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PNNL-39048

Enabling depth resolved temporal resolved soil microbial sampling with novel vadose zone diffusion sampler

March 2026

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U.S. DEPARTMENT of ENERGY

Prepared for the U.S. Department of Energy
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Abstract

To address the difficulty in Earth system science in making time-course measurements of molecular signatures in soil biochemistry, we developed a soil stake system to sample and replace a defined soil analog medium, connected through hydraulic connectivity via perforated casings and modular inserts. We deployed these stakes to a site in Prosser, WA and measured microbial colonization of sterile sand-clay inserts enriched with N-acetyl-glucosamine at different depths over spring and summer. DNA and RNA analyses revealed distinct microbial recruitment and activity patterns. Inserts showed lower microbial diversity but higher abundance of Proteobacteriota and Bacteriota compared to native soils, alongside seasonal shifts in taxonomic and functional profiles. The soil stake system offers a novel approach for studying microbial dynamics across temporal and spatial scales.

Summary

To address the difficulty in Earth system science in making time-course measurements of molecular signatures in soil biochemistry, we developed a soil stake system to sample and replace a defined soil analog medium, connected through hydraulic connectivity via perforated casings and modular inserts. We deployed these stakes to a site in Prosser, WA and measured microbial colonization of sterile sand-clay inserts enriched with N-acetyl-glucosamine at different depths over spring and summer. DNA and RNA analyses revealed distinct microbial recruitment and activity patterns. Inserts showed lower microbial diversity but higher abundance of Proteobacteriota and Bacteriota compared to native soils, alongside seasonal shifts in taxonomic and functional profiles. The soil stake system offers a novel approach for studying microbial dynamics across temporal and spatial scales.

Keywords: Hydraulic connectivity, repeat sampling, microbial communities, soil moisture, dispersal, diversity, meta-transcriptomics

Acknowledgments

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1.0 Report

As currently practiced, earth science research relies on a variety of emplaced sensors and measurement devices in order to capture the spatial and temporal dynamics of a field site. However, greater integration of these measures with molecular biology (genomes, transcriptomes, proteomes, metabolomes) requires new approaches for sampling – since the extraction of these molecules from the soil is necessarily destructive.

To achieve repeat time sampling of microbial communities in Earth science research, we developed the "soil micro-stake". The stake is designed to connect investigator-introduced samples to the surrounding soil biota by way of hydraulic connectivity (Tecon and Or, 2017). The soil stake is 30 cm with an outer shell perforated with micro-laser etchings as well as larger pores to creating range of openings from 60 – 300 µm in diameter. An inner removable structure allows investigators to place samples at the same depth repeatedly (Figure 1).

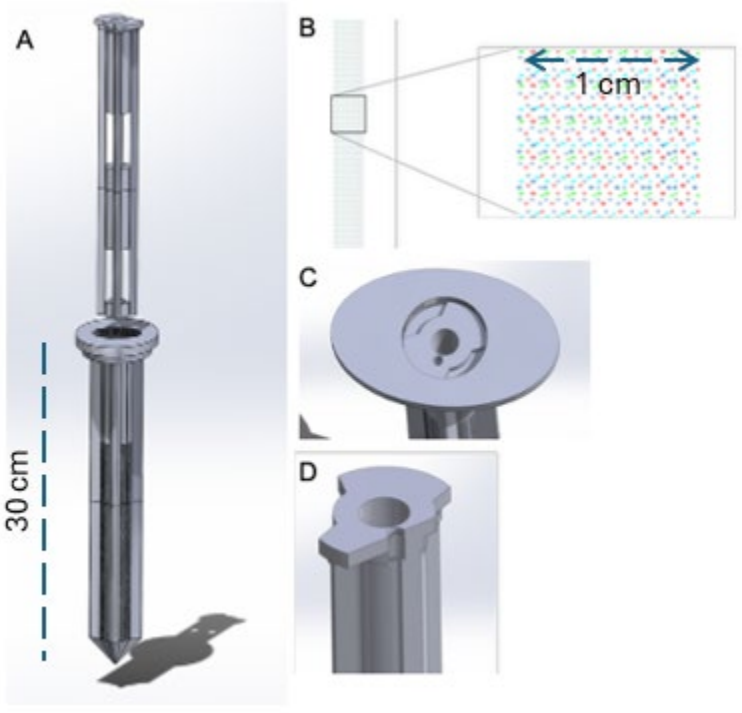

Figure 1. Visualization of soil stake design. A) Overall design. B) Micropore etchings: Red = 60 µm, Blue = 90 µm, Green = 135 µm, Cyan = 300 µm, with an overall ratio of R:B:G:C = 3:3:1:1. C) Top view of stake with insert. D) View of water addition channel for lower depth insert.

To test the capacity of soil stakes to capture members of the soil community, we measured the movement of microorganisms into sterile sand mixture samples from the native soil of Prosser, WA during the spring and summer. We tested two hypotheses. H1: community composition in the soil inserts

at both depths will be more similar to the 10-15 cm native soil than the 25-30 cm native soil, given the greater presence of active motility in surface soil layers, as well as the role of motile organisms as early colonizers (Ramoneda et al., 2024; Wang et al., 2025). H2: that soil inserts will have similar species composition but will differ in function, given the importance of soil moisture and temperature as controls on soil biotic function [ref]. Prior to field tests, we confirmed the ability of the micropore channels to conduct water from the surrounding soil into our mesh-bag-enclosed soil inserts (data not shown). In the field, stakes were placed into root free, non-planted alleyway of a tall wheatgrass field plot at the Washington State University Irrigated Agriculture Research and Extension for a Resilient Future (IAREC) (6.2571ºN, 119.7419ºW). The soils are characterized as Warden very fine sandy loam (Xeric Haplocambid).

Soil inserts were prepared using 30 g of autoclaved sterile sand with 3.3 g of kaolinite clay, enclosed in a 54 µm nylon mesh bag. To attract microorganisms, 28 mg of N-acetyl-glucosamine (NAG) was added, mixed, and the mesh bag was heat sealed. As a microbial food source, NAG is readily dissolvable, bioavailable, and contains both carbon and nitrogen. It is abundant in soils, a common food source to many soil microorganisms, and is the chief monomer of fungal chitin (Tharanathan and Kittur, 2003). Prior studies from the field site measured dissolved nutrients at 115 µg C and 25 µg N from a K_2SO_4 salt extraction (per g dry soil). As such, NAG concentration was made to be greater than the typical amount of dissolved carbon of the system, but not so great that a biased assemblage of microorganisms became established within the inserts. NAG is 221.21 g / mol, with 43.4% C by mass and 6.3% N by mass. Thus, for the 33.3 g of sand and kaolinite clay, an addition of 28 mg of NAG is equivalent to 364 µg of C and 52 µg N (per g dry soil) – roughly 3X as much carbon and 2X as much N as the native soil.

Five soil stakes were placed on May 1, 2025, arranged surrounding a central moisture soil TEROS 54 soil probe within a radius of 0.5 m (Figure 2A, 2B). A 3 cm diameter auger was used to remove a sampled soil profile from which two cores were saved at 10-15 cm depth and 25-30cm depth – (here and hereafter reported by the deepest depths) the same depth positions as eventual soil inserts as

designed for the soil stakes. Soil cores were stored in 50 mL polypropylene tubes on ice and until returned to the laboratory and stored at –20ºC. Following this initial sampling, soil was removed using an impact corer to allow for insertion of the soil stake. Sterile sand and clay inserts were added to the inner structure and placed into the soil stakes. Following the addition of insert samples area around soil stakes was irrigated daily using a total volume of 50 L. Sample inserts were removed on June 17, 2025 (47 days in soil) and placed in 50 mL polypropylene tubes on ice for transport to the laboratory before storage at –20ºC. A second round of inserts was immediately placed into position and remained in the soil before sampling in the same manner on July 30, 2025 (43 days in soil). Over this time period, soil moisture decreased in both the 15 cm and 30 cm depths concurrent with an increase in temperature (Figure 2B, 2C)

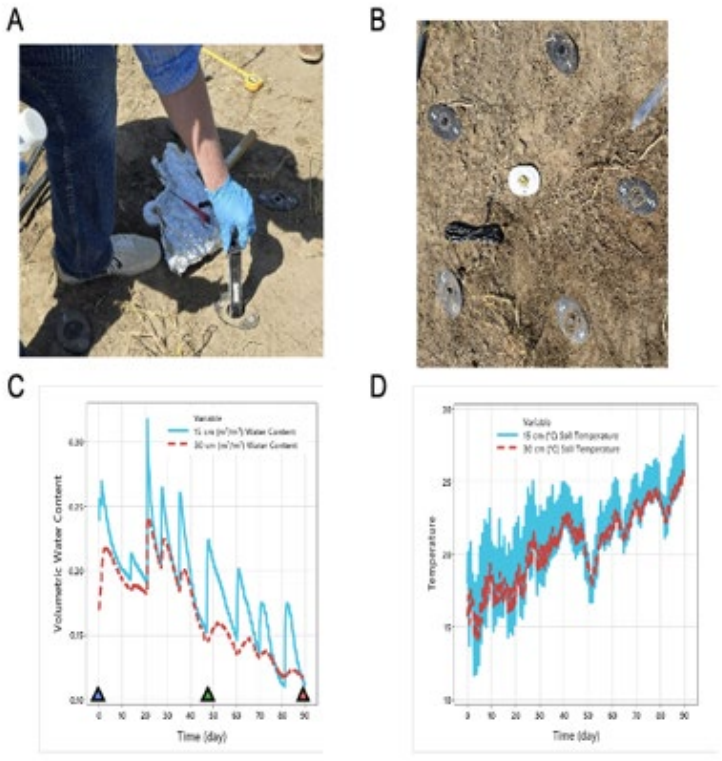

Figure 2. Field deployment of stake system. A) Image of stake interior insert being placed inside porous exterior shell. B) Image of stakes inserted into soil, positioned concentrically around a Teros soil moisture meter. C) Soil moisture trends for depths 10-15 cm and 25-30 cm. Colored triangles represent dates of sampling during the spring and summer (blue = May 1, green = June 17, red = July 30). D) Soil temperature (ºC) trends for depths 10-15 and 25-30 cm.

Soil samples and sample inserts (n = 30; five stakes by two depths by three timepoints) were subjected to DNA extraction using Zymo Quick-DNA Fecal/Soil Microbe kits following standard protocols with a final elution volume of 50 µL (starting mass: 0.25 g). Duplicate extractions were performed for each sample which were combined (total volume: 100 µL), and subject to ethanol

precipitation for cleaning and concentrating to a final volume of 50 µL. RNA was extracted using Qiagen RNeasy PowerMax Soil Pro kits (starting mass: 12-15 g) following standard protocols except that DNA degradation was performed at the end of the extraction using the TURBO DNA-free kit (FisherSci).

To understand the abundance, identity, and activities of the microbial populations in soils and soil inserts, DNA and RNA material was analyzed using several methods. DNA samples were used to generate 16S V4 rRNA amplicon libraries by Azenta generally following the Earth Microbiome Project protocols (Gilbert et al., 2014). RNA samples were analyzed for the total transcription profile by Azenta. Prior to amplicon library generation, DNA was used to quantify bacterial 16S rRNA gene abundances using qPCR on a BioRad CFX384 Real-Time PCR system. Two separate reactions were performed as DNA from soil inserts was of noticeably lower concentration than DNA from native soils. Both reactions used 5 µL of Applied Biosystems SYBER Green Master Mix, and 0.25 µL of the Eubacterial 338F and 518R primer set. The first reaction used 4.5 µL of DNA template on soil insert samples, prepared in duplicate. The second reaction used 1 µL of DNA template on native soils, prepared in triplicate. Reactions were amplified using the following conditions: 95ºC for 2 minutes, 40 cycles of [95ºC for 10 s, 60ºC for 10 s, 72ºC for 20 s].

Composition of the marker gene amplicons and transcripts from soil stakes and adjacent soil demonstrated that a subset of the community was recruited as colonizers and that these organisms were seasonally distinct. Bacterial abundances (per g dry soil) were significantly greatest in the native soils ($F = 137.5$, $df = [1, 24]$, $p < 0.001$) (Figure 3). In native soils, bacterial abundance was greater at 10 cm depth (285,302 ± 73,292, mean ± 1 SE) than at 25 cm (4,489 ± 2,549), a very common pattern of soil microbiology (Sokol et al., 2022). In soil inserts, abundances did not differ between depths but abundances were significantly lower in the second set of inserts where soil moisture was also lowest (insert 1 = 1,519 ± 284; insert 2 = 408 ± 192) ($F = 18.6$, $df = [1, 24]$, $p < 0.001$). Correspondingly, alpha diversity was lower in the inserts than in the native soil. The composition of 16S rRNA gene amplicons differed significantly between native stakes and soils and was less consistent across stakes in the newly colonized medium (Figure 3A, 3B). Broadly, the Proteobacteriota and Bacteriota were more abundant in

the soil stakes than in the native soil with specific lineages especially noticeable: the Oxalobacteraceae, Burkholderiales, and Betaproteobacteriota. The composition of expressed genes also differed significantly between soil stakes and native soil but were also more functionally constrained – as observed from the lower statistical dispersion in soil stake transcription profiles (Figure 3C, 3D).

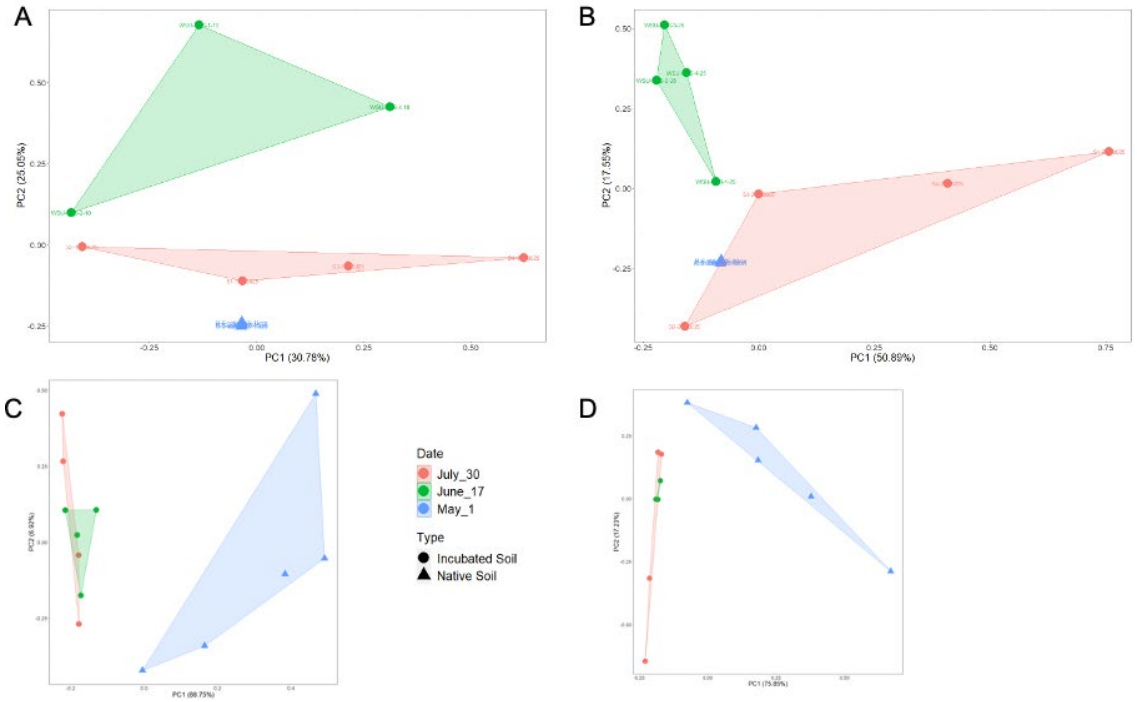

Figure 3. Soil taxonomic and transcriptional profiles between soil stakes and native soils. Points represent individual samples, depicted with colored hulls to better show differences or overlap across treatments. A) Composition of 16S rRNA amplicon sequences across treatments 10-15 cm depth. B) Composition of 16S rRNA amplicon sequences across treatments 25-30 cm depth. C) Composition of transcript sequences across treatments 10-15 cm depth. D) Composition of transcript sequences across treatments 25-30 cm depth.

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