

Video Article

# Soil Lysimeter Excavation for Coupled Hydrological, Geochemical, and Microbiological Investigations

Aditi Sengupta<sup>1</sup>, Yadi Wang<sup>2</sup>, Antonio A. Meira Neto<sup>3</sup>, Katarena A. Matos<sup>3</sup>, Katerina Dontsova<sup>1,2</sup>, Rob Root<sup>2</sup>, Julie W. Neilson<sup>2</sup>, Raina M. Maier<sup>2</sup>, Jon Chorover<sup>1,2</sup>, Peter A. Troch<sup>1,3</sup>

<sup>1</sup>Biosphere 2, University of Arizona

<sup>2</sup>Department of Soil, Water and Environmental Science, University of Arizona

<sup>3</sup>Department of Hydrology and Water Resources, University of Arizona

Correspondence to: Aditi Sengupta at [asengupta@email.arizona.edu](mailto:asengupta@email.arizona.edu)

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## Abstract

Studying co-evolution of hydrological and biogeochemical processes in the subsurface of natural landscapes can enhance the understanding of coupled Earth-system processes. Such knowledge is imperative in improving predictions of hydro-biogeochemical cycles, especially under climate change scenarios. We present an experimental method, designed to capture sub-surface heterogeneity of an initially homogeneous soil system. This method is based on destructive sampling of a soil lysimeter designed to simulate a small-scale hillslope. A weighing lysimeter of one cubic meter capacity was divided into sections (voxels) and was excavated layer-by-layer, with sub samples being collected from each voxel. The excavation procedure was aimed at detecting the incipient heterogeneity of the system by focusing on the spatial assessment of hydrological, geochemical, and microbiological properties of the soil. Representative results of a few physicochemical variables tested show the development of heterogeneity. Additional work to test interactions between hydrological, geochemical, and microbiological signatures is planned to interpret the observed patterns. Our study also demonstrates the possibility of carrying out similar excavations in order to observe and quantify different aspects of soil-development under varying environmental conditions and scale.

## Video Link

The video component of this article can be found at <https://www.jove.com/video/54536/>

## Introduction

Soil and landscape dynamics are shaped by the complex interaction of physical, chemical, and biological processes<sup>1</sup>. Water flow, geochemical weathering, and biological activity shape the overall development of the landscape into a stable ecosystem<sup>2,3</sup>. While surface changes are the most conspicuous features of landscape<sup>4</sup>, understanding cumulative effects of hydrological, geochemical, and microbiological processes in the subsurface region is crucial to understanding the underlying forces that shape a landscape<sup>2</sup>. Future climate perturbation scenarios further confound the predictability and pattern of landscape evolution<sup>5</sup>. It thus becomes a challenge to link small-scale processes to their large-scale manifestation on the landscape-scale<sup>6</sup>. Traditional short-run laboratory experiments or experiments in natural landscapes with unknown initial conditions and time-variable forcing fall short in capturing the intrinsic heterogeneity of landscape evolution. Also, due to strong nonlinear coupling, it is difficult to predict biogeochemical changes from hydrological modeling in heterogeneous systems<sup>7</sup>. Here, we describe a novel experimental method to excavate a fully controlled and monitored soil hillslope with known initial conditions. Our excavation and sampling procedure is aimed at capturing the developing heterogeneity of the hillslope along its length and depth, with the goal of providing a comprehensive dataset to investigate hydro-bio-geochemical interactions and their impact on soil formation processes.

Hydrologic systems found in nature are far from being static in time, with changes in hydrological responses taking place over a wide range of spatial and temporal scales<sup>3</sup>. The spatial structure of flow pathways along landscapes determines the rate, extent and distribution of geochemical reactions and biological colonization that drive weathering, the transport and precipitation of solutes and sediments, and the further development of soil structure. Thus, incorporating knowledge from pedology, geophysics, and ecology into theories and experimental designs to assess hydrologic processes and improve hydrologic predictions has been suggested<sup>8,9</sup>. Landscape evolution is also impacted by subsurface biogeochemical processes in conjunction with water dynamics, elemental migration during soil development, and by mineralogical transformations brought about by reaction of mineral surfaces with air, water, and microorganisms<sup>10</sup>. Consequently, it is important to study development of geochemical hotspots within an evolving landscape. Additionally, it is critical to relate geochemical weathering patterns to hydrological process and microbiological signatures during incipient soil formation in order to understand the dynamics of complex landscape development. The specific processes of soil genesis are governed by the combined influence of climate, biological inputs, relief and time on a specific parent material. This experiment was designed to address heterogeneities in the weathering of parent material governed by hydrological

and geochemical variations associated with relief (including slope and depth) and the associated variability in microbial activity that is driven by environmental gradients (*i.e.*, redox potential) under conditions where parent material, climate and time are held constant. With respect to microbial activity, soil microorganisms are critical components and have a profound impact on landscape stability<sup>11</sup>. They play a crucial role in soil structure, biogeochemical cycling of nutrients, and plant growth. Therefore, it is necessary to understand the significance of these organisms as drivers of weathering, soil genesis, and landscape formation processes, while simultaneously identifying the reciprocal effects of hydrological flow-paths and geochemical weathering on microbial community structure and diversity. This can be achieved by studying spatial heterogeneity of microbial community diversity over an evolving landscape whose hydrological and geochemical characteristics are also being studied in parallel.

Here, we present an excavation procedure of a soil lysimeter, operationally named miniLEO, designed to mimic the large-scale zero-order basin models of the Landscape Evolution Observatory (LEO) housed at Biosphere 2 (University of Arizona). The miniLEO was developed to identify small-scale landscape evolution patterns arising from cumulative heterogeneous hydro-bio-geochemical processes. It is a lysimeter 2-m in length, 0.5-m in width, and 1-m in height, and slope of 10° (**Figure 1**). Additionally, the walls of the lysimeter are insulated and coated with non-biodegradable two-part epoxy primer and an aggregate filled aliphatic urethane coat to avoid potential contamination or leaching of metals from the lysimeter frame into the soil. The lysimeter was filled with crushed basalt rock that was extracted from a deposit of late Pleistocene tephra associated with Merriam Crater in northern Arizona. The loaded basalt material was identical to the material used in the much larger LEO experiments. The mineral composition, particle size distribution, and hydraulic properties are described by Pangle *et al.*<sup>12</sup>. The downslope seepage face was lined with a perforated plastic screen (0.002-m diameter pores, 14% porosity). The system is fitted with sensors such as water content and temperature sensors, two types of water potential sensors, soil-water samplers, hydraulic weight balance, electrical conductivity probes, and pressure transducers to determine water table height. The lysimeter was irrigated for 18 months prior to the excavation.

The excavation was meticulous in its approach and was aimed at answering two broad questions: (1) what hydrological, geochemical, and microbial signatures can be observed across the length and depth of the slope with respect to simulated rainfall conditions and (2) whether relationships and feedbacks between hydro-bio-geochemical processes occurring on the hillslope can be deduced from the individual signatures. Alongside the experimental setup and excavation procedure, we present representative data and suggestions on how to apply similar excavation protocols for researchers interested in studying coupled earth-system dynamics and/or soil development processes.

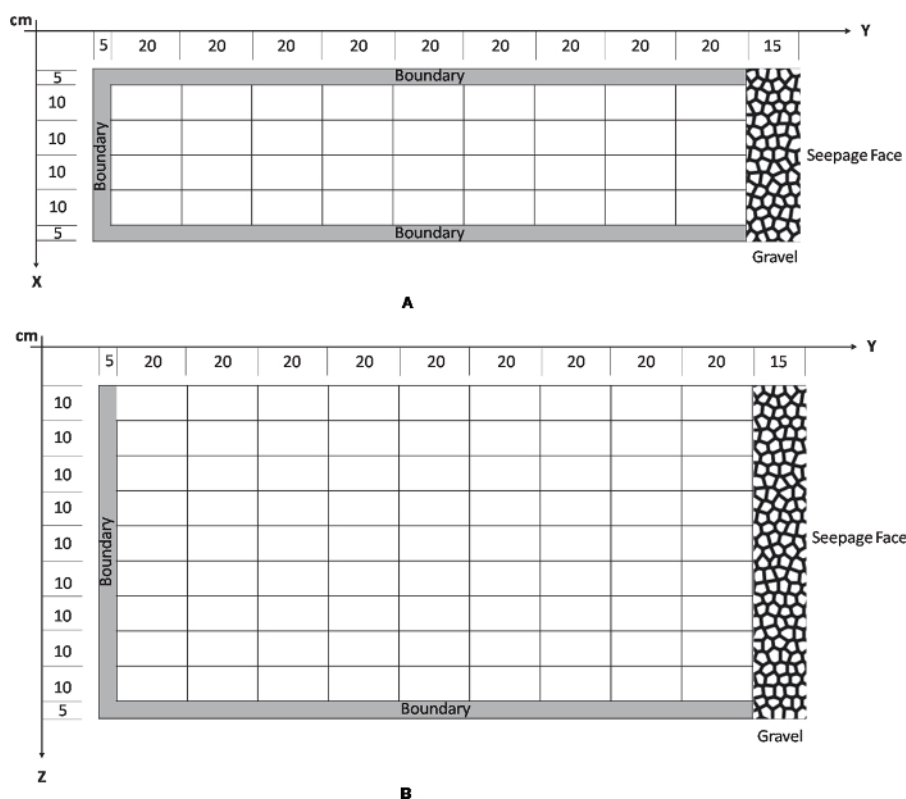
## Protocol

### 1. Devise a Sampling Matrix to Ensure Systematic and Comprehensive Sampling of Lysimeter

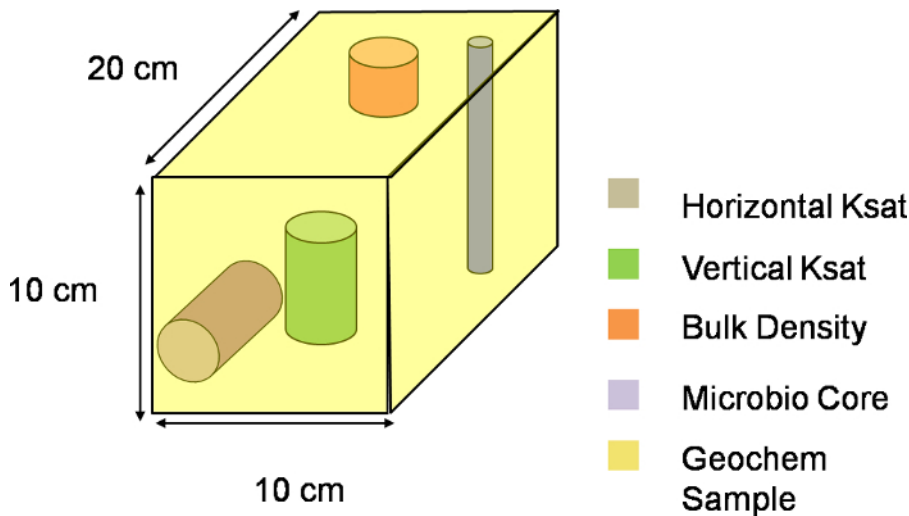
1. Divide lysimeter into voxels of fixed length, width, and depth.
  1. Use a Euclidean space coordinate system and divide the total distance along each direction (X, Y and Z) into an adequate number of equally spaced intervals. Consider discarding the soil near the walls of the lysimeter to avoid boundary effects.  
NOTE: A buffer of 5 cm along the four walls is adopted in this experiment to avoid boundary effects, while ensuring that the volume of soil collected is sufficient.
  2. Assign each sample a unique XYZ location and identify as a voxel.  
NOTE: In this excavation, X denotes the location along the width of the slope, Y denotes location along the length of the slope, while Z denotes location along the depth of the slope. The size of the intervals within each dimension determines the width, length, and depth of the voxels. **Figure 2** shows the division of the lysimeter after determining spacing intervals along with the chosen origin for the XYZ system. The division in the current excavation scheme has 9 intervals along both Y and Z directions and 4 intervals along the X direction, producing a total of 324 voxels of 10 cm x 20 cm x 10 cm dimensions (**Figure 3**).  
NOTE: The sampling strategy chosen ensures that the entire system is evenly sampled with minimal damage to the sensors. Boundaries of each voxel (1-2 cm) are discarded to limit cross contamination from neighboring voxels. Additionally, voxel dimensions ensure that enough soil material is available for microbiological, geochemical, and hydrological sample collection in each voxel.



**Figure 1. Side-view of lysimeter.** View of lysimeter from the seepage face. Also visible are three sensor regions (white PVC tubes) along the slope and sprinkler system at the four corners.



**Figure 2. Sampling Scheme.** Sampling scheme of lysimeter along XYZ. **A.** The X dimension divides the width into 4 sections each of 10 cm while Y divides the length into 20 cm. **B.** The Z dimension indicates depth and was divided into 9 layers of 10 cm depth. A boundary of 5 cm all along the edges of the lysimeter was identified to prevent collection of samples that can potentially exhibit boundary effect. [Please click here to download this file.](#)



**Figure 3. Three-dimensional representation of a voxel.** Visual schematic of one voxel along the XYZ plane of the lysimeter. The entire slope was divided into 324 such voxels, with each voxel depicting a single sampling unit. [Please click here to download this file.](#)

## 2. Add Brilliant Blue FCF Dye to Track Water Infiltration in the Slope

1. Apply brilliant blue dye at the surface of the soil, enough to cover top 105 cm of the surface along the Y direction. Cover the remaining soil with plastic sheets.
  1. Choose a concentration (here 10 g/L) to guarantee contrast against black basaltic soil. Add the dye to irrigation system tanks and dilute with water to the desired concentration.
  2. Decide the duration of the irrigation based on the desired depth of the infiltration front and the rate supplied by the irrigation system. NOTE: For this study, an irrigation rate of 30 mm/hr for 20 min (**Figure 4**) prior to excavation is considered sufficient in order to identify heterogeneous patterns of water infiltration during the first few centimeters.
  3. After dye application, give time for the infiltration to stop and the moisture states within the lysimeter to equilibrate. For this study, a period of 10 hr (overnight) between dye application and excavation was appropriate.

## 3. Demarcation of Voxels

1. Attach measuring tape along the length of the slope to provide an *in-situ* reference system for guidance during demarcation of voxels.
2. Mark the dimension of each soil voxel with the help of the measuring tape. Draw grid lines for each layer using aluminum-blade shields and plastic putty knives (**Figure 4**). Discard the boundary materials (5 cm from each wall to prevent boundary effects).



**Figure 4. Top view of lysimeter.** This view shows the dyed surface of layer 2 (10 cm deep). Grids drawn on the soil surface to aid sampling are also visible, along with core holes regions at each voxel after microbiological sample collection.

#### 4. Microbiology Sample Collection

1. Collect microbiology samples aseptically from each voxel prior to hydrological and geochemical analyses to prevent cross contamination of samples. Ensure that new gloves are worn by all members carrying out the excavation to reduce contamination from human skin.
2. Use a soil corer of 1 cm diameter and 20 cm height, and a thin spatula for microbiological sample collection. Clean the corer and the spatula with distilled water, wipe dry with clean wipes, and rinse with 75% ethanol using a spray bottle. Allow corer and spatula to air dry.
3. Note the collection time of each sample. Use the corer to core to a depth of 10 cm at each voxel location, and the spatula to empty the soil sample into pre-sterilized plastic bags (**Figure 5**). Take care to open the bag just prior to depositing the sample. Homogenize the sample pouches by hand.
4. Store the sample bag in an ice cooler during sampling, and transfer as soon as possible to the -80 °C freezer.



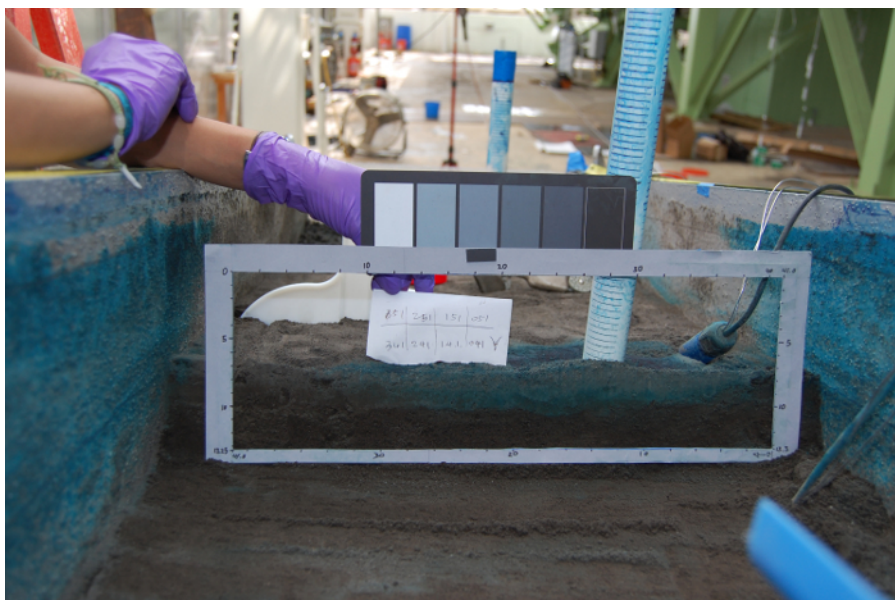
**Figure 5. Microbiology sample collection.** A small handheld corer of 20 cm x 1 cm, sterile bags, and spatula is shown here during microbiological sampling. [Please click here to download this file.](#)



## 5. Geochemistry and Hydrology Sample Collection

1. Photograph dyed regions in X and Y planes during excavation for depths where the dye is observed. Use a color card to provide reference for the color observed (**Figure 6**). Ensure proper natural lighting is present to correctly document color intensity.
2. Calibrate portable x-ray fluorescence spectrometer (pXRF) daily before starting measurements. For calibration and measurement details, see manufacturer's instructions<sup>13</sup> (**Figure 7**). Briefly, place the instrument on the holder and point the beam window directly to the factory metal bead. Select 'Cal' and wait for 30 sec to allow the calibration to be completed.
  1. Clean the beam window before taking every measurement. Measure the surface of each voxel in triplicate at three different locations. Place the pXRF instrument on the soil surface and wait for 90 sec to allow the measurement to be completed.

NOTE: X-ray can penetrate through a long distance in the direction of the beam. Therefore, ensure that only a trained personnel handles the equipment and maintains proper safety protocols.



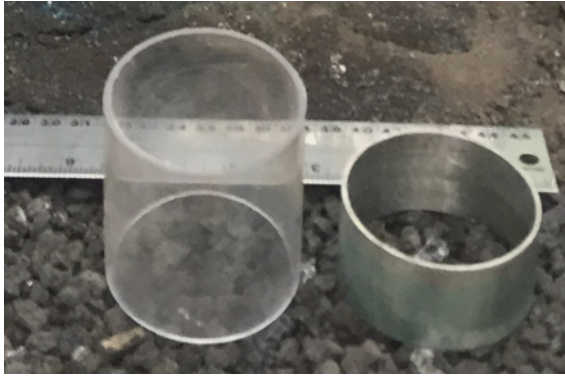
**Figure 6. Color card to follow dye infiltration.** Each location with visible dye penetration was photographed with a color card serving as reference. [Please click here to download this file.](#)



**Figure 7. Portable X-ray Fluorescence Spectrometer.** Handheld pXRF positioned on surface of a voxel. Measurements were recorded at three different locations on the surface of each voxel and then averaged.

3. Clean metallic cores (height = 3 cm, dia. = 5.7 cm) and polycarbonate cores (height = 6 cm, dia. = 5.7 cm) for bulk densities (BD) and hydraulic conductivity measurements (Ksat) of desired voxels, respectively (**Figure 8**).
4. Vertically insert metal cores and polycarbonate cores (vertical Ksat) into desired voxels taking care not to damage sensors or sensor wires. Do this by gently hammering the cores into the soil, taking care to use a flat surface like block of wood between the core and the hammer in order to minimize disturbance to the soil. Additionally, once the core is halfway into the soil, place a second core on top of the first core. Place the wooden block on top of the second core and gently hammer the block until the first core is embedded in the soil with the core rim still visible.

5. Insert cores for horizontal Ksat as the lateral face of the voxel opens up with sequential excavation. Use the wooden block and second core as mentioned in step 5.4 to minimize compaction.
6. Take care to ensure that the voxel being sampled is isolated from boundaries and neighboring voxels prior to geochemical sample collection. Use plastic putty knives for this purpose, followed by hand-held trowels to collect soil samples around metal or polypropylene cores into labeled geochemical (GC) sample bags until cores can be easily removed (e.g., **Figure 9a, b**).

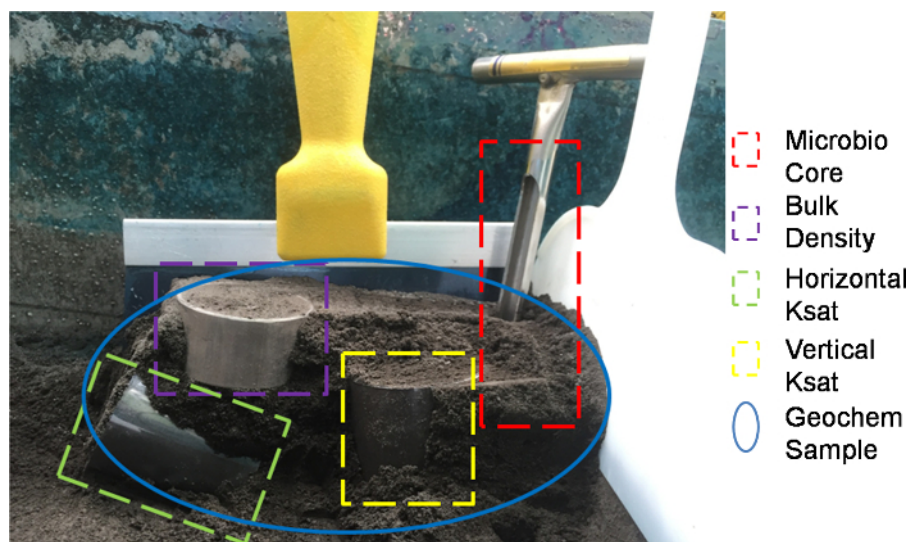


**Figure 8. Bulk density and hydraulic conductivity cores.** Polypropylene cores (left) were used for collecting vertical and horizontal hydraulic conductivity samples while metal cores (right) were used for collecting bulk density samples.



**Figure 9. Voxel demarcation.** Plastic putty knives were used to (A) isolate voxel boundaries prior to (B) geochemical, bulk density, and hydraulic conductivity core collection. [Please click here to download this file.](#)

7. Remove the metallic core, brush off excess material from both ends, and transfer sample from core to a labeled BD sample bag. Weigh each sample bag with sample and record the total weight.
8. Remove the polypropylene cores. Cover both sides with red plastic caps and label vertical polypropylene core as "V" and horizontal polypropylene core as "H" followed by the sample ID.
9. Collect remaining material from the voxel into the GC sample bag, leaving behind a couple of centimeters of soil at all four sides to prevent cross-contamination with the next voxel.
10. Repeat from steps 5.1 to 5.9 for the rest of the voxels in one layer.
11. Once all voxels from one layer have been completed, repeat steps from 3.2 to 5.10 for the subsequent layer.  
NOTE: Step 5.1 needs to be performed only for the voxels that have visible dye. Refer to **Figure 10** to visualize representative diagram of a voxel highlighting all samples collected from each voxel.
- 12.



**Figure 10. Representative voxel.** The red dashed line indicates core collected for microbiology sample, the green dashed line indicates horizontal hydraulic conductivity core, the yellow dash line indicates vertical hydraulic conductivity core, the purple dashed indicates bulk density core, and the blue oval boundary indicates remaining sample from the voxel being used for geochemical analysis. [Please click here to download this file.](#)

## 6. Sample Analysis

1. Use samples collected for microbiological analyses for molecular (soil microbial DNA extraction)<sup>14</sup> and cultured (heterotrophic plate counts)<sup>15</sup> analyses. Use extracted DNA for quantitative polymerase chain reactions (qPCR)<sup>16</sup>, and high-throughput gene sequencing experiments<sup>17,18</sup>.
2. Use samples collected for geochemical analyses to measure a multitude of geochemical properties including pH (US EPA method 150.2), electrical conductivity (EC) (US EPA method 120.1), carbon and nitrogen content (US EPA method 415.3, sequential extraction of elements)<sup>19</sup>, and X-ray diffraction (XRD) and extended X-ray absorption fine structure (EXAFS) spectroscopy as per specifications of Stanford Synchrotron Radiation Laboratory, to investigate mineral transformations.
3. Use core samples collected for hydrological analyses for laboratory experiments such as bulk density<sup>20</sup> and hydraulic conductivity<sup>21</sup>.

## Representative Results

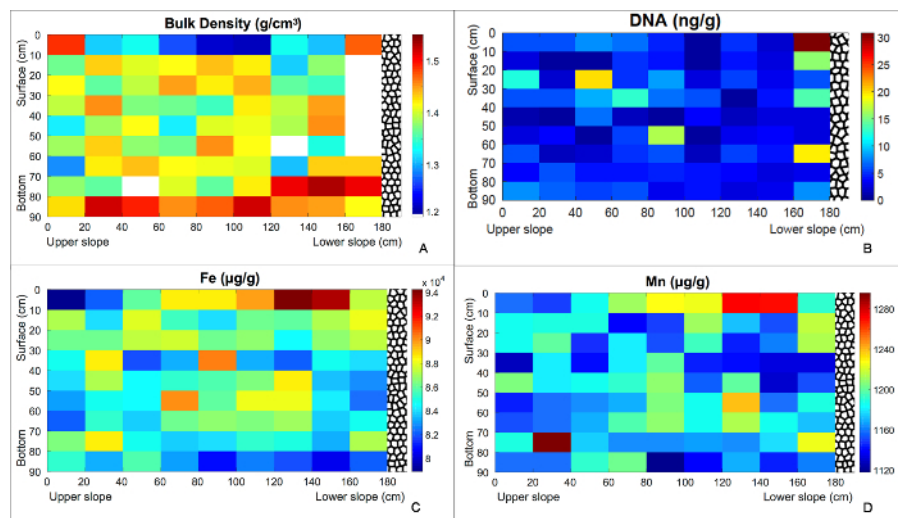
The dimensions of voxels ensured collection of samples for hydrological, geochemical, and microbiological measurements. The excavation procedure yielded 324 cores for microbiological analysis, 972 pXRF data points, 324 geochemical sample bags, 180 Ksat samples (128 vertical and 52 horizontal), and 311 bulk density samples. Preferential flow of Brilliant Blue dye was also observed to a depth of 30 cm below the surface. A representative set of 81 samples from a single vertical slice of the lysimeter were chosen for preliminary analysis. The samples chosen were from X=2 position on the slope while Y and Z voxels ranged from 0-8. Preliminary results from DNA concentration, bulk density, and pXRF Fe (Iron) and Mn (Manganese) measurements are presented here as isopleth heatmaps on a 2-D plot (**Figure 11**).

Preliminary analysis of bulk density measurements (**Figure 11a**) showed that depth 0-10 cm had the lowest value of about  $1.2 \text{ g cm}^{-3}$  while the deepest three layers (70-100 cm) had considerably higher values of 1.4 to  $1.5 \text{ g cm}^{-3}$ . Bulk density also increased from the upper slope to the seepage face. Compaction of the system as well as accumulation of particles carried by converging flow can result in greater quantity of soil particles per unit volume of soil, which in turn can explain the higher bulk density values observed at the deeper layers and at the seepage face. The likelihood of movement of finer particles down the slope with water flow can potentially alter the local environment, and explain the patterns observed.

Microbial DNA was extracted from the representative cores. The concentrations of the recovered DNA were heterogeneous and ranged from those below detection limit to a high of 30 ng/g of dry soil. Highest average concentrations were localized in layer Z=3 (20-30 cm) with a one-way ANOVA showing significantly higher concentration in this layer ( $p=0.013$ ,  $\alpha=0.05$ ). Average concentrations along the Y scale Y=8 (seepage face region representing 160-180 cm along the length of the lysimeter) recorded the highest value. However, one-way ANOVA was not significant ( $\alpha=0.05$ ) along the length. A single voxel in layer Z= 6 (50-60 cm) recorded a high concentration even though layer Z=6 on average had low DNA concentrations. Most of the other regions recorded concentrations in the range of 2-10 ng/g of soil (**Figure 11b**). It thus appears that microbial presence is more heterogeneous across depth of the lysimeter than along the length of the slope. From preliminary analysis, layer Z=3 was indicative of higher microbial presence. It is likely that a potential redox boundary zone with intermittent aerobic-anaerobic pockets exists in this layer, yielding environmental conditions conducive to the presence of both facultative aerobic and anaerobic microorganisms. The DNA recovery patterns also showed patches of high and low concentration in the deeper layers. Comparatively, higher concentrations were intermittently observed at the toe slope, possibly due to deposition of particles in this region. The regions with DNA concentrations below the detection limit reveal low biomass pockets that can be attributed to the fact that the system under study is highly oligotrophic. A clear understanding of the total microbial community will be achieved with further experiments including qPCR quantification of bacterial, archaeal, and fungal populations and high-throughput gene sequencing analysis.



Qualitative total elemental Fe and Mn concentrations showed similar patterns (**Figures 11 c and d** respectively). For both elements, higher concentrations were observed on the surface of mid-slope, and toe-slope. This likely implies that dissolution of elements occurs at the upper slope. Dissolved ions and fine particles can then potentially flow down the slope and precipitate or deposit at the lower-slope. However, Fe concentrations showed greater variability than Mn concentrations. Fe ranged from 80-94 mg kg<sup>-1</sup>, while Mn ranged from 1.12 to 1.28 mg kg<sup>-1</sup>. Since the parent material was generally homogeneous, the larger variation in per voxel Fe concentration is attributed to mobilization and precipitation of secondary phases from weathering reactions of Fe with air and water. The lower DNA concentrations observed on the surface across the entire slope may indicate lower capability of chemoautotrophs to utilize primary minerals (basalt) whereas high biomass patches observed in the lower layers and seepage face may correlate with secondary mineral accumulation as suggested by the high biomass value (Z=3, Y=8) that corresponds to elevated bulk density and Mn concentration. This pattern suggests potential precipitation of secondary minerals (e.g., iron hydroxides) by autotrophic microorganisms. Future profiling of microbial diversity will further elucidate the observed relationships. Indeed, literature reports limited microbial growth on oligotrophic tephra basaltic media, with weathering-induced reduced substrates acting as metabolic and growth inputs for microbes<sup>22</sup>. High elemental responses observed in the middle layers of mid-slope region may also reflect the formation of redox boundary in this region.



**Figure 11. Two-dimensional isopleth heatmaps. (A) Bulk Density Bulk.** Density values were obtained by transferring samples to aluminum weighing dishes and oven drying them for 48 hr at 105 °C. Cells left in blank represent voxels where sample collection was not possible due to presence of sensors and lack of space to accommodate bulk density cores. **(B) DNA concentration.** For microbiological cores, 2 g of soil was subsampled to extract microbial DNA, representative of each voxel. **(C) Elemental Fe and (D) Elemental Mn.** For elemental analysis of Fe and Mn, a pXRF data for a total of 81 samples were measured in triplicates. Average of each element in each voxel was calculated and plotted. [Please click here to download this file.](#)

## Discussion

Landscape evolution is the cumulative effect of hydrological, geochemical, and biological processes<sup>12</sup>. These processes control flow and transport of water and elements, and biogeochemical reactions in evolving landscapes. However, capturing the interactions simultaneously requires precisely coordinated experimental design and sampling. Additionally, studying incipient landscape evolution is difficult in natural systems, with limited capabilities to identify "time zero" conditions. Literature reports one destructive lysimeter study which was carried out to measure plant root density<sup>23</sup> while field based approaches of irrigation and excavation are reported by Graham *et al.*<sup>24</sup> and Anderson *et al.*<sup>25</sup> However, none of the studies incorporated a method for studying hydrological-geochemical-microbiological heterogeneity of a simulated landscape. A key component of our study was to ensure that a scale was defined for experiments and sampling procedures chosen to ensure that heterogeneity of the chosen scale was captured efficiently. The question of scale is particularly important when studying Earth-system processes and have been noted by researchers in the respective fields of hydrology<sup>26</sup>, geochemistry<sup>27</sup>, and microbiology<sup>28</sup>. The methodology outlined in this study is aimed at studying a range of hydro-geochem-microbiological processes relevant to our research questions, while at the same time providing flexibility to modify the protocol according to individual research questions.

Our preliminary representative results suggest that a homogeneous starting environment will develop heterogeneous properties. Bulk density results indicate the presence of a region with higher values at the deeper layers close to the seepage face, which might represent a result of the accumulation of fine particles due to the flow processes within the lysimeter as well as a compaction caused by the overlaying weight of the wetted soil. These two hypotheses might be elucidated with the investigation of additional parameters. For example, by performing particle size analysis of voxels, it is possible to obtain the actual proportions of finer versus coarser particles. The preliminary total Fe and Mn concentrations indicate the occurrence of elemental dissolution and re-precipitation as a consequence of applying several watering cycles to the lysimeter before the excavation. Such results can be explained in two ways: (1) water translocates clay-and finer-sized particles, enriched in Fe and Mn, down slope where they can accumulate at the lower slope<sup>29</sup> (this assumes that physical movement is more important than chemical reactions); (2) water dissolves fine particles and soluble trace ions, such as Fe and Mn, precipitate at lower slope (this scenario presumes chemical reactions are the principal driving forces). In order to confirm the mechanisms of elemental lability, more evidence is needed. The DNA concentration measurements confirm a heterogeneous distribution of microbial life in the lysimeter. Despite the low nutrient condition of the basalt hillslope, the ability to detect the presence of microbial life indicates microbial colonization under oligotrophic conditions is possible. This finding is consistent with reports of basalt-hosted microbial communities and concurrent biologically mediated weathering in diverse environments like volcanic soil<sup>30</sup>, ocean floor<sup>31</sup>, and tropical watershed<sup>32</sup>. Further analysis of the microbial diversity present in each voxel is

needed to address hypotheses concerning the potential contributions of microbes to weathering processes. Upon complete analysis of our samples and results, we will be able to interpret the hydrological, geochemical, and microbiological interactions occurring during incipient landscape evolution.

The methodology presented in this article is more a suggestion of steps rather than a rigid scheme for excavating a soil lysimeter to explore hydrological and biogeochemical interactions. Some steps may be more or less relevant depending on the objectives of the study. It is also important to stress the time needed to perform such an excavation. Our excavation required a team of 3 people at all times with the eventual addition of 1 or 2 other people during some days of work. The excavation lasted for 10 days, with daily working hours ranging between 8 to 10 hours. Therefore, carefully choosing the intended steps is very important when time constraints are to be taken into consideration. Additionally, some steps outlined in the protocol are critical to the success of excavation and research questions being asked. During the dye application, special care must be taken to ensure that areas which have been marked to remain unstained are covered properly to prevent the dye from leaking into the unstained regions. A good estimation of the voxel size is also crucial to the success of this experiment. The voxel size determines the scale of sample collection: greater number of voxels imply finer sample resolution at the cost of increased time spent at carefully excavating each voxel as opposed to less number of voxels and coarser sample resolution. Preventing cross-contamination of samples using hand-held trowels and plastic putty knives is also crucial, both for microbiological and geochemical sample collection and analysis.

A number of modifications to the protocol may be carried out, based on research questions. First, referring to the question of scale, one can choose to develop a sampling strategy that is finer than the protocol described here or opt for a coarser scale; however the scale chosen for this experiment confirms that we have captured significant physical, chemical and biological heterogeneities in the hillslope. These choices have to be made based on the research questions being asked, scale of the hillslope or lysimeter that can be constructed, and the logistics to conduct analyses. Second, many such mini-lysimeters can be set up to study soil-development processes. For example, researchers may want to look at weathering of different soil materials when subjected to a varied precipitation regime, or development of soil profile on hillslopes that have the same parent material but are treated differently with respect to slope, precipitation, temperature, etc. Additionally, the duration of the study can also be modified based on research questions and researchers may want to construct identical lysimeters followed by destructive excavation of each lysimeter temporally.

Third, vegetation can be introduced to study the effect of plant growth on hydrological flow-path, geochemical weathering, and microbial community development.

Additionally, researchers who wish to study existing processes and features of a landscape, instead of focusing on the developmental stages, may apply our method to soil monoliths in a natural setting. Traditional soil mounting procedures can be followed to obtain a soil monolith, followed by partitioning of the monolith into clearly defined regions of interest. This approach can overcome the limitations associated with carrying out intensely destructive sampling of lysimeters in the field. The sections chosen can then be excavated in a similar manner to observe hydrological, geochemical, and microbiological characteristics specific to the monolith.

A limitation of this method is obtaining all sample sets from voxels that were located near sensors. Pre-embedded sensors in some areas of the hillslope prevented collection of hydrological samples. Additionally, to negate the influence of preferential flow paths due to the presence of sensors, some samples from these locations were discarded. Moreover, the excavation was carried out in two phases over a period of ten days, with a gap of three days between phases. While care was taken to cover the exposed surface between the excavation phases, the exposed layer could potentially exhibit altered microbial activity due to changing vapor pressure and oxidation conditions. An excavation of this length is thus time consuming, which in turn may introduce additional time-sensitive variation.

Capturing landscape heterogeneity as influenced by hydrological, geochemical, and microbiological processes is a challenge. The synergistic effect of these processes on each other compounds the complexity. An excavation of a simulated landscape presented at this scale and intensity is novel. The ability to coordinate collection of hydrological, geochemical, and microbiological samples without compromising the integrity of either sample presents an excellent approach for conducting multi-disciplinary studies of earth-system processes. The techniques outlined are simple, repeatable, and flexible to accommodate multiple research questions, thereby allowing implementation of alternate experimental designs. Future outcomes of this method may include potentially developing theoretical frameworks and models of landscape evolution to answer complex questions of earth-system dynamics.

## Disclosures

The authors have nothing to disclose.

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