helpful in reducing uncertainty of results. For example, in an initial sampling, a glaciated soil, heavily leached by acidic deposition, showed base saturation to be minimum in the upper B horizon then increase with depth. In a repeated sampling, this pattern should also occur even if concentrations of individual layers change. If a different pattern is observed in the repeat sampling, there is a strong possibility that the two samplings were not done in comparable soil. Ideally, the sample should be collected over the full horizon thickness. However, in excessively thick horizons vertically integrating sample collection may be difficult over the entire thickness. In this situation, samples of equal volume can be collected at equally spaced intervals from the bottom to the top of the horizon. If sampling is not done over the full horizon thickness, record the sampling depth interval within that horizon.

Soil Sample Processing and Analysis – Background Information

The process of removing a soil sample from the profile alters that sample by severing roots, and causing changes in factors such as temperature, moisture, oxygen and other gas concentrations. Therefore, some measurements must be done quickly without the ability to preserve the sample, making them difficult to use in long-term monitoring programs. However, for most common physical and chemical measurements such as texture, bulk density, total C and nitrogen (N), and concentrations of total and exchangeable metals, air-drying the sample after collection provides a relatively consistent method for stabilizing the chemistry before analysis. In almost all cases, soil measurements are operationally defined, reflecting both the conditions of the soil *in situ*, and the consequences of the sample collection, preparation, and analysis employed. Artifacts are minimized by selection of the best methods for the goals of the program, and consistency in methodology over time. Once dried, further changes in the soil sample are minimized, and with most of the moisture removed, the sample can be sieved to break up clods and remove stone and root fragments. These steps enable the sample to be homogenized prior to subsampling for chemical analysis. Just as the consistency of sample collection and processing methods must be maintained over time, potential bias from the chemical analysis must also be controlled. Documentation of the standard operating procedure (SOP) for the chemical analysis used each time samples are collected and analyzed is essential, and ideally, the same SOP is used for all sample collections. The success of the chemical analysis needs to be verified with a quality assurance program that involves the use of internal reference samples and inter-laboratory exchange samples, as well as standard internal quality control procedures. For information on comparability of commonly used chemical analysis methods see Ross et al. [8].

When resampling is done over five to ten-year intervals, some changes are likely to occur in one or more aspect of the chemical analysis such as the SOP, laboratory instrumentation, laboratory personnel, or the laboratory doing the analysis. These factors create the possibility of analytical bias between the collections. To control for analytical bias, unused portions of samples from each collection should be archived for future use. Samples from the previous collection can be analyzed with the newly collected samples, and by comparing data, the possibility of analytical bias can be addressed. This approach is based on the assumption that chemical changes do not occur in the archived sample during the storage period. Loss-on-ignition and concentrations of exchangeable bases, exchangeable Al, total C, and total N have been shown to be stable in various studies that have extended up to 30 years [9-11]. However, storage of air-dried soils has been shown to lower soil pH [12] and manganese oxides [13]. The mass of soil collected from each horizon or depth interval should be sufficient to complete one full set of planned chemical analyses plus additional mass for at least four sets of analyses in the future. A variety of methods have been used to archive soil samples. The method described herein follows the storage procedures used by the New York State Museum.

PROTOCOL:

1. Study unit selection and description

1.1) Locate a forested area with the characteristics desired for monitoring. Establish the boundaries of the study unit within this area, ensuring that (1) the study unit is representative of the area to be monitored, and (2) that the area is large enough to accommodate the planned sampling and resamplings, but not so large that an excessive amount of replicate pits are needed to represent the variability within the unit.

1.2) Record the location of the study unit with a global positioning system (GPS) unit. Record the center and corners if the study unit is rectangular, or center and ends of perpendicular diameters if the study unit is circular. Record written site coordinates on a field form, in addition to storing them electronically in the GPS unit. If permissible, mark key locations with permanent monuments such as an iron rod.

1.3) Record the slope by hanging flagging or some other marker at eye level at the study unit center and at the lowest elevation edge of study site. Measure the slope with a clinometer from (1) the highest elevation edge of the study unit to the study unit center (slope up), and (2) from the study unit center to the lowest edge (slope down). Record the compass reading along the predominant downslope direction (slope aspect) from the highest elevation edge of the study unit.

1.4) Record the slope position as summit, shoulder, backslope, footslope or toeslope if the study area is on a hillslope, or flat plain if the study unit is in an area of low relief. See pages 1-7 and 1-10 in Shoeneberger et al. [6] to verify identification of the slope position.

1.5) Identify the dominant vegetation species by vertical strata. For example, record the dominant herb species in the understory below 1 m, the dominant sapling species taller than 1 m but not reaching the canopy, and the dominant tree species in the canopy (those that reach the top of the canopy). How to define the strata will depend on the type of forest being worked on. Take a digital photo of the understory from the lowest elevation edge of the study unit looking upslope and from the highest elevation edge looking downslope.

1.6) Select locations for pits, avoiding land surfaces that are of minor importance within the selected study unit, and therefore not representative of the study unit. Also avoid land surfaces where sampling methods are not possible because of perennial wetness, excessive rocks at or near the surface or excessive density of trees, or of a condition that is counter to the objectives of the soil monitoring project.

2. Excavation and profile description

2.1) Lay out a tarp (approximately 10 ft by 12 ft or 3.1 m by 3.7 m) adjacent to the location where a pit is to be excavated. Choose one side of the planned pit (upslope side if possible) to protect from trampling and contamination during pit digging by covering with plastic garbage bag or something similar (Figure 2). This side will then be used for the profile description and sampling.

[Place Figure 2 here]

2.2) Begin excavating the pit by removing the forest floor (O horizon) with the shovel. If possible, keep the forest floor intact and place where it will not be mixed with mineral soil being removed from the pit. Excavate the pit with the smallest footprint possible (usually about 0.5 to 1 m^2) until reaching the desired depth determined by the monitoring design.

2.3) Prepare a vertical pit face for description and sampling by lightly scraping downward with a hand trowel to remove any loose soil resulting from the excavation. Prune roots with hand snippers where necessary.

Note: If excessive rocks or roots preclude the clearing of a pit face for description and sampling, or reaching the desired depth, the pit may need to be expanded somewhat.

2.4) Record (in a field notebook or electronic recording device) any observations of water seeping into the pit from a pit face or the bottom of the pit.

2.5) Visually evaluate the pit face from top to bottom for differences in color, texture and structure. Remove small amounts of the differing soil and place side by side on a white piece of paper (such as the back side of the field form) to assist in identifying horizon boundaries, as shown in Figure 3.

[Place Figure 3, 4 and 5 here]

2.6) Record the horizon designations following pages 2-2 to 2-5 of the NRCS Field Book [6].

2.7) Mark horizon boundaries with T shaped pins or similar objects (Figures 2, 4, 5). Take a digital photo of the profile with horizon markers and a tape in place showing scale.

2.8) Measure and record the depth of the top and bottom of each horizon with a metric tape relative to the interface between the air and soil surface.

2.9) Record the distinctness class and topography code for the boundaries of each horizon following pages 2-6 to 2-7 of the NRCS Field Book [6].

2.10) Record the color of each horizon using the Munsell Soil Color Book following pages 2-8 to 2-11 of the NRCS Field Book[6].

2.11) For each horizon, record the texture class (pages 2-36 to 2-37), structure type (pages 2-52 to 2-54), and visually inspect the pit face to make a rough estimate of the quantity of rocks (as percent volume) following the instructions in the NRCS Field Book[6]. Also for each horizon, indicate whether fine roots (<2 mm diameter) are abundant, common, few or none.

3. **Sample Collection**

3.1) Select the horizons and/or depths to be sampled based on the study design and requirements.

Note: Collect by horizon if: (1) boundaries can be clearly identified in the field, and (2) horizons are sufficiently thick to remove soil without contamination from horizons above or below. Collect by depth interval if: (1) horizon boundaries are too thin to sample, or (2) horizon boundaries are irregular and or broken.

3.2) Collect the soil from the selected horizons or depth intervals, starting with the deepest sample and working upward. To remove the sample from the pit face, insert the gardening trowel near the bottom of the layer that is being sampled. Then insert a flat trowel above the gardening trowel to loosen the soil so that it can be removed with the bottom trowel (Figure 3).

Note: The mass of soil collected should equal the total mass required by the planned chemical analyses plus the mass needed for archiving (at least four additional complete analyses).

3.3) Place samples in sealable plastic bags and double-bag samples if soils are stony. For both horizon and depth sampling, collect soil across the breadth of the pit face where the horizon can be sampled (i.e. where the horizon is thick enough to sample and rocks and roots don't occur).

3.4) Label the sample bag with study unit, date, pit identification, horizon or depth interval, and sampler name.

3.5) Once sampling is completed, backfill the pit with the mineral soil and coarse fragments. Place forest floor on top of the mineral soil, keeping the organic material as intact as possible. Record the location of the pit with respect to the study unit monument (distance and aspect).

3.6) Excavate additional pits within the study unit to provide the replication called for in the sampling design. At each pit, follow steps 2.1 through 2.8, and if profile descriptions are required at all pits, also follow steps 2-9 through 2-11. Then collect the samples following steps 3.1 through 3.5.

4. Sample processing

4.1) Within 24 h of collection, pour samples out of the plastic bags into pans that will facilitate air drying of the samples. Air-dry at approximately room temperature in a secure location that is protected from air-borne contaminants such as dust. Mix the samples in the pans every few days, depending on wetness. Inspect each sample for visual and tactile evidence of dryness to determine if air-drying is near completion.

4.2) Verify the completion of air-drying, by weighing subsamples (approximately 5 grams) from several samples (a minimum of 3). Then oven-dry these subsamples for 24 h (organic soil at 60 °C; mineral soil at 105 °C), and reweigh. Calculate the mass of moisture lost through drying as a percent of the total mass (soil plus moisture) before drying.

4.3) After 2 days, repeat step 4.2 and compare the moisture lost from the first oven drying, to that lost in the second oven drying. If the moisture lost in each oven drying is within 2 percent, the soil can be considered air-dried. Once air drying is complete, place samples in plastic bags that can be sealed after expelling as much air as possible.

4.4) To remove coarse fragments and roots, sieve all collected soil. Pass the organic samples through a sieve with an opening of approximately 4-6 mm; pass mineral soil samples through a sieve with an opening of 2 mm. Additional sieving through smaller openings may be required for specific chemical analyses. For resampling, make sure that the sieving procedure matches that of the previous sampling.

Caution: People doing the sieving should be protected from inhaling dust either by sieving in an exhaust hood or wearing a National Institute for Occupational Safety and Health (NIOSH) approved N95 Particulate Filtering Facepiece Respirator.

5. Chemical analyses

5.1) Choose chemical analysis methods that are consistent with those being used in similar forest soils, such as those in Ross et al.[8]. The U.S. Environmental Protection Agency Soil Methods Manual [14] also provides a compendium of methods that continue to be commonly used for analysis of forest soils. If deviations are necessary, the data comparability must be verified. Ensure that the SOP is fully documented for each analysis.

5.2) Include reference soil samples with similar properties to soil samples collected in the monitoring program in all analysis batches to maintain quality control. Also include samples from inter laboratory exchanges [8] to determine data comparability with other laboratories.

6. Archiving soil samples

6.1) Archive the soil that remains after chemical analyses for future use. Select the mass of soil to be saved on the basis of (1) how much soil was used for the full suite of measurements, (2) the anticipated number of times samples will be reanalyzed in the future, and (3) available long-term storage space.

6.2) With a permanent marker, write the following information on an appropriately-sized tin tie (twistable wire attached to the bag for sealing) poly lined paper bag: (1) sample identification information including horizon or depth increment, (2) sieve size, (3) date collected, and (4) any necessary laboratory information such as sample serial number.

6.3) Weigh and record the mass of the soil that is being archived for each sample, and place in the tin tie bag. Place the tin tie bag in an appropriately-sized plastic bag (Figure 6).

[Place Figure 6 here]

6.4) Store the bags in cardboard storage containers configured to the available shelving (such as the method shown in Figure 7). Label the box with information on the samples contained within to enable samples to be located efficiently. Keep the archive room at a stable temperature.

[Place 7 here]

6.5) Store information on each archived sample in a digital database that is routinely backed up. Include (1) sample identification, (2) each date that the sample was analyzed, (3) the laboratory in which the sample was analyzed, (4) the analyses done on each date, (5) the mass of sample remaining (update this each time that a portion of the sample is removed for analysis), and (6) the name of the institution with custodial responsibility for the archived samples.

7. Verifying consistency of chemical analyses over time

7.1) Reanalyze a minimum of twelve archived samples from each horizon or depth increment along with the analyses of the newly collected samples.

7.2) Run a two tailed t-test (or Mann-Whitney rank sum test if data normality is disproven) to determine if chemical analysis results differed significantly ($P < 0.10$) between the previous analysis and the current reanalysis.

Note: If a significant difference (or a clear bias that is not statistically significant) is observed, then the relationship between the original data and the reanalysis data should be evaluated. If most of the variability ($R^2 > 0.9$) can be explained by this relationship, then it can be used to adjust the data to remove bias. However, if the $R^2 < 0.9$, the remainder of the archived samples should be rerun to ensure that there is no analytical bias when comparing data from the previous sampling results and results obtained from the newly collected samples.

REPRESENTATIVE RESULTS:

Data collected in the study of Lawrence et al. [9] can be used to demonstrate the effect of sampling replication on statistical power for detecting change in Oa horizon samples from 12 pits in a red spruce (*Picea rubens*) forest in eastern ME. More information on this study site (referred to as Kossuth) is available in Lawrence et al. [9]. The soil (classified as a Spodosol) had a relatively thin Oa horizon (average thickness equaled 2.5 cm and 3.7 cm in 1992-93 and 2004, respectively) that overlaid an E horizon with an abrupt boundary. With a sample size of 12, significant changes ($P < 0.05$) between samples collected in 1992-93 and 2004 were detected in measurements of pH, organic C, and exchangeable calcium (Ca), sodium (Na) and aluminum (Al), whereas no change was observed for exchangeable magnesium (Mg) (Table 1). When 8 of the 12 samples were randomly selected for statistical analysis, significant differences ($P < 0.001$) were observed for exchangeable Na and Al, and at the $P < 0.10$ level for organic C. With 4 of 12 samples randomly selected, significant differences were only observed for exchangeable Al and Na at the $P \leq 0.05$ level.

[Place Table 1 here]

Data from Oa horizons and the upper 10 cm of B horizons collected in the North and South Tributary watersheds of Buck Creek (western Adirondack region of New York) provide examples of the value of archived soil in reducing uncertainly when comparing data from different time periods. Of the 55 samples collected, analyzed, and archived in 1997-2000, 15 were randomly selected for reanalysis in 2013-14. Analyses in both time periods were done in the laboratory of the U.S. Geological Survey New York Water Science Center, Troy, NY, following the same SOP. Values for exchangeable Ca in the original and reanalysis of 15 Oa horizon samples showed no difference ($P > 0.10$) for exchangeable Ca concentrations (Figure 8a). Plotting against the 1:1 line also showed little or no bias and the R^2 value indicated little unexplained variation. The lack of a difference between original data and data from reanalysis after storage indicates that neither analytical bias nor storage effects during 14-16 years caused erroneous differences in the Ca data. On this basis, reanalysis of the additional 40 Oa samples collected in 1997-98 for exchangeable Ca concentrations was determined to be unnecessary.

[Place Figure 8 here]

A different result was obtained when archived soils were reanalyzed for exchangeable Ca in B horizons (Figure 8b), also using the same SOP. A significant difference ($P < 0.10$) was obtained between the original analysis (mean = 0.40 $cmol_c/kg$) and reanalysis (mean = 0.33 $cmol_c/kg$), although linear regression showed a highly significant linear relationship between the two data sets ($P < 0.001$; R^2=0.99). With this strong relationship, the regression model was used to adjust the original values of the 40 samples not reanalyzed to remove bias with respect to the newly collected and analyzed samples.

A change in the SOP for determining exchangeable Al concentrations resulted in differing results between the original analysis in which Al was measured by titration [15] and the reanalysis in which Al was measured by inductively coupled plasma (ICP) following Blume et al. [14]. Comparison of exchangeable Al measurements of 15 Oa horizon samples (Figure 9a) between original values (mean = 11.5 $cmol_c/kg$) and reanalysis (mean = 7.8 $cmol_c/kg$) revealed a strong linear relationship ($P < 0.001$; R^2=0.96) and significant bias ($P < 0.05$). As was done for B horizon Ca concentrations, the regression model was used to adjust the original values of the 40 samples not reanalyzed to remove analytical bias.

[Place Figure 9 here]

Original and reanalyzed data for exchangeable Al in the B horizon were also significantly different ($P < 0.001$) and linear regression indicated a significant relationship between the two data sets ($P < 0.10$). In contrast to the Oa horizon Al data, the relationship was weak (Figure 9b) and the regression model could only account for a small fraction of the variability (R^2=0.23). Because the model could not be used to remove the bias, all of the samples collected and analyzed in 1997-2000 needed to be reanalyzed with the recently collected samples.

A change in analysis method may result in a bias in the data so testing must be done to verify that the data is unbiased. For example, results of archived mineral soils collected at the Turkey Lake Watershed, Ontario, Canada, in 1986 and reanalyzed in 2005 [10] are presented in Figure 10. The analysis showed that the two methods produced unbiased data with little unexplained variability (Figure 10). The original analysis was done using the Walkley-Black wet digestion method and the archived samples were analyzed by combustion analyzer. In this case, the comparison between results of the original analysis and analysis of archived samples demonstrated that the data produced by the two methods were directly comparable.

The examples shown in Figures 8-10 demonstrate that the use of consistent analysis methods does not eliminate the possibility of unbiased data, but also shows that a method change does not necessary result in bias. These conclusions emphasize the importance of archived samples to reduce the uncertainty of results by controlling for analytical bias.

[Place figure 10 here]

Studies that have demonstrated the value of soil monitoring to detect soil changes at individual sites or watersheds are growing, and recently, soil monitoring has been applied to assess effects of acidic deposition decreases in a large regional study[14]. At all of these sites, acidic deposition had decreased over the past three decades, although the acidic deposition levels and rate of decrease varied among sites. A large number of changes were identified in that study, which were generally consistent across the large study region, over differing time periods, using varying resampling designs (Table 2). By linking multiple resampling studies, responses of forest soils to changes in a major environmental driver were identified over an extensive region (Figure 11). The study of Lawrence et al. [5] demonstrated that results of soil resampling studies with differing designs can be aggregated to address broad regional problems.

[Place Table 2 here]

[Place figure 11 here]

Figure 1: Example study design.
A generalized resampling study design. Note that the study unit is located to avoid the riparian areas of two stream channels.

Figure 2: Completed pit excavation.
Soil pit excavation showing the removed mineral soil and intact forest floor on a tarp to minimize site disturbance, along with pins marking horizons on the pit face.

Figure 3: Sample removal technique.
Technique used to remove soil from the pit face. Also shown are samples of differing colors removed from the pit face, aligned in order, to help identify horizon boundaries.

Figure 4: Example of horizon expression.
A soil profile with horizon boundaries that have distinctness classes of abrupt or clear and topography that is smooth or wavy.

Figure 5: Example of horizon expression.
A soil profile with horizon boundaries that have distinctness classes of clear or gradual and topography that is wavy or irregular.

Figure 6: Soil samples packaged for archiving.
Internal packaging of archived soil samples.

Figure 7: Exampling or archived shelving.
Space-efficient shelving of archived soil samples.

Figure 8: Reanalysis results for Ca.
The relationship between exchangeable Ca measurements in the Oa horizon (a), and the upper 10 cm of the B horizon (b), made in 1997-2000 (original analysis) and measurements of the archived samples reanalyzed in 2013-2014 (reanalysis). The 1:1 line is shown on the plot. The equation represents the best-fit line determined by linear regression.

Figure 9: Reanalysis results for Al.
The relationship between exchangeable Al measurements in the Oa horizon (a), and the upper 10 cm of the B horizon (b), made in 1997-2000 (original analysis) and measurements of the archived samples reanalyzed in 2013-2014 (reanalysis). The 1:1 line is shown on the plot. The equation represents the best-fit line determined by linear regression.

Figure 10: Reanalysis results for C.
The relationship between organic C measurements of mineral soils made in 1986 (original analysis) and measurements of archived samples analyzed in 2005. The dotted line is the 1:1 line; the solid line is the linear regression describing the relationship between initial and archived analysis.

Figure 11: **Map of resampling sites**.
Locations of soil resampling investigations in eastern Canada and the northeastern United States presented in Table 2.

Figure 12: Spodosol profile.
A Spodosol horizon from the Adirondack region of New York showing the distinctive E horizon that separates the forest floor (Oa and Oe horizon) from the B horizon.

Table 1: Sample size effects.
Statistical results of using sample sizes of 12, 8 and 4 to detect significant differences in chemical measurements of soil samples collected 10 to 11 years apart. P values considered statistically significant are shown in red italics.

Table 2: Examples of resampling results.
Mean values (initial – final) and results of tests (T-tests or Mann-Whitney tests) for differences between initial and final measurements for O, and upper B horizons for soil investigations in the northeastern US and Eastern Canada (locations shown in Figure 11). P values > 0.10 are indicated as ns (not significant). Analyses with P < 0.1 are shown in yellow to indicate significant differences observed in these measurements for sites located across the northeastern U.S. and eastern Canada. Boxes with dashed lines indicate no data. BB stands for Bear Brook, ME; TMT stands for BB sites that received experimental additions of $(NH_4)_2SO_4$ annually. REF refers to untreated sites at BB. Some sites had different study units based on forest type. CF stands for northern conifer stands; HW northern hardwood stands; MF stands for mixed conifer-hardwood stands.

DISCUSSION:
Selection of which horizons or depth increments to sample is guided by the objectives of the monitoring, but is ultimately dependent upon the characteristics of the soil. The decision of where and how to sample the profile is therefore a critical step in soil monitoring. For example, the Spodosol shown in Figure 12 has a forest floor with a boundary between the Oe (moderately decomposed organic matter) and Oa (black humified organic matter) that is abrupt and the two horizons are sufficiently thick to enable them to be sampled separately. This profile also has a well-defined E horizon with an abrupt boundary separating the organic Oa horizon from the mineral E horizon. These colorful horizons with abrupt boundaries enable collection of the same horizon material to be consistently repeated, making these horizons excellent candidates for soil monitoring. If the boundary between the mineral and organic layers is not clearly seen or is gradual relative to the horizon thickness, repeated sampling of layers directly above and below this interface will likely include varying amounts of soil from the adjacent layers. This characteristic adds uncontrolled variation and would therefore make these horizons less desirable for repeated sampling.

In some instances, sampling by depth interval can provide a consistent sampling approach in soils where certain horizons are mixed or intermingled, if this mixing is a consistent feature of the soils being monitored. In Figure 12, the upper 10 cm of the B horizon has an abrupt boundary with the E horizon, but color variation suggests the presence of Bh and Bhs horizons that are intermixed. In this situation, sampling the upper 10 cm of the B horizon would be the most repeatable collection method. This approach has proven successful in Spodosols such as shown in Figure 12[7].

[Place Figure 12 here]

Full profile descriptions are extremely useful in reducing the chance of sampling bias and interpreting the data, but collecting this information is time consuming and could limit the time available for available sampling replication, depending upon project resources and available field time. An alternative to full profile descriptions of each pit would be to make a full description of a primary pit (with photo), then limit descriptions for replicate pits to measurements of horizon thickness along with profile photographs. This information would be sufficient to verify that resampling was done in the same soil in a manner consistent with the prior sampling. High quality images are extremely valuable for maintaining sampling consistency when resampling profiles to determine chemical changes over time.

Assessment of potential bias from sampling inconsistencies can be evaluated through comparisons of measurements among horizons. For example, lower concentrations of organic carbon were observed in the Oa horizon in a second sampling than in the initial sampling conducted 10-12 years earlier [9]. This might have resulted from a sampling bias—more of the underlying mineral E horizon may have been collected in the second sampling than in the first sampling. This would lower the organic carbon concentration, and likely lower the exchangeable Ca concentration because E horizon Ca concentrations in the soil being studied were at least an order of magnitude lower in than in the Oa horizon. The lack of a decrease in the E-horizon Ca concentrations observed in this study provides evidence to support the interpretation that lower organic C concentrations in the second sampling were not a result of sampling bias. This type of comparison among horizons provides valuable information for evaluating sampling consistency. Therefore sampling additional horizons not specifically needed for project objectives is warranted to help reduce uncertainly in results.

Reanalysis of archived soil samples is a key practice in reducing uncertainty. However, archiving of soils requires resources to manage the archive and storage space that can be difficult to acquire on a permanent basis. Therefore, the mass of archived soil must be used judiciously. Reanalyzing all archived soil samples for a particular resampling study is generally the most effective approach for reducing chemical analysis uncertainty, but selective reanalysis of archived samples, where possible, will help to conserve the irreplaceable soil for future uses. Reanalysis of all archived samples should not be done unless necessary. A variety of methods for archiving soil are currently in use and have been shown to be effective. The method and materials recommended in this article are based on the experience of curators of the New York State Museum, who have found that this highly space-efficient packaging design protects the sample in unbreakable, water resistant, easily labeled materials, which are stable for many decades.

Protecting archived soil samples is a key step in soil monitoring because it not only enables analytical consistency between samplings, it also provides the opportunity for future analysis with methods that have not yet been developed. Furthermore, the archived samples can provide information to address new questions as they will undoubtedly arise in the future. Had archived soil samples predating acid rain been available, effects of this disturbance on soils would have been identified within years rather than decades after its discovery. Instead, pre-acid rain soil chemistry remains uncertain as we now monitor the recovery of soils from declining acid rain levels.

Soil monitoring is somewhat limited by the time-frame over which change can be detected (generally 5 years or more), and with a reliance on destructive sampling, the sampling area needed for monitoring increases over time. Nevertheless, without soil monitoring, soil changes must be inferred from indirect approaches, such as chronosequences (space for time substitution), watershed mass balances, dendrochemistry, short-term manipulations and modeling. These approaches provide coarse estimates of soil change, and all require assumptions that increase uncertainty that can be best reduced through direct measurements of soil through time. The procedures of repeated soil sampling can also be applied to long-term controlled manipulation experiments, such as the watershed Ca-addition experiment at the Hubbard Brook Experimental Forest, NH, lasting more than 12 years[16] and the Calhoun, SC, long-term soil experiment lasting more than 50 years[2].

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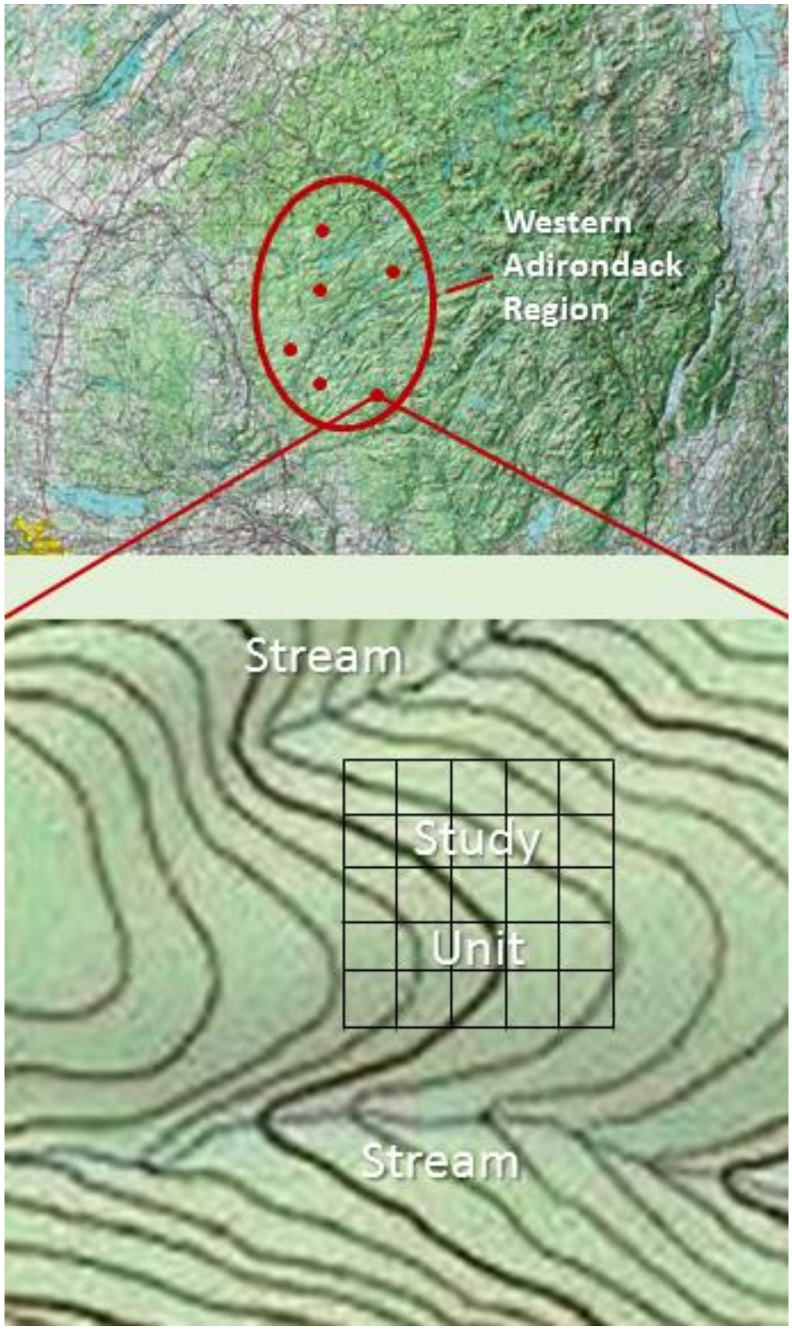
DISCLOSURES:
The authors have nothing to disclose.

REFERENCES

1 Walker, T. W. & Syers, J. K. The fate of phosphorus during pedogenesis. *Geoderma* **15**, 1-19 (1976).
2 Lawrence, G. B. *et al.* Measuring environmental change in forest ecosystems by repeated soil sampling: a North American perspective. *J. Environ. Qual.* **42**, 623-639, doi:10.2134/jeq2012.0378 (2013).
3 Lawrence, G. B. & Bailey, S. W. Workshop establishes the Northeastern Soil Monitoring Cooperative (http://www.uvm.edu/~nesmc/). *EOS* **23**, 247, doi:10.1029/2007EO230006 (2007).
4 Desaules, A. Measurement instability and temporal bias in chemical soil monitoring: sources and control measures. *Environ. Monit. Assess.* **184**, 487-502, doi:10.1007/s10661-011-1982-1 (2012).
5 Lawrence, G. B. *et al.* Declining acidic deposition begins reversal of forest-soil acidification in the Northeastern U.S. and Eastern Canada. *Environ. Sci. Technol.* **49**, 13103-13111, doi:10.1021/acs.est.5b02904 (2015).
6 Schoeneberger, P. J., Wysocki, D. A., Benham, E. C. & Staff, S. S. Field book for describing and sampling soils, Version 3.0. Natural Resources Conservation Service, National Soil

Survey Center, Lincoln, NE., (2012).
7 Soil Science Staff, Keys to Soil Taxonomy. 12th ed. USDA Natural Resources Conservation Service. Washington, D.C. (2014).
8 Ross, D. S. *et al.* Inter-laboratory variation in the chemical analysis of acidic forest soil reference samples from eastern North America. *Ecosphere* **6**, 73, doi:10.1890/ES14-00209.1 (2015).
9 Lawrence, G. B. *et al.* Early indications of soil recovery from acidic deposition in U.S. red spruce forests. *Soil Sci. Soc. Am. J.* **76**, 1407-1417 (2012).
10 Hazlett, P. W., Curry, J. M. & Weldon, T. P. Assessing decadal change in mineral soil cation chemistry at the Turkey Lakes Watershed. *Soil Sci. Soc. Am. J.* **75**, 287-305, doi:10.2136/sssaj2010.0090 (2011).
11 Bailey, S. W., Horsley, S. B. & Long, R. P. Thirty years of change in forest soils of the Allegheny Plateau, Pennsylvania. *Soil Sci. Soc. Am. J.* **69**, 681-690, doi:10.2136/sssaj2004.0057 (2005).
12 Johnson, A. H., Moyer, A. J., Bedison, J. E., Richter, S. L. & Willig, S. A. Seven decades of calcium depletion in organic horizons of Adirondack forest soils. *Soil Sci. Soc. Am. J.* **72**, 1824-1830, doi:10.2136/sssaj2006.0407 (2008).
13 Ross, D. S., Hales, H. C., Shea-McCarthy, G. C. & Lanzirotti, A. Sensitivity of manganese oxides: dryng and storage cause reduction. *Soil Sci. Soc. Am. J.* **65**, 736-743 (2001).
14 Blume, L. J. *et al. U.S. Environmental Protection Agency, EPA/600/4-90/023*, Washington, D.C., 1990).
15 Thomas, G. W. in *Agronomy No. 9* (ed A.L. Page) 159-166 (ASA, 1982).
16 Johnson, C. E., Driscoll, C. T., Blum, J. D., Fahey, T. J. & Battles, J. J. Soil chemical dynamics after calcium silicate addition to a northern hardwood forest. *Soil Sci. Soc. Am. J.* **78**, 1458-1468, doi:10.2136/sssaj2014.03.0114 (2014).


Western
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ACID BUCK
BS 2mm
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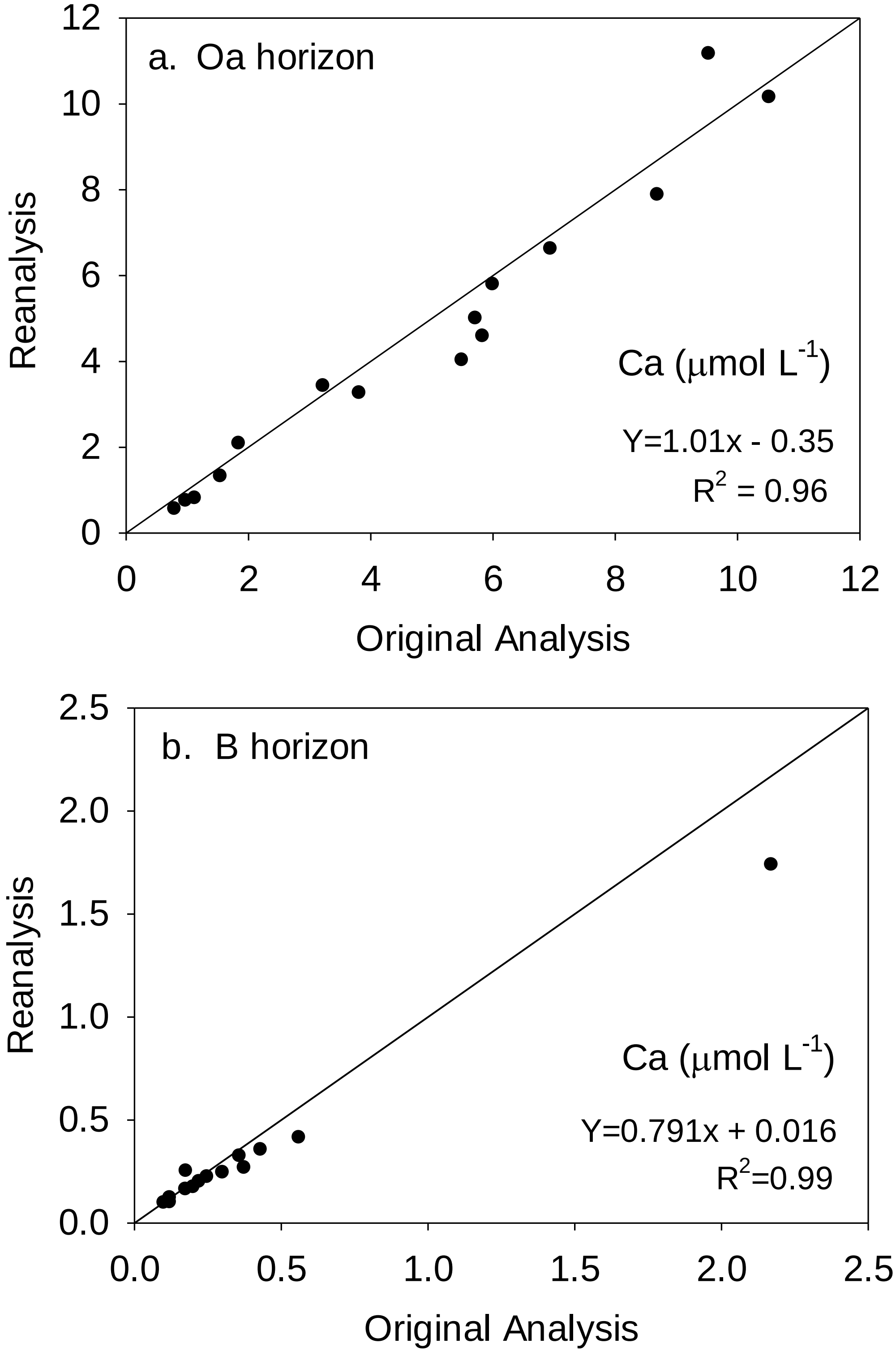
a. Oa horizon
Ca (μmol L-1)
Y=1.01x - 0.35
R2 = 0.96
Reanalysis
Original Analysis
0
2
4
6
8
10
12
b. B horizon
Ca (μmol L-1)
Y=0.791x + 0.016
R2=0.99
Reanalysis
Original Analysis
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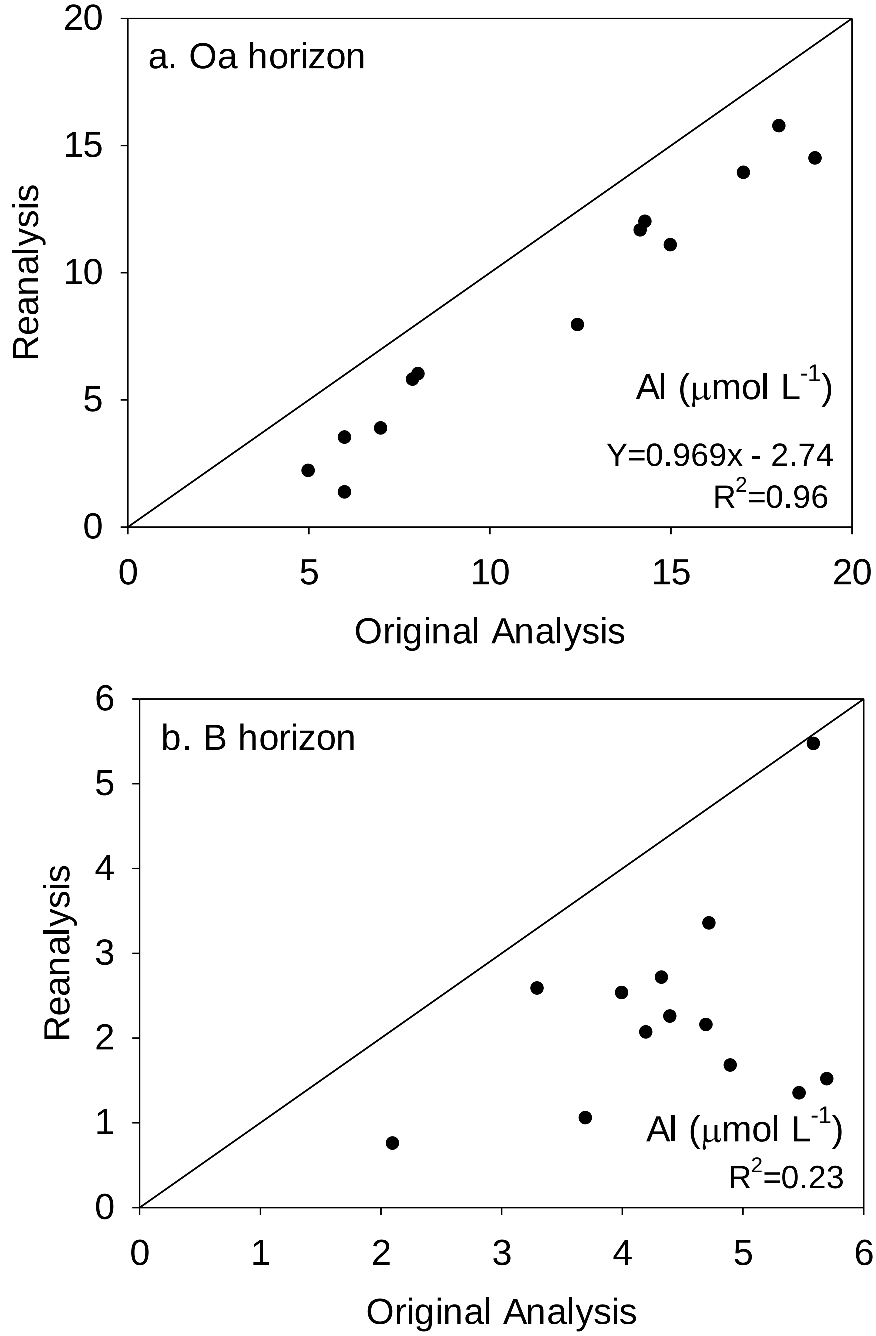
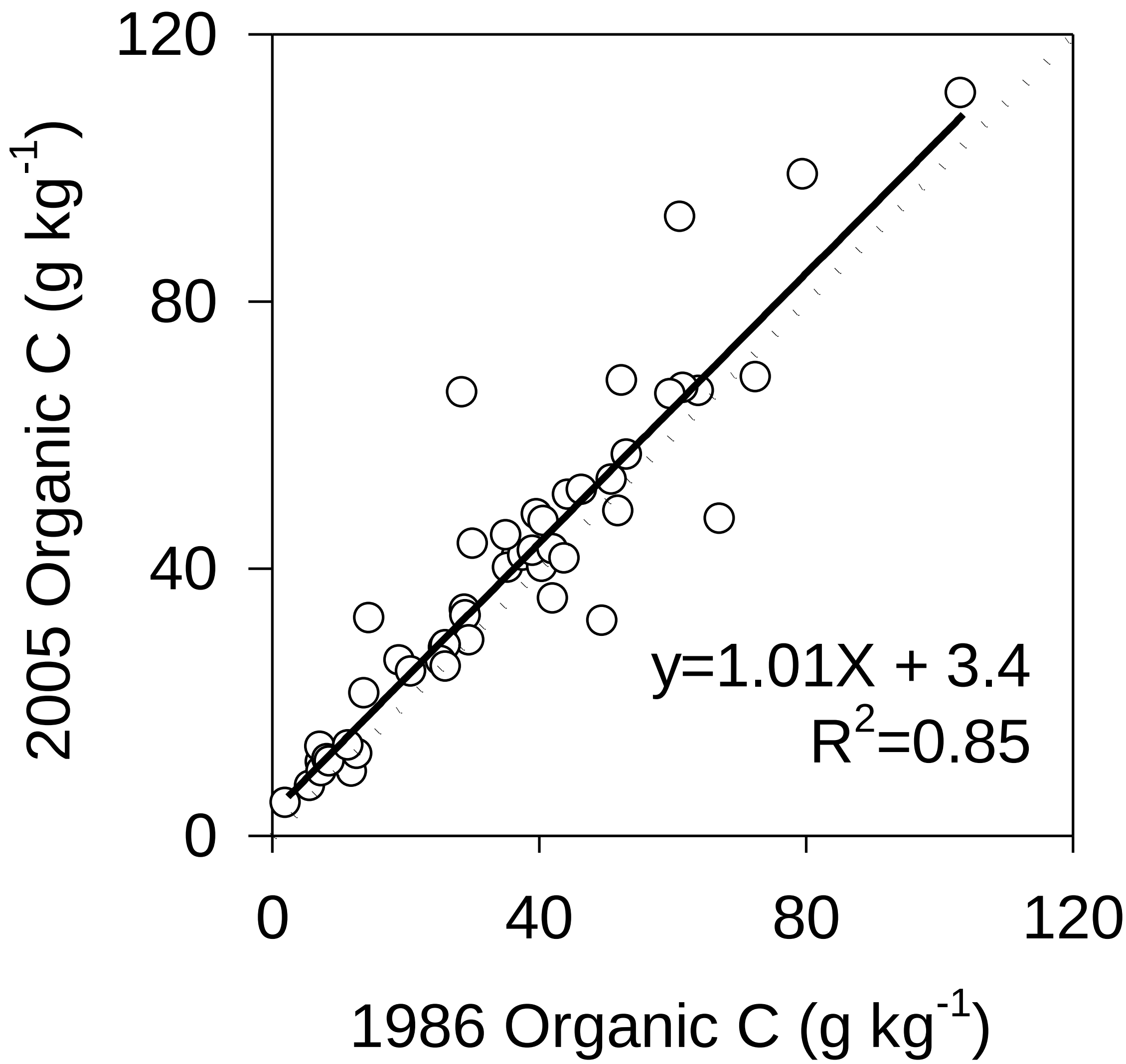
0.5
1.0
1.5
2.0
2.5


a. Oa horizon
Reanalysis
Original Analysis
Al (μmol L-1)
Y=0.969x - 2.74
R2=0.96
b. B horizon
Reanalysis
Original Analysis
Al (μmol L-1)
R2=0.23


120
80
40
0
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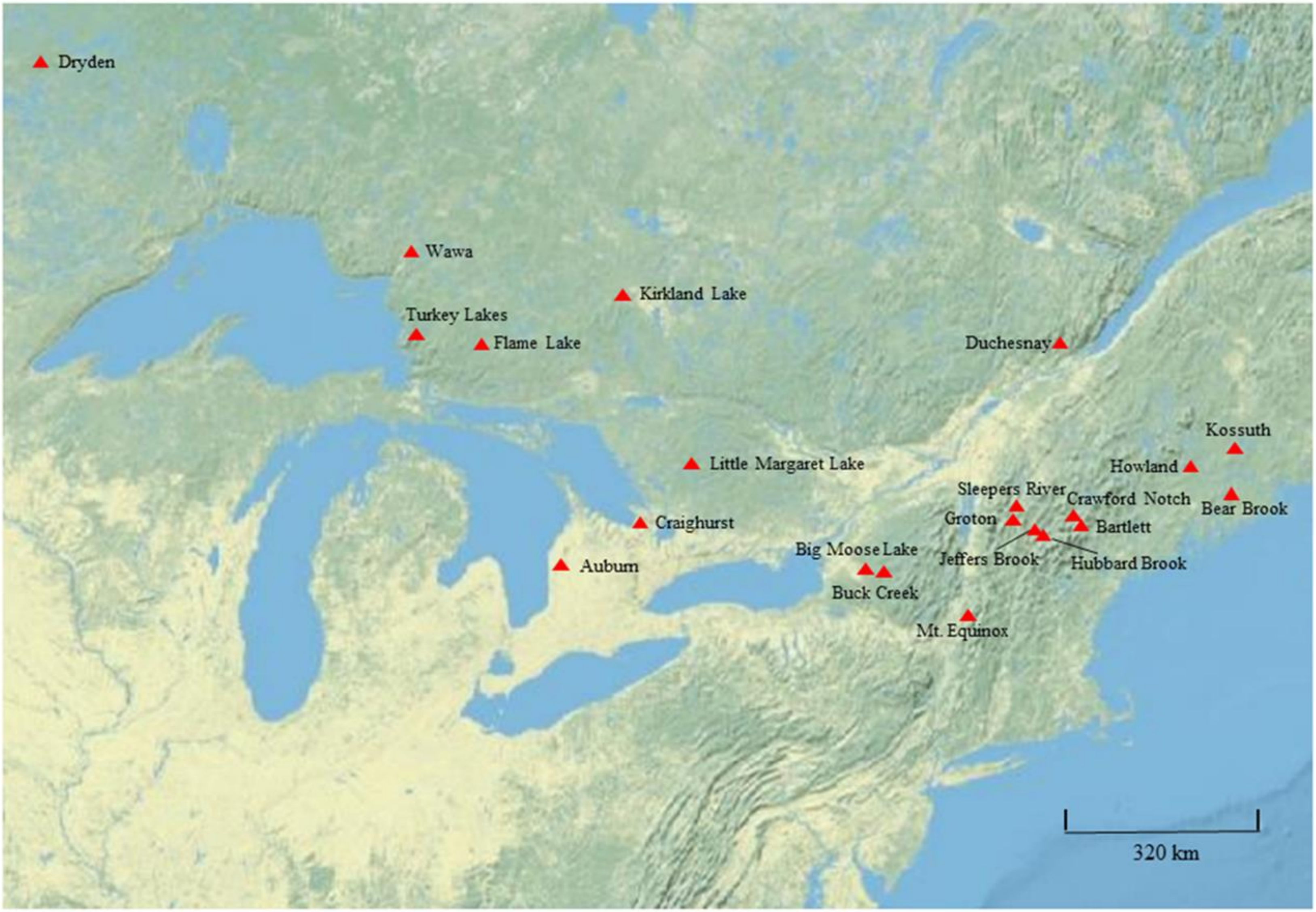
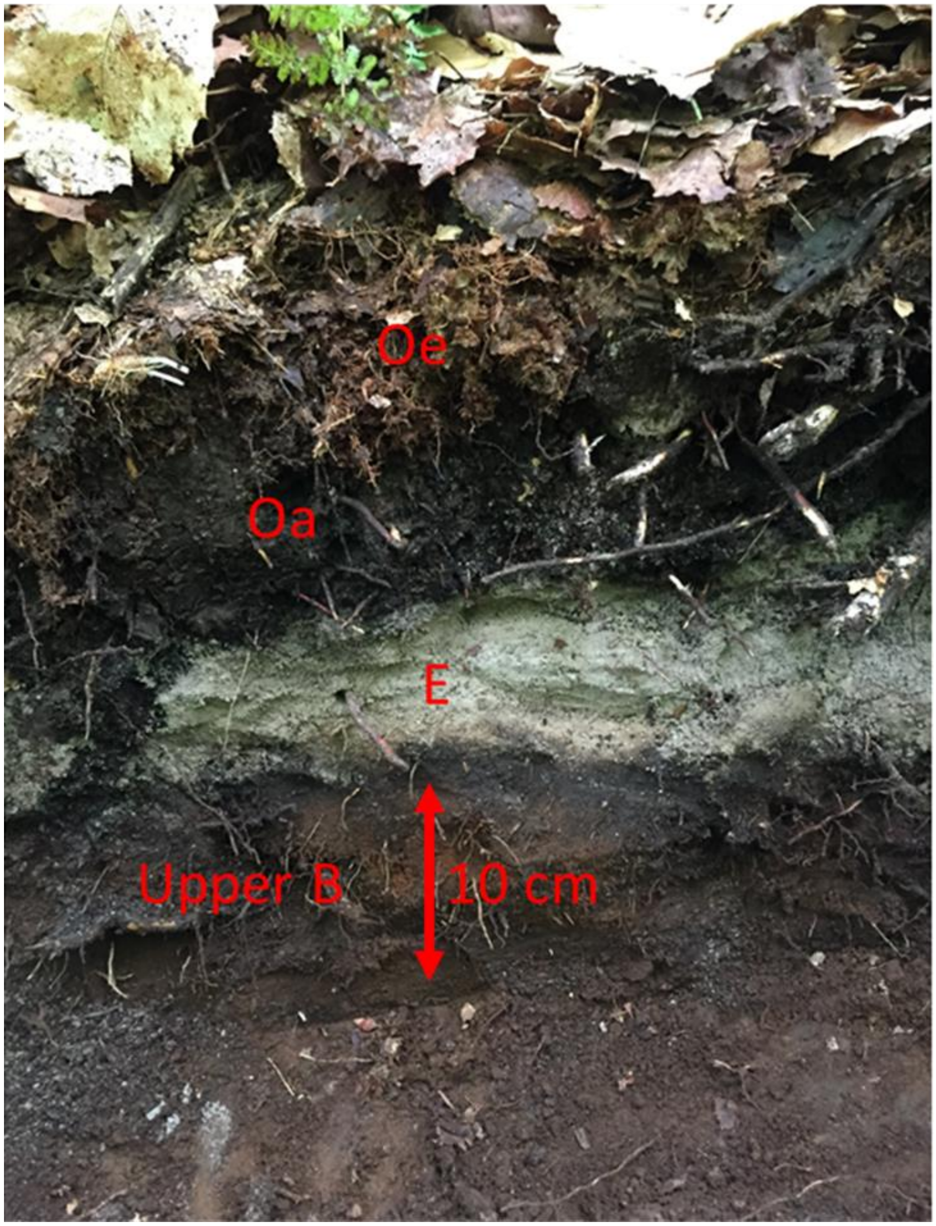
0
40
80
120
1986 Organic C (g kg-1)
y=1.01X + 3.4
R2=0.85


Dryden
Wawa
Kirkland Lake
Turkey Lakes
Flame Lake
Duchesnay
Kossuth
Howland
Little Margaret Lake
Sleepers River
Crawford Notch
Bear Brook
Groton
Bartlett
Craighurst
Big Moose Lake
Jeffers Brook
Hubbard Brook
Auburn
Buck Creek
Mt. Equinox
320 km


Oe
Oa
E
Upper B
10 cm

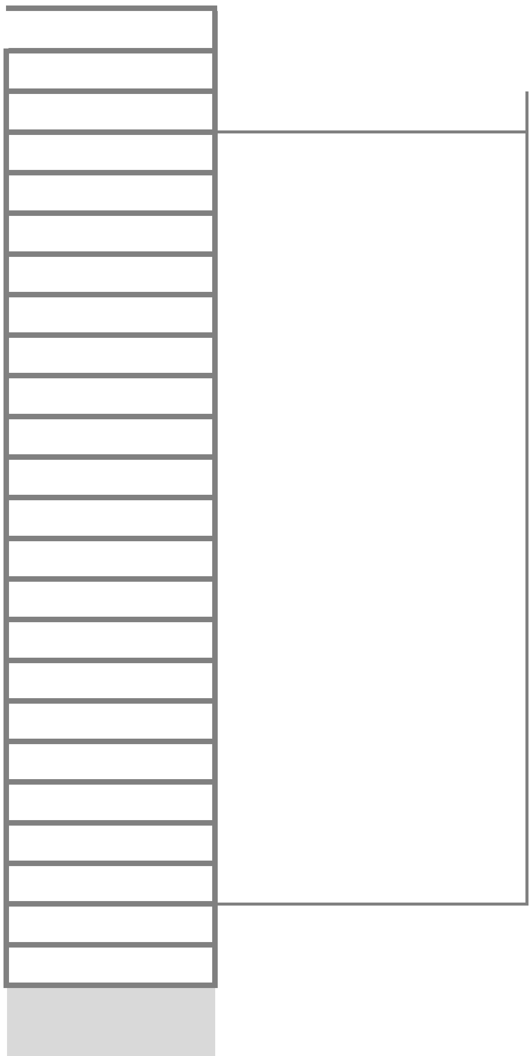
Kossuth, ME	Oa Horizon	2-tailed T test	
n	Measurement	p	power at $p < 0.05$
12	pH in $CaCl_2$	*0.004*	0.87
	C	*0.033*	0.46
	Exch. Ca	*0.008*	0.79
	Exch. Na	*< 0.001*	1.00
	Exch. Al	*< 0.001*	0.97
	Exch. Mg	0.161	0.16
8	pH in $CaCl_2$	0.111	0.36
	C	*0.063*	0.47
	Exch. Ca	0.115	0.35
	Exch. Na	*< 0.001*	1.00
	Exch. Al	*< 0.001*	0.79
	Exch. Mg	0.180	0.26
4	pH in $CaCl_2$	0.49	0.96
	C	0.271	0.18
	Exch. Ca	0.237	0.20
	Exch. Na	*0.013*	0.70
	Exch. Al	*0.050*	0.54
	Exch. Mg	0.246	0.19

Table 2 Click here to download Table Table 2.xlsx

	n	Ca (cmolc kg^{-1})		Na (cmolc kg^{-1})	
		O	B	O	B
BB-TMT, *CF* ; 1998-06	8	3.4-3.2; ns	0.19-0.24; ns	0.22-0.31; ns	0.07-0.09; ns
BB-TMT, *HW* , 1998-06	8	11-6.6; ns	0.81-0.35; ns	0.14-0.15; ns	0.06-0.06; ns
BB-REF, *CF* ; 1998-2006	8	6.1-5.8; ns	0.31-0.14; P < 0.10	0.27-0.33; ns	0.08-0.09; ns
BB-REF, *HW* ; 1998-06	8	9.7-10.4; ns	0.48-0.82; ns	0.17-0.20; ns	0.077-0.061; ns
Duchesnay, QC	4-16	9.1-9.0; ns	0.37-0.34; ns	0.04-0.06; P < 0.05	0.040-0.033; ns
Kossuth, ME	12	6.3-9.9; P < 0.01	0.15-0.10; ns	0.35-0.13; P < 0.01	0.06-0.03; P < 0.01
Howland, ME	12	9.5-9.2; ns	0.10-0.12; ns	0.37-0.28; P < 0.05	0.05-0.02; P < 0.01
Crawford Notch, NH	12	6.2-6.0; ns	0.17-0.19; ns	0.38-0.13; P < 0.01	0.08-0.02; P < 0.01
Bartlett, NH	12	9.5-8.1; ns	0.10-0.04; P < 0.05	0.27-0.11; P < 0.01	0.04-0.02; P < 0.01
Sleepers River, VT, *MF*	12	18-15; ns	0.41-0.54; ns	0.19-0.05; P < 0.05	0.073-0.015; P < 0.01
Groton, VT	12	12.7-11.6; ns	0.11-0.08; ns	0.28-0.10; P < 0.01	0.04-0.02; P < 0.01
Buck Creek, NY, *HW*	27	7.9-6.0; ns	0.34-0.26; ns	0.12-0.055; P < 0.01	0.047-0.018; P < 0.01
Buck Creek, NY, *MF*	28	7.6-8.1; ns	0.38-0.41; ns	0.14-0.042; P < 0.01	0.028-0.023; P < 0.01
Big Moose Lake, NY	12	5.8-7.1; ns	0.21-0.21; ns	0.27-0.11; P < 0.01	0.08-0.02; P < 0.01
Little Margaret Lake, ON	5	----	0.71-0.20; P < 0.05	----	0.05-0.01; P < 0.01
Craighurst	5	----	1.1-0.76; ns	----	0.02-0.01; ns
Auburn, ON	5	----	13.6-14.0; ns	----	0.03-0.02; p < 0.05
Turkey Lakes, ON	5	----	3.8-1.5; ns	----	0.054-0.027; P < 0.01
Kirkland Lake, ON	5	14.2-12.5; ns	0.28-0.21; ns	0.13-0.11; ns	0.033-0.019; P < 0.01
Flame Lake, ON	5	12.2-13.8; ns	0.15-0.09; ns	0.20-0.13; P < 0.01	0.041-0.018; P < 0.01
Wawa, ON	5	7.2-25.3; P < 0.01	0.17-0.12; ns	0.14-0.13; ns	0.019-0.016; ns
Dryden, ON	5	12.5-13.5; ns	0.15-0.21; ns	0.17-0.12; P < 0.05	0.024-0.015; P < 0.01

Two-tailed T -test, unless normality is disproven or unequal variance, then Mann Whitney rank-sum test

pH		Al (cmolc kg^{-1})			
O	B	O	B		
3.62-3.83; ns	4.16-4.44; P < 0.05	10.8-9.1; ns	7.0-7.1; ns		
4.00-3.96; ns	4.33-4.37; ns	3.4-6.0; ns	5.6-7.3: P < 0.05		
3.62-3.61; ns	4.09-4.28; ns	7.3-3.1; P < 0.05	8.1-6.7; ns		
4.00-4.15; P < 0.10	4.30-4.49; P < 0.05	4.2-1.8; p < 0.05	6.2-5.9; ns		
3.53-3.89; P < 0.01	4.28-4.71; P < 0.01	2.3-2.2; ns	6.3-7.1; ns		
2.87-3.01; P < 0.05	4.15-4.02; ns	6.4-4.6; P < 0.05	4.4-3.4; p < 0.01		
2.77-2.80; ns	4.13-4.27; ns	7.2-4.4; P < 0.01	2.9-2.2; ns		
2.62-2.98; P < 0.01	3.91-3.83; ns	8.0-6.3; P < 0.01	3.6-3.8; ns		
2.63-2.89; P < 0.01	3.93-3.94; ns	7.5-4.7; P < 0.01	2.9-2.6; ns		
2.75-3.03; P < 0.01	3.95-3.81; ns	3.6-2.9; ns	4.3-7.4; P < 0.01		
2.87-3.06; P < 0.10	4.46-4.33; ns	5.8-3.5; P < 0.01	1.3-1.4; ns		
3.17-2.98; ns	3.80-3.74; ns	6.0-3.2; P < 0.01	2.4-3.9; P < 0.01		
2.69-2.74; ns	3.35 to 3.53; P < 0.05	10.4-7.4; P < 0.05	3.3-8.6; P < 0.01		
2.56-2.38; P < 0.01	3.58-3.44; ns	7.6-8.5; ns	3.6-6.4; P < 0.01		
----	4.22-4.51; P < 0.01	----	2.0-1.1; P < 0.10		
----	4.78-5.17; P < 0.05	----	0.35-0.06; ns		
----	6.46-7.10; P < 0.05	----	Below RL		
----	4.45-4.34; ns	----	1.6-2.2; ns		
3.12-3.82; P < 0.01	4.16-4.64; P < 0.01	3.3-0.3; P < 0.01	1.7-0.45; ns		
3.48-3.23; P < 0.05	4.73-5.32; P < 0.01	6.1-1.7; P < 0.01	0.20-0.03; P < 0.01		
3.07-3.98; P < 0.01	4.37-4.78; P < 0.01	4.6-0.4; P < 0.01	0.53-0.27; ns		
3.23-3.54; P < 0.05	4.46-5.01; P < 0.05	3.5-1.6; P < 0.10	0.44-0.17; P < 0.01		



*Note, several of the authors are government scientists and are therefore not allowed to endorse the products of private companies.

Name of Reagent/ Equipment	Company	Catalog Number
Equipment Required in the Field		
global positioning system	outdoor suppliers such as Forestry Suppliers	
water-proof paper	Forestry Suppliers	49450
iron rod (approximately 3 ft length)	Available at any hardware store	
vinyl flagging	Available through any outdoor supplier	
clinometer	outdoor suppliers such as Forestry Suppliers	
plastic tarp	Available at any hardware store	
round-pointed shovel or sharpshooter shovel for digging	Available at any hardware store	
hand pruner for cutting small roots	Available at any hardware store	
Lesche digging tool	Forestry Suppliers	33488
gardening trowel		
T-pins	Forestry Suppliers	53851

a copy of "Field Book for Describing Soils"	Currently available only online at: http://www.nrcs.usda.gov/Internet/FSE_DOCUMENTS/nrcs142p2_052523.pdf; Reprinting by the National Resource Conservation Service is expected in October 2026.	
Munsell Soil Color Book	Forestry Suppliers	77321
digital camera	Widely available	
metric tape with 3 to 5 meter length	Available through any outdoor supplier such as Forestry Suppliers	
sealable plastic bags with a non-clear panel for labeling	Available at any grocery store	
Indelible felt markers for bag labeling and pencils for field recording forms	Widely available	
Materials Needed to Process and Archive Samples in the Laboratory		
testing sieves	Duel Manufacturing Co., Inc.	2 mm: 200MM-2MM 4 mm: 200MM-4MM 6 mm: 200MM-6.3MM
National Institute for Occupational Safety and Health (NIOSH) approved N95 Particulate Filtering Facepiece Respirator	MSA Safety Works, model number 10102483	available through multiple suppliers
kraft tin tie bags with poly liner	Papermart	7410100
2 ml gussetted poly bag	Associated Bag	64-4-53

200-lb kraft literature mailers	Uline	s-2517

Comments/Description
A wide variety of makes and models of GPS systems would be suitable. Available through any outdoor supplier
A wide variety of makes and models of clinometers would be suitable.
A variety of hand trowels available at hardware and gardening stores would be suitable.

With flash and minimum resolution 8 megapixels

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Author(s): LAWRENCE, G.B., Fernandez, I.J. et al.

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CORRESPONDING AUTHOR:

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Department:	NEW YORK WATER SCIENCE CENTER
Institution:	U.S. GEOLOGICAL SURVEY
Article Title:	Methods of Soil Resampling to Monitor Changes in Chemical Concentrations of Forest Soils
Signature:	[signature] Date: 3/31/16

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Done

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Done

3. Please abbreviate all journal titles.

Done

4. Please include volume, issue numbers, and DOIs for all references.

Done

5. Please define all abbreviations before use.

Done

6. Please use focused images of uniform size/resolution (at least 300 dpi).

Done

7. Please revise the table of the essential supplies, reagents, and equipment. The table should include the name, company, and catalog number of all relevant materials in separate columns in an xls/xlsx file.

Done

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

Done

9. Please remove URLs from the manuscript text and place them in the References.

Done

10. Please revise the text to avoid the use of any personal pronouns (e.g., "we", "you", "our" etc.).

Done

11. Please label the photos as Figures and number accordingly.

Done

12. 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.). The actions should be described in the imperative tense in complete sentences wherever possible. Avoid usage of phrases such as “could be,” “should be,” and “would be” throughout the Protocol. Any text that cannot be written in the imperative tense may be added as a “Note.” However, notes should be concise and used sparingly. Please include all safety procedures and use of hoods

Done

13. The Protocol should contain only action items that direct the reader to do something. Please move the discussion about the protocol to the Discussion.

Done

14. Please add more details to your protocol steps. Please ensure you answer the “how” question, i.e., how is the step performed? Alternatively, add references to published material specifying how to perform the protocol action.

Done

15. The Protocol should be made up almost entirely of discrete steps without large paragraphs of text between sections. Please simplify the Protocol so that individual steps contain only 2-3 actions per step and a maximum of 4 sentences per step.

Done

16. 2.1: What is the size of the tarp?

Done

17. Please do not use contractions in the text.

Done

18. How large are the samples?

Done

19. Please note that in order to film, we need explicit details on how to perform the action/technique written in the protocol text. This cannot be referenced out.

Done. Note the reference to the NRCS Field Book remains because the book is used in the field.

20. Please see the attached manuscript as an example.

Done

21. Please highlight 2.75 pages or less of the Protocol (including headings and spacing) that identifies the essential steps of the protocol for the video, i.e., the steps that should be visualized to tell the most cohesive story of the Protocol. The highlighted steps should form a cohesive narrative with a logical flow from one highlighted step to the next. Remember that non-highlighted Protocol steps will remain in the manuscript, and therefore will still be available to the reader.

Redone

22. Please ensure that the highlighted steps form a cohesive narrative with a logical flow from one highlighted step to the next. Please highlight complete sentences (not parts of sentences). Please ensure that the highlighted part of the step includes at least one action that is written in imperative tense.

Done

23. As we are a methods journal, please revise the Discussion to explicitly cover the following in detail in 3-6 paragraphs:
a) Critical steps within the protocol
b) Any modifications and troubleshooting of the technique
c) Any limitations of the technique

d) The significance with respect to existing methods
e) Any future applications of the technique

The Discussion Section has been rewritten to add this material.

Nam Nguyen <em@editorialmanager.com>
Jun 3 (2 days ago)
to me

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.

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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.

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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 R^2 *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