Organic co-amendments reshape soil microbial community responses to enhanced rock weathering in croplands

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Abstract

Soil microbial responses to enhanced rock weathering (ERW) in agricultural systems remain poorly characterized, despite the known role of microbes in biogeochemical processes and mineral weathering. We evaluated how a crushed andesitic metavolcanic rock amendment, applied alone or with organic co-amendments (compost and/or biochar), affected bacterial and fungal communities over a three-year maize field trial. Adding rock alone altered bacterial alpha diversity in the second year, with only isolated differences in the third. Fungal alpha diversity shifted only under organic co-application in year two and was otherwise unaffected by rock. Differential abundance analysis showed that rock and organic additions selectively enriched microbial taxa with reported roles in mineral weathering, organic matter decomposition, and nutrient cycling. This compositional shift tracked shifts in soil chemistry: canonical correspondence analysis showed organic carbon and nitrogen as the strongest correlates early on, and pH and ions later. Together, these results show how ERW reshapes microbial community composition over time as environmental conditions change. Individual taxa were replaced by others, but this turnover did not cause a lasting drop in overall diversity. The changes depended on whether organic co-amendments were present, showing that ERW's effects on soil microbes are altered by organic inputs.

Keywords

Enhanced rock weathering (ERW),
Organic amendments (compost, biochar),
Soil microbial communities,
Functional redundancy

1. Introduction

The rapidly growing field of enhanced rock weathering (ERW) typically involves application of crushed silicate rock to croplands as a strategy to modify soil chemistry and fertility while contributing to climate mitigation [1,2]. Yet agricultural soils are biologically active ecosystems rather than chemical substrates. Contemporary soil management increasingly seeks to leverage ecological processes to sustain productivity, regulate nutrient availability, and maintain soil carbon (C) stocks [3]. Central to all these processes are the microbial communities that drive mineral weathering, organic matter decomposition, and nutrient cycling [4–6]. Introducing crushed rock into managed soils therefore represents not only a geochemical intervention but also a biological perturbation [7]. Despite the rapid expansion of interest in ERW deployment, it remains unclear how rock amendments interact with resident microbial communities within agricultural systems [1].

Microbial–mineral interactions are inherently reciprocal: microbes contribute to mineral weathering, and mineral amendments reshape microbial habitats and communities. Microorganisms influence mineral dissolution through the production of organic acids, extracellular enzymes, and respiration-derived CO_2, thereby affecting nutrient mobilization and soil C dynamics [4,5]. At the same time, mineral amendments alter soil pH, ionic strength, and resource availability, reshaping the physicochemical environment for microbes [8,3,9,10]. From a community ecology perspective, such environmental change can differentially affect community stability. Communities may be resistant, showing little compositional change; resilient, exhibiting temporary shifts followed by recovery to the original state; functionally redundant, where species turnover occurs but ecosystem function is retained; or fundamentally restructured in both composition and function [11]. These concepts provide a framework for evaluating whether ERW induces transient microbial community reorganization or longer-term restructuring as soil chemistry evolves under repetitive field-scale deployment.

Early ERW research in agricultural systems largely evaluated rock application as a standalone intervention. However, emerging work suggests that co-application with organic amendments—critical for sustaining microbial activity and soil health—may be necessary to optimize weathering processes and downstream C stabilization [12,13]. Organic inputs alter C availability and nutrient pools, thereby reshaping microbial community assembly and activity [14,15]. Because microbial communities are sensitive to shifts in resource supply and physicochemical conditions [8,10], organic amendments may modulate how communities respond to rock-induced changes in pH and ionic gradients [16]. Experimental evidence further suggests that C sources can regulate microbially mediated silicate dissolution [17], implying that ERW outcomes may depend on organic management practices such as incorporation of compost, biochar or crop residues. However, the identity of responsive taxa and the stability of bacterial and fungal communities under repeated rock application—particularly in combination with organic inputs—remain poorly resolved.

Here, we evaluated how soil bacterial and fungal communities respond to crushed rock applied with and without organic co-amendments (compost and/or biochar) in a conventionally managed, irrigated maize-cropping system. We hypothesized that the crushed rock addition would lead to shifts in specific soil microbial taxa, with differential responses of bacterial and fungal communities. We further hypothesized that organic co-amendments would exert distinct and

synergistic effects on microbial diversity and composition relative to rock-only treatments, resulting in community responses indicative of resilience and redundancy under repeated amendment.

2. Materials and methods

2.1 Field site and experimental design

The study was conducted at the University of California, Davis Campbell Tract Agricultural Research Station (38°31'53.96"N, 121°46'54.15"W), Davis, California, USA. The site has a Mediterranean climate with a mean annual temperature of approximately 16°C and annual precipitation of ~450 mm [13]. Soils are classified as fine-silty, mixed, superactive, nonacid, thermic Mollic Xerofluvents (Yolo series) with mixed clay mineralogy (smectite, vermiculite, mica, kaolinite). Surface soil horizons (0–10 cm) are silt loam (~14% sand, 61% silt, 25% clay) formed from Holocene alluvial deposits [18]. Baseline soil pH ranged from 6.79 to 6.89 across treatments prior to amendment (table S8).

The 2.07-ha field followed a randomized complete block design with three blocks and eight treatment combinations (n = 24). Treatments consisted of factorial combinations of crushed andesitic metavolcanic rock, compost, and biochar: control, rock, compost, biochar, compost+biochar, rock+compost, rock+biochar, and rock+compost+biochar (figure S1). Amendments were surface-applied after corn harvest and incorporated into the upper 15 cm of soil. Crushed rock, compost, and biochar were sourced from Specialty Granules Incorporated (Ione, CA), West Marin Compost (Nicasio, CA), and Oregon Biochar Solutions (White City, OR), respectively. Crushed rock was applied annually from 2019-2021 at 40, 50, and 50 t ha^{-1}, respectively, and compost at a constant 9 t ha^{-1}, whereas biochar (10 t ha^{-1}) was applied once at study initiation; the field was maintained under continuous corn monoculture with no crop rotation. In addition to a granular starter fertilizer, the crop was fertigated with UAN-32 and irrigated by subsurface drip, with irrigation volume differing between years for reasons unrelated to this study [13]. Full amendment composition and characteristics and additional management details are provided in Supplementary Materials and Sohng et al. (2025) [13].

2.2 Soil sampling and analyses

Baseline soil samples were collected in October 2019 prior to the first amendment application. Post-amendment samples (0–10 cm) were collected at the end of Year 2 (October 2021, post-harvest), mid-Year 3 (March 2022, pre-planting), and the end of Year 3 (October 2022, post-harvest) following initial rock application in fall 2019. For sampling, 12 subsamples from each plot were combined into a single homogenized composite sample. Sampling points were randomly distributed across central rows of each plot.

Microbial DNA analyses were performed for all post-amendment samples only. In the field, homogenized subsamples designated for microbial DNA analysis were immediately placed on dry ice and transported to the laboratory, where they were stored at −80 °C until DNA extraction.

Soil analyses were conducted for all sampling dates across treatments, including baseline. Gravimetric soil water content was determined prior to further processing. Remaining soil was air-dried to constant mass and sieved (<2 mm) for physicochemical analyses. Measured soil

properties included pH, electrical conductivity (EC), and exchangeable cations (K^+, Ca^{2+}, Mg^{2+}, Na^+, Ni^{2+}, Ba^{2+}, Mn^{2+}, and Sr^{2+}). Carbon fractions were quantified, including microbial biomass carbon (MBC), inorganic carbon (IC), and water-extractable organic carbon (WEOC). C and nitrogen (N) were quantified in size-fractionated particulate organic matter (POM) and mineral-associated organic matter (MAOM). Baseline values (October 2019) were subtracted from each post-amendment measurement to generate baseline-corrected change (Δ) values. Analytical procedures followed standardized protocols as detailed in Sohng et al. (2025) [13].

2.3 Microbial DNA extraction and amplicon sequencing

Soil DNA was extracted from 0.25 g of frozen soil using the DNeasy PowerSoil kit (QIAGEN, Hilden, Germany) following the manufacturer's protocols [19]. DNA concentration was quantified fluorometrically using Qubit dsDNA High Sensitivity assay (Thermo Fisher Scientific).

Amplicon libraries were prepared and sequenced on an Illumina MiSeq platform at the UC Davis Genome Center. Bacterial communities were profiled using 16S rRNA gene primers 341F/806R; fungal communities were profiled using ITS2 primers ITS86F/ITS4R [20]. Sequencing reads were demultiplexed and trimmed using dbcAmplicons [21].

Raw sequences were processed using the DADA2 pipeline [22]. Taxonomy was assigned using SILVA for 16S sequences [23] and the UNITE database for ITS sequences [24]. Non-target sequences were removed. Filtering parameters were maxEE = 5, truncQ = 2 for 16S (250/195 bp truncation), and maxEE = 2, truncQ = 8 for ITS.

2.4 Bioinformatic analyses

Alpha diversity metrics (Observed richness, Shannon index, Simpson index, and Pielou's evenness) for bacterial and fungal groups were assessed at the ASV level with *phyloseq* and *vegan* packages in R [25,26].

To evaluate relationships between microbial composition and soil properties, canonical correspondence analysis (CCA) based on chi-square distances was conducted separately for each sampling date. Environmental constraints included pH, EC, Ca^{2+}, Mg^{2+}, MBC, WEOC, ΔPOM-C, ΔPOM-N, ΔMAOM-C, and ΔMAOM-N. Analysis was performed using the ordinate function in the *phyloseq* package, with validation of model, axis, and term significance conducted via permutation tests in the *vegan* package. Multicollinearity among CCA constraints was evaluated using variance inflation factors (vif.cca, vegan). For the soil variables most directly associated with rock weathering (pH, EC, Ca, and Mg), VIF values ranged from 1.70 to 5.47 across sampling dates, below the commonly used threshold of 10 for problematic multicollinearity.

Differential abundance at the genus-level was assessed using DESeq2 [27] integrated with *phyloseq* to identify specific microbial taxa whose relative abundances significantly shifted in response to rock addition. Pairwise contrasts compared treatments with and without rock within each amendment category for each timepoint: control vs. rock, compost vs. rock+compost, biochar vs. rock+biochar, and compost+biochar vs. rock+compost+biochar. Statistical significance was defined at $\alpha = 0.05$ for 16S data and $\alpha = 0.01$ for ITS data, with p-values

adjusted using the Benjamini–Hochberg procedure to control the false discovery rate (FDR). Differentially abundant taxa were identified based on log2-transformed fold changes (LFC).

2.5 Statistical analysis

Statistical analyses and data visualization were performed using R version 4.5.1 [28]. Observed richness and phylogenetic diversity were compared using analysis of variance (ANOVA), followed by the Tukey honestly significant difference (HSD) post hoc test. Assumptions of normality and homogeneity of variance were evaluated prior to analysis using Shapiro–Wilk tests and diagnostic plots; log transformation was applied when necessary. Linear mixed-effects models (lme4) evaluated rock addition (fixed effect) within amendment categories (soil, compost, biochar, and compost-biochar), with block as a random effect across amendment treatments.

Pairwise differences between rock addition levels were assessed using Tukey's HSD post-hoc test. Statistical significance was defined as $p < 0.05$, and marginal significance ($0.05 < p < 0.1$) was reported to indicate trends.

3. Results

3.1 Effect of rock and organic amendments on soil microbial alpha diversity

Rock addition influenced bacterial alpha diversity in a treatment- and time-dependent manner (table 1; table S1). Compared to the control, the rock amendment reduced the observed richness and Shannon diversity at the end of Year 2 (October 2021). Simpson diversity increased at the end of Year 2 but decreased by the end of Year 3 (October 2022), while no differences were detected at mid-Year 3 (March 2022).

Bacterial responses to co-application varied by amendments. Under compost treatments, the addition of rock increased the Simpson diversity at mid-Year 3 and Pielou evenness at the end of Year 3, without consistent effects on richness or Shannon diversity. Under rock+compost+biochar, the Simpson diversity increased at the end of Year 2, but with lower evenness. Increases in observed richness and Shannon diversity at mid-Year 3 were not sustained by the end of Year 3. Under biochar treatments, bacterial diversity did not differ with rock addition.

Fungal alpha diversity was comparatively stable. The rock-only amendment did not affect fungal richness, diversity, or evenness across sampling periods. In contrast, co-application effects were detectable only at the end of Year 2: rock+compost reduced fungal richness, whereas rock+biochar increased fungal richness and Shannon diversity. These differences were not sustained.

3.2 Relationships between soil properties and microbial community composition

Soil physicochemical variables structured microbial community composition, with dominant drivers shifting over time (figure 1).

Bacterial community (16S)

At the end of Year 2, the bacterial community composition was strongly constrained by soil chemical variables, and both canonical axes were significant (figure 1a). EC was the strongest predictor, followed by ΔPOM-C and MBC; ΔPOM-N and Ca showed marginal associations. At mid-Year 3, the overall model remained significant, although individual axes were not; pH and ΔMAOM-N were significant predictors and WEOC was marginal. By the end of Year 3, the overall model and the first canonical axis were significant, with EC and pH emerging as dominant drivers.

Fungal community (ITS)

At the end of Year 2, the overall fungal CCA model was marginally significant, although the first canonical axis was not (figure 1d). ΔMAOM-N significantly constrained fungal composition, with WEOC marginally associated. At mid-Year 3, no environmental variables significantly structured fungal communities, although ΔPOM-C showed marginal association. By the end of Year 3, the overall fungal model was marginal, while the first canonical axis was significant. Soil pH emerged as the only significant predictor.

3.3 Soil bacterial and fungal taxa driving community differences

Differential abundance analysis at the genus level identified taxa contributing to compositional differences among treatments (control, compost, biochar, and compost+biochar) with and without rock amendment. Across all comparisons, 17 bacterial genera (3.4 ± 0.4% of 16S sequences) and 48 fungal genera (16.5 ± 2.7% of ITS sequences) were identified as differentially abundant (figure 2). Specific bacterial genera showed no consistent recurrence across amendment conditions or sampling periods, whereas in fungal communities, a small number of genera recurred across either amendment conditions or timepoints (figure 3).

Bacterial communities (16S)

Few bacterial genera were enriched by the end of Year 2, and Streptomyces was enriched in rock-only treatments (table S4). At mid-Year 3, *WCHB1-32* and *Citrifermentans* were enriched under rock-only conditions, and *Angustibacter* under rock+biochar amendments. By the end of Year 3, *Intrasporangium*, *Anaeromyxobacter*, and *Clostridium sensu stricto* were enriched in rock-only treatments. *Rhizobiaceae* family (unclassified genus) and *Caulobacter* were associated with rock+compost and rock+compost+biochar treatments, respectively.

Fungal communities (ITS)

At the end of Year 2, rock-only treatments were associated with an increased abundance of five fungal genera, including *Pseudeurotium* and *Chaetomium* (table S5). *Pseudeurotium* was also enriched under rock+compost and rock+compost+biochar treatments, while *Chaetomium* was enriched under rock+biochar. *Arachnomyces*, which showed lower abundance under rock-only treatment, was enriched under rock+compost and rock+compost+biochar. Among treatments, rock+biochar showed the fewest differentially abundant genera relative to biochar alone. At mid-Year 3, five fungal genera were enriched under the rock-only treatment, including *Tubaria*, which was also enriched under the rock+biochar treatment (table S6). By the end of Year 3, *Chaetomium* reappeared in the rock-only treatment, and *Naviculispora* was enriched in both the rock+compost and rock+compost+biochar treatments (table S7).

4. Discussion

4.1 Contrasting impacts of treatments on bacterial and fungal diversity

Alpha diversity describes the number and evenness of taxa within a sample. Crushed silicate rock amendment acted as a physicochemical disturbance where responses of bacterial alpha diversity were largely early and treatment dependent. Under the rock-only treatment, bacterial richness and Shannon diversity declined by the end of Year 2, reflecting early sensitivity of the bacterial community to mineral inputs (Table 1). Similar early reductions in bacterial diversity following rock application have been attributed to disruptions in resource availability and soil microstructure immediately following incorporation into soil [29]. Such disturbances may temporarily destabilize established bacterial niches by altering pore networks and chemical gradients.

However, these changes in diversity were not sustained. By mid-Year 3, bacterial alpha diversity no longer differed from control plots, suggesting community resilience [11]. This stabilization likely reflects maintenance of microbial richness and evenness under evolving geochemical conditions, including shifts in pH and nutrient release from weathering products [4,8]. During mineral weathering, an initial chemical perturbation is followed by gradual equilibration, creating conditions that allow microbial recolonization and niche reorganization [5].

The addition of organic co-amendments modified these dynamics. Compost co-application attenuated early diversity declines and, in some cases, enhanced dominance-weighted diversity in later sampling periods. Compost supplies labile C and nutrients that buffer physicochemical disturbance and expand the niche space for heterotrophic taxa [15]. In contrast, the biochar amendment—characterized by recalcitrant C forms—had limited effects on bacterial diversity when combined with rock. While biochar can modify soil structure and pH, it often contributes limited bioavailable C content [14], which is consistent with the fact that initial changes in microbial diversity were not sustained. Together, these findings are strong evidence that organic C availability mediates microbial responses to mineral disturbance.

Fungal alpha diversity showed a contrasting pattern. In the rock-only treatment, neither fungal richness nor diversity were significantly altered across time, indicating strong resistance to the perturbation. With co-application of organic amendments with rock, compost temporarily reduced fungal richness, whereas biochar increased fungal richness and Shannon diversity at the end of Year 2. Fungi differ fundamentally from bacteria regarding their growth strategy and resource utilization. Their broad metabolic capabilities and slow turnover rates can intensify competition under high-labile-C conditions [30,31], potentially explaining the early decline in richness under compost treatments. Conversely, the more recalcitrant biochar may provide stable microsites and slowly available C pools that initially favor fungal colonization [32].

4.2 Shifts from carbon pools to pH as biogeochemical drivers of microbial community

The environmental variables most strongly associated with microbial community composition shifted over time from early associations with organic C and N fractions to later associations with soil chemical properties under repeated annual rock addition. Whereas alpha diversity stabilized after the initial sampling period (Section 4.1), community composition continued to

differentiate throughout the trial. Importantly, community differentiation was not explained solely by treatment (e.g., rock vs. no rock) but instead aligned with continuous soil physicochemical gradients. At the end of Year 2, variation in both bacterial and fungal communities was most strongly associated with POM, MBC, MAOM and WEOC (figure 1). These early responses suggest that compost and biochar amendments exerted immediate influence by modifying labile C and N availability, likely stimulating resource-based microbial interactions and competitive dynamics.

By mid-Year 3, soil pH and ΔMAOM-N became dominant predictors of bacterial composition. The observed shift from C and N fractions to pH suggests bacterial communities were responding to cumulative changes in soil physicochemical conditions driven by mineral dissolution. Such responses are consistent with high sensitivity of bacteria to environmental shifts due to their shorter generation times and metabolic flexibility [10,33]. In contrast, fungal communities exhibited a slower transition. ΔPOM-C remained influential at mid-Year 3, while pH emerged as the primary driver by the end of Year 3. This delayed shift likely reflects differences in growth strategies and slower metabolic flexibility, which allow fungi to respond to environmental changes over longer timescales [31].

pH emerged as the dominant driver of microbial community composition, supporting field observations that pH served as a primary weathering indicator [13]. This pattern is consistent with extensive evidence that pH is a major determinant of bacterial community structure, as shifts in pH selectively constrain or promote microbial taxa based on physiological tolerances [8]. The increasing influence of pH over time suggests that cumulative mineral dissolution progressively reshaped soil niches. EC further structured bacterial communities, particularly at the end of Year 2 and Year 3, consistent with microbial sensitivity to ionic strength and nutrient availability associated with weathering products. Changes in EC can modify microbial activity through osmotic regulation and altered ion-mediated nutrient acquisition [9,34]. Importantly, these results show that rock-driven shifts in fundamental soil properties, such as pH and EC, can become dominant determinants of microbial community composition over time, even in the presence of organic amendments.

Microbial community composition in agricultural soils is also influenced by management practices that modify these same physicochemical conditions. In the context of ERW, fertilization and irrigation are particularly relevant because N inputs can alter soil pH and nutrient availability, whereas water supply can influence mineral dissolution, solute transport, EC, and microbial activity. At our site, soil pH did not progressively decline over the study period and generally increased across treatments, including in plots without rock or organic amendments, consistent with the relatively high buffering capacity of this soil. Irrigation also varied substantially between years, with approximately 2000 mm applied in 2021 and 1000 mm in 2022, and this difference may have contributed to temporal variation in soil chemistry and microbial community composition. However, because fertilization and irrigation were applied uniformly across plots and were not experimentally manipulated, their contributions cannot be separated from cumulative amendment effects or other seasonal changes in the present design.

4.3 Effects of rock amendment on differential abundance of soil microbial taxa

4.3.1 Bacterial community

The addition of rock to the plots selectively enriched or suppressed specific bacterial taxa without fundamentally restructuring the community. This taxon-specific response aligns with the concepts of resistance and resilience in the context of diversity metrics: community composition shifted, yet the overall community structure remained relatively stable across time (figure 2).

By the end of Year 2, *Streptomyces* was more abundant in rock-only treatments despite low relative abundance (table S4). This metabolically versatile genus produces organic acids and extracellular enzymes that promote mineral dissolution [35]. These bacteria are adept at thriving in alkaline environments, where they have been shown to enhance basalt weathering, increasing soil fertility and mineral substrate availability [35].

By the end of Year 3, the rock-only treatments were enriched in *Clostridium sensu stricto 10*, *Anaeromyxobacter*, and *Intrasporangium*—taxa associated with reductive metabolism and adaptation to low-oxygen, nutrient-limited microsites. *Clostridium* spp. are obligate anaerobes that ferment organic matter in anoxic microsites [36]. *Anaeromyxobacter* performs aromatic compound dechlorination, metal reduction and denitrification under facultatively anaerobic conditions [37,38]. *Intrasporangium* is adapted to neutral-to-alkaline soils and contributes to nitrate reduction and organic matter turnover [39]. Their enrichment suggests that the addition of rock amendments may enhance microscale redox heterogeneity, potentially through aggregation and ion-mediated structural changes that modify water retention and gas diffusion [40], or alternatively through increased microbial respiration that consumes oxygen and promotes localized oxygen depletion. Such conditions can favor fermentation and metal-reducing pathways; for instance, both *Citrifermentans* (mid-Year 3) and *Clostridium sensu stricto 10* (end of Year 3) are capable of autotrophic acetate production [36].

Under co-application of organic amendments with rock amendment, differential enrichment was less pronounced. By the end of Year 2, no taxa were significantly enriched relative to organic-only treatments. By mid-Year 3, *Angustibacter* was enriched in rock+biochar plots relative to biochar-only treatments. By the end of Year 3, the *Rhizobiaceae* family (unclassified genus) and *Caulobacter* showed significant differential abundance in the rock+compost and rock+compost+biochar treatments, respectively. These Alpha-proteobacterial taxa are associated with C and N cycling [41], suggesting that organic amendments moderate rock-induced selection and redistribute functional roles across taxa.

4.3.2 Fungal community

Fungal communities showed stronger compositional shifts than bacteria in response to the addition of rock, for both rock-only and rock+organic amendments (figure 3). Several genera consistently appeared across time points and amendments (tables S5–S7), suggesting consistent responders within the silicate weathering environment.

Genera associated with mineral weathering were enriched following the addition of rock. By the end of Year 2, *Humicola* was enriched in rock-only plots, followed by enrichment of *Aspergillus* by mid-Year 3. Both genera are widely recognized for their roles in fungal-mediated mineral dissolution through organic acid production and enzymatic activity. *Humicola* species have been reported in humid tropical regions contributing to weathering of sandstone and marble, as well as in arid and semi-arid environments associated with quartzite and andesite breakdown [42].

Similarly, *Aspergillus* species are implicated in biodeterioration of sandstone, marble, granite, limestone and andesite [42], and can dissolve metal-bearing minerals [43].

Chaetomium emerged repeatedly in the rock-only and rock+biochar treatments, indicating temporal persistence. As a cellulose-degrading genus associated with mineral biodeterioration, it links organic matter decomposition with rock weathering processes. Species within this genus degrade cellulosic substrates, including wood and synthetic materials, suggesting its metabolic versatility and potential synergistic activity with biochar decomposition [44]. *Chaetomium* has also been reported in the biodeterioration of sandstone and limestone, emphasizing its role in mineral weathering processes [42]. Its recurrence across time points suggests adaptability to sustained changes in soil chemistry and substrate availability [45].

Additional saprotrophic taxa, including *Pseudeurotium* (rock-only, rock+compost, Rock+Compost+Biochar) and *Tubaria* (rock-only, rock+biochar), were enriched in response to rock amendment. Ascomycetous *Pseudeurotium* species function as both endophytes and saprotrophs with versatility to metabolize volatile aromatic hydrocarbons as C and energy sources [46]. Similarly, *Tubaria* contributes to litter decomposition and degradation of complex organic substrates, including polyaromatic hydrocarbons, making their association with biochar particularly relevant [47].

Several bacterial and fungal taxa that responded to rock addition have previously been associated with overlapping biogeochemical processes, including mineral weathering, organic-matter turnover, fermentation and nutrient cycling. Although only direct measures of process rates, enzyme activities or biogeochemical fluxes can decidedly establish the level of preservation of ecosystem functioning, this overlap is consistent with functional redundancy among responsive taxa, and it aligns closely with the observed diversity maintenance as well as the general absence of a detectable treatment effect on microbial biomass carbon in the companion study [13].

5. Conclusion

This study shows that ERW is a moderate, not destabilizing, disturbance to soil microbial communities in agricultural soils, mediated by shifts in soil chemistry and organic inputs. Rock-only treatments were associated with initial reductions in bacterial diversity in Year 2, but these differences were no longer detected by Year 3. Fungal diversity, however, remained largely stable when rock-only was added, but notably, showed treatment-specific responses when organic inputs were co-applied. In contrast, microbial community composition shifted from early resource-driven influences to later pH- and ion-mediated filtering, consistent with cumulative mineral dissolution shaping soil chemical gradients. Bacterial communities responded more rapidly to these shifts, whereas fungal communities adjusted more gradually, highlighting distinct temporal strategies between domains. Although specific taxa were selectively enriched over the study period and across amendment types, many have reported roles in mineral weathering, organic matter decomposition, and nutrient cycling. The turnover among taxa with overlapping literature-reported functional capacities is consistent with the hypothesis of functional redundancy, suggesting that changes in microbial community composition under rock amendment may occur without an associated loss of ecosystem functioning.

Together, these results suggest that ERW reorganizes microbial community composition without evidence of sustained diversity loss. Pairing rock amendments with organic amendments may further moderate early physicochemical disturbance and maintain C-mediated niche diversity in managed soils.

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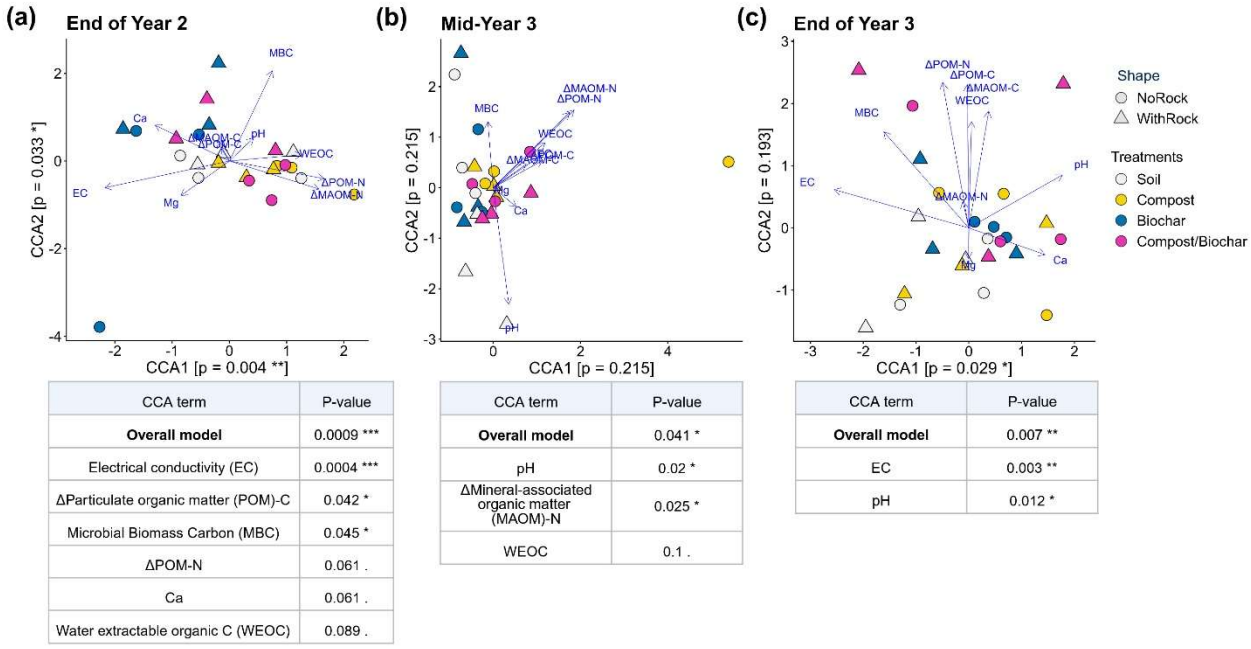
Table 1. Directional effects of crushed rock addition on alpha diversity indices—observed richness, Shannon diversity, Simpson index, and Pielou's evenness—across three sampling times for bacterial and fungal communities. Arrows indicate significant increases (↑) or decreases (↓) under rock addition relative to treatments without rock; dashes (–) denote non-significant effects. Significance was assessed using linear mixed-effects models with rock co-application as a fixed effect and block as a random effect. Full regression coefficients are provided in Table S1.

Treatment Comparison (No rock - With rock)	Alpha diversity index	Bacterial community			Fungal community		
		End of Year 2	Mid-Year 3	End of Year 3	End of Year 2	Mid-Year 3	End of Year 3
control vs. rock	Observed	↓	–	–	–	–	–
	Shannon	↓	–	–	–	–	–
	Simpson	↑	–	↓	–	–	–
	Pielou	–	–	–	–	–	–
compost vs. rock+compost	Observed	–	–	–	↓	–	–
	Shannon	–	–	–	–	–	–
	Simpson	–	↑	–	–	–	–
	Pielou	–	–	↑	–	–	–
biochar vs. rock+biochar	Observed	–	–	–	↑	–	–
	Shannon	–	–	–	↑	–	–
	Simpson	–	–	–	–	–	–
	Pielou	–	–	–	–	–	–
compost+biochar vs. rock+compost+biochar	Observed	–	↑	–	–	–	–
	Shannon	–	↑	–	–	–	–
	Simpson	↑	–	–	–	–	–
	Pielou	↓	–	–	–	–	–

Figure 1. Canonical correspondence analysis (CCA) of microbial community composition constrained by soil physicochemical variables across three sampling times. Panels (a–c) show bacterial (16S) communities and panels (d–f) show fungal (ITS) communities. Ordinations were based on normalized genus-level relative abundance data. Points represent samples colored by amendment treatment and shaped by rock addition. Blue arrows indicate environmental variables associated with community structure. Axis labels show the proportion of constrained variation explained and permutation-based significance; insets summarize overall model significance (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$).

Bacterial community

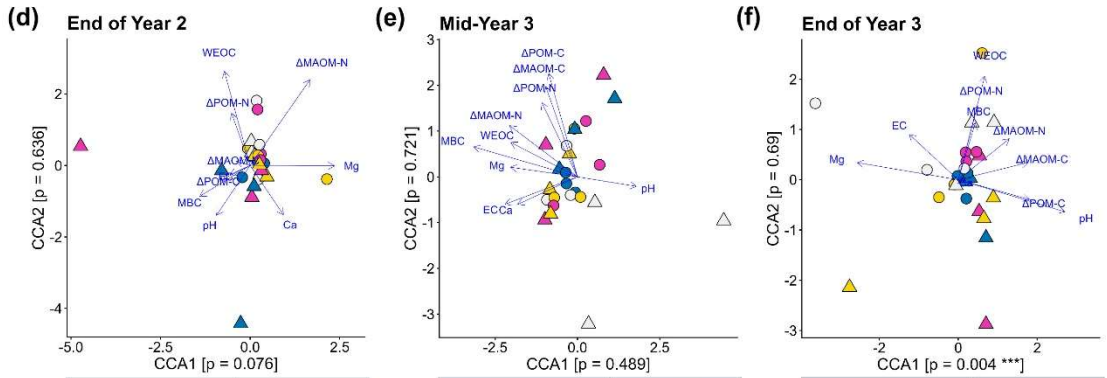


CCA term	P-value
Overall model	0.0009 ***
Electrical conductivity (EC)	0.0004 ***
ΔParticulate organic matter (POM)-C	0.042 *
Microbial Biomass Carbon (MBC)	0.045 *
ΔPOM-N	0.061 .
Ca	0.061 .
Water extractable organic C (WEOC)	0.089 .

CCA term	P-value
Overall model	0.041 *
pH	0.02 *
ΔMineral-associated organic matter (MAOM)-N	0.025 *
WEOC	0.1 .

CCA term	P-value
Overall model	0.007 **
EC	0.003 **
pH	0.012 *

Fungal community



CCA term	P-value
Overall model	0.056 .
ΔMAOM-N	0.03 *
WEOC	0.078 .

CCA term	P-value
Overall model	0.309
ΔPOM-C	0.091 .

CCA term	P-value
Overall model	0.054 .
pH	0.002 **

Figure 2. Differentially abundant bacterial genera between treatments with and without crushed rock across amendment types and sampling times. Columns represent amendment contrasts (as labeled in the column headers), and rows represent sampling times. Bars indicate log2 fold changes; only taxa with adjusted $P < 0.05$ (DESeq2) are shown. Taxa labeled as "Unclassified" are displayed at the highest resolved taxonomic level. Full differential abundance statistics are provided in Table S4.

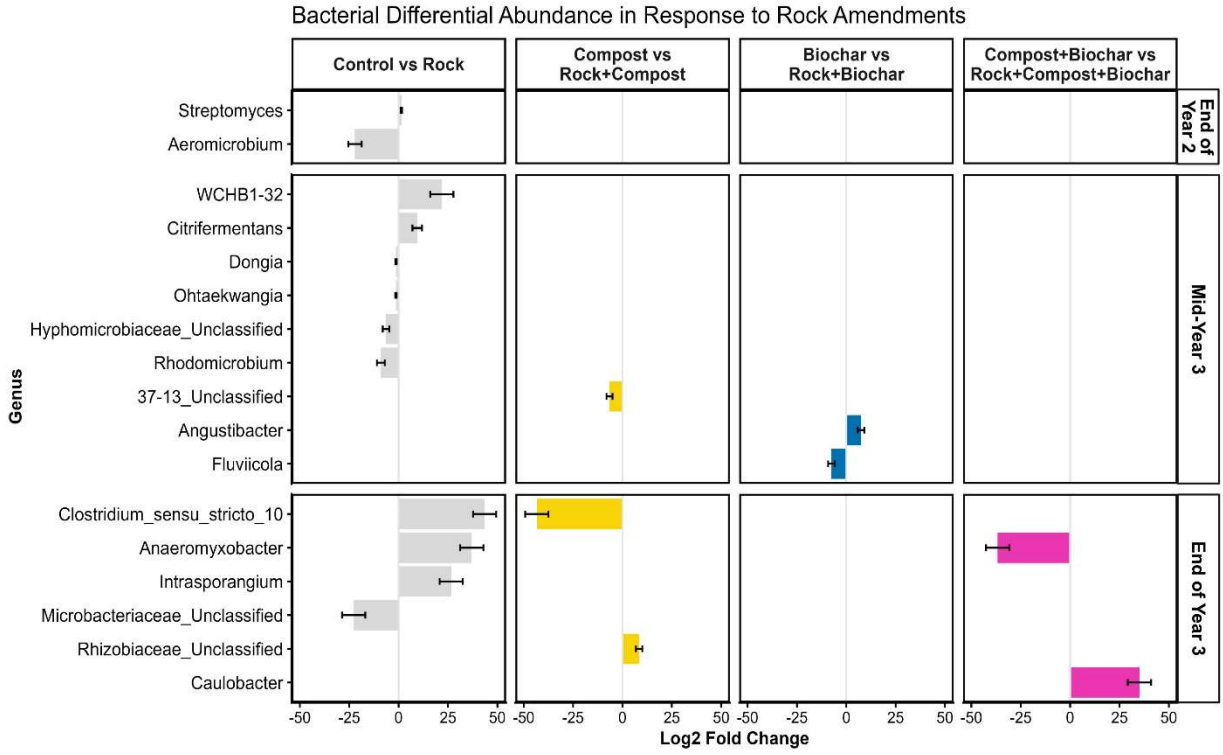
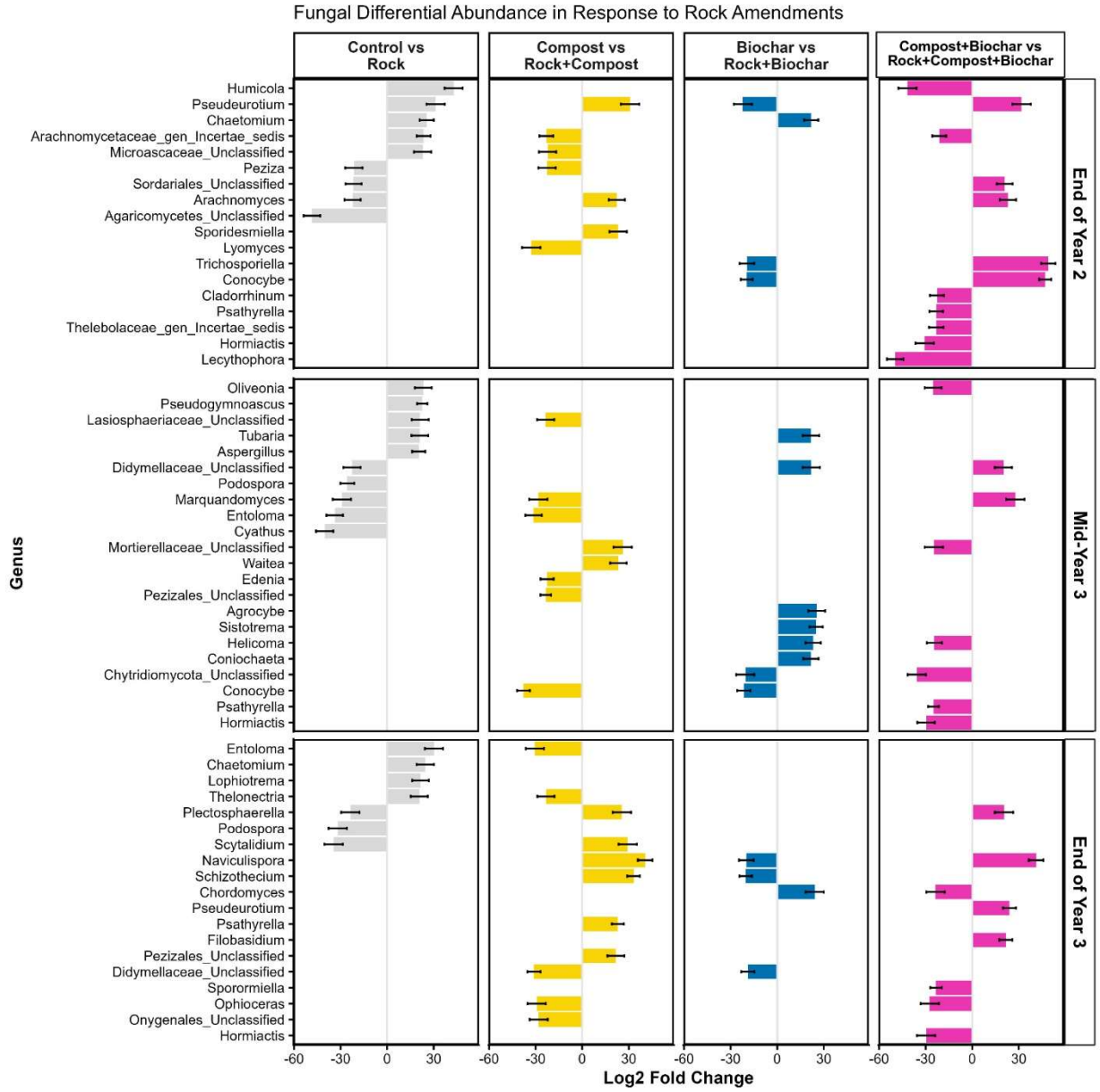


Figure 3. Differentially abundant fungal genera between treatments with and without crushed rock across amendment types and sampling times. Columns represent amendment contrasts (as labeled in the column headers), and rows represent sampling times. Bars indicate log2 fold changes; only taxa with adjusted $P < 0.01$ (DESeq2) are shown. Taxa labeled as "Unclassified" are displayed at the highest resolved taxonomic level. Full differential abundance statistics are provided in Table S5 (end of Year 2), S6 (mid-Year 3), and S7 (end of Year 3).



Supplementary Materials

Title: Organic co-amendments reshape soil microbial community responses to enhanced rock weathering in croplands

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Supplementary Information: Methods, Figures S1, Tables S1-S9

Supplementary Information Addition

Methods

Amendment sourcing and characterization

Crushed rock was obtained from Specialty Granules Incorporated (Ione, CA) and consisted of a mixed metavolcanic material, predominantly andesitic in composition, comprising 33% plagioclase feldspar, 32% quartz, 15% epidote, 5% chlorite, and 4.5% muscovite, with particle sizes between 150 µm and 2 mm and no measurable organic carbon. Crushed rock was applied from 2019 to 2021 at 40, 50, and 50 t ha^{-1}, respectively [1].

Compost was obtained from West Marin Compost (Nicasio, CA) and consisted of the Nicasio Blend, a mixture of recycled yard trimmings, dairy manure, and horse manure (16% organic carbon). This is a finished, mature compost product, with maturity moisture content typically maintained below 50%. The reported application rate therefore reflects an as-applied, field-moist basis rather than an oven-dry basis. Compost was included as a recurring organic amendment and nutrient input and was applied annually at a constant rate of 9 t ha^{-1} from 2019 to 2021 [1].

Biochar (Rogue Biochar) was obtained from Oregon Biochar Solutions (White City, OR) and was produced by pyrolysis of mixed softwood feedstock (Douglas fir, pine, and cedar) at 870°C (77% organic carbon; initial pH 10.58). In contrast to compost, biochar was included as a persistent, C-rich amendment rather than a recurring nutrient input. Given its expected persistence in soil, it was applied once at study initiation in 2019 at 10 t ha^{-1} [1]. This application strategy avoided cumulative annual biochar loading and allowed its interaction with repeated rock additions to be evaluated over time as the biochar aged in situ.

Management descriptions

The field was maintained under continuous corn (*Zea mays* L.) monoculture with no crop rotation throughout the 2019-2022 study period, with spring tillage (15-cm disc) conducted twice annually for bed preparation [1]. In addition to a granular 8-24-6 (N-P-K) starter fertilizer was applied prior to planting each year at 28 kg ha^{-1}, the crop was fertigated with UAN-32 (32% urea ammonium nitrate) four times per growing season at 56 kg N ha^{-1} per application (~224 kg N $ha^{-1}yr^{-1}$ total) [1]. Both were applied uniformly to all plots.

Irrigation was supplied from April to July using subsurface drip lines with emitters positioned at 30 cm depth. Irrigation frequency and duration increased as the growing season progressed, from 4-6 h every other day in April to 9-14 h per day from May through July, corresponding to a field-wide flow rate of approximately 26,000-45,000 L h^{-1} across the 2.07-ha field. Total irrigation differed between years, with approximately 2000 mm applied in 2021 and 1000 mm in 2022. The greater water input in 2021 was associated with the needs of unrelated experiments conducted at the same site rather than with the design of the present study [1].

1. Sohng J *et al.* 2025 Combining organic amendments with enhanced rock weathering shifts soil carbon storage in croplands. *Sci. Total Environ.* **998**, 180179. (doi:10.1016/j.scitotenv.2025.180179)

Figure S1. Study location and experimental layout. a) Location of study site at the University of California, Davis Campbell Tract Agricultural Research Station (38°31'53.96"N, 121°46'54.15"W), Davis, California, USA. b) Plot layout. The 2.0 ha research site was divided into three Blocks of 8 plots each – 24 plots total. Treatments were assigned randomly to each plot within a Block. Amendments were surface-applied after harvest and incorporated into the upper 15 cm of soil. Crushed rock (40 t ha^{-1}) and compost (9 t ha^{-1}) were applied annually from 2019 to 2021, whereas biochar (10 t ha^{-1}) was applied once at study initiation in 2019. Corn (Zea mays L.) was grown continuously under conventional management with subsurface drip irrigation and synthetic fertilization (8–24–6 N–P–K formulation).

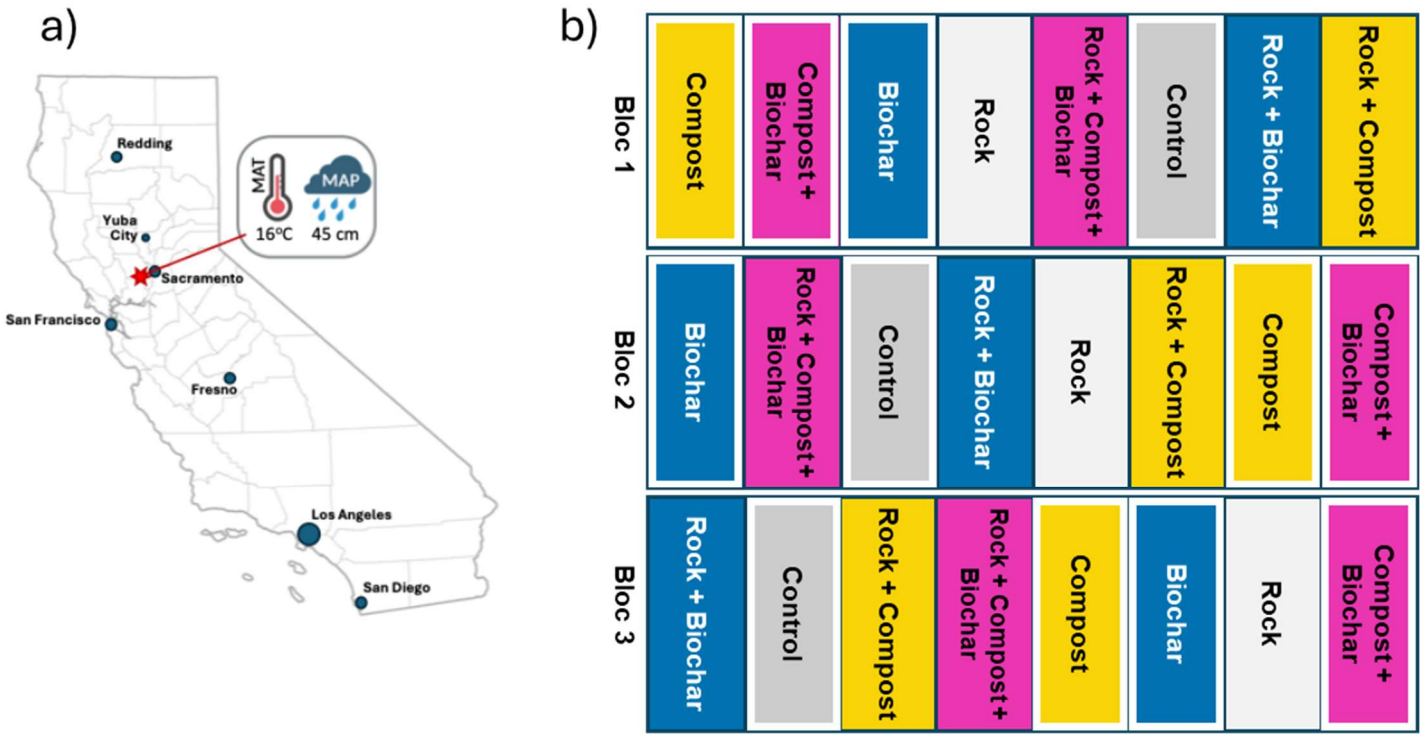

Table S1 Regression coefficients (± SE) from linear mixed-effects models evaluating the effects of crushed rock treatment and sampling time (end of Year 2, mid-Year 3, and end of Year 3) on alpha diversity metrics, including observed richness (species counts), Shannon diversity (sensitive to both richness and relative abundance), Simpson diversity (weighted toward dominant taxa), and Pielou's evenness (relative distribution of taxa). The effect of crushed rock was assessed relative to treatments without rock under the following conditions: control vs. rock, compost vs. rock+compost, biochar vs. rock+biochar, and compost+biochar vs. rock+compost+biochar. Rock co-application (+Rock) was treated as a fixed effect, while block was included as a random effect. Statistical significance is indicated as follows: *P < 0.05, **P < 0.01, ***P < 0.001.

Bacterial community		End of Year 2 (October 2021)		Mid-Year 3 (March 2022)		End of Year 3 (October 2022)	
Treatment Comparison (No rock - With rock)	Alpha diversity index	Coefficient estimate ± SE	P-value	Coefficient estimate ± SE	P-value	Coefficient estimate ± SE	P-value
control vs. rock	Observed	-47.33 ± 18.48	0.010 *	-196.0 ± 149.8	0.191	-117.67 ± 80.94	0.146
	Shannon	-0.083 ± 0.003	<2e-16 ***	-0.249 ± 0.157	0.113	-0.176 ± 0.105	0.094 .
	Simpson	0.0009 ± 0.0003	0.004 **	-0.0006 ± 0.0003	0.067 .	-0.0008 ± 0.0004	0.047 *
	Pielou	-0.004 ± 0.003	0.275	-0.004 ± 0.003	0.306	0.001 ± 0.004	0.751
compost vs. rock+compost	Observed	28.33 ± 67.02	0.672	95.67 ± 137.82	0.488	-63.33 ± 44.18	0.152
	Shannon	0.065 ± 0.042	0.126	0.192 ± 0.146	0.187	-0.018 ± 0.081	0.823
	Simpson	-0.0003 ± 0.0003	0.29	0.001 ± 0.001	0.049 *	0.001 ± 0.0006	0.0625 .
	Pielou	0.005 ± 0.006	0.45	0.013 ± 0.011	0.223	0.013 ± 0.003	1.68e-06 ***
biochar vs. rock+biochar	Observed	111.00 ± 60.19	0.065 .	76.33 ± 147.12	0.604	36.67 ± 25.04	0.143
	Shannon	0.160 ± 0.119	0.181	0.1385 ± 0.1478	0.349	0.041 ± 0.043	0.336
	Simpson	-0.0004 ± 0.002	0.884	0.0005 ± 0.0003	0.089 .	0.00008 ± 0.0004	0.84
	Pielou	0.0005 ± 0.01	0.959	0.005 ± 0.005	0.301	-0.002 ± 0.003	0.566
compost+biochar vs. rock+compost+biochar	Observed	33.67 ± 78.64	0.669	165.33 ± 58.81	0.005 **	-182.0 ± 204.7	0.374
	Shannon	-0.009 ± 0.082	0.916	0.148 ± 0.041	0.0003 ***	-0.215 ± 0.287	0.455
	Simpson	0.001 ± 0.0005	0.016 **	0.00007 ± 0.0001	0.63	-0.001 ± 0.001	0.307
	Pielou	-0.009 ± 0.003	0.006 **	-0.005 ± 0.004	0.213	-0.005 ± 0.006	0.376

Fungal community		2021 October		2022 March		2022 October	
Treatment Comparison (No rock - With rock)	Alpha diversity index	Coefficient estimate ± SE	P-value	Coefficient estimate ± SE	P-value	Coefficient estimate ± SE	P-value
control vs. rock	Observed	-6.333 ± 5.696	0.266	-8.333 ± 8.131	0.305	1.667 ± 11.479	0.885
	Shannon	-0.039 ± 0.068	0.57	-0.032 ± 0.067	0.632	0.003 ± 0.08	0.975
	Simpson	-0.004 ± 0.004	0.377	0.004 ± 0.005	0.387	0.000007 ± 0.003	0.998
	Pielou	0.008 ± 0.012	0.515	0.015 ± 0.022	0.508	-0.004 ± 0.008	0.605
compost vs. rock+compost	Observed	-6.333 ± 2.906	0.029 *	5.667 ± 6.386	0.375	-8.333 ± 5.077	0.101
	Shannon	-0.112 ± 0.085	0.185	0.011 ± 0.218	0.959	-0.295 ± 0.172	0.086 .
	Simpson	-0.005 ± 0.008	0.52	-0.005 ± 0.015	0.768	-0.024 ± 0.016	0.131
	Pielou	-0.007 ± 0.015	0.616	-0.012 ± 0.036	0.733	-0.049 ± 0.029	0.091 .
biochar vs. rock+biochar	Observed	10.333 ± 2.603	0.00007 ***	-4.00 ± ± 7.18	0.577	4.333 ± 3.930	0.27
	Shannon	0.252 ± 0.117	0.031 *	-0.131 ± 0.14	0.351	0.059 ± 0.067	0.38
	Simpson	0.015 ± 0.011	0.181	-0.011 ± 0.011	0.294	0.003 ± 0.004	0.555
	Pielou	0.029 ± 0.023	0.215	-0.021 ± 0.02	0.293	0.006 ± 0.013	0.674
compost+biochar vs. rock+compost+biochar	Observed	2.667 ± 4.055	0.511	2.667 ± 6.515	0.682	-16.67 ± 17.72	0.347
	Shannon	0.013 ± 0.115	0.91	-0.113 ± 0.184	0.544	-0.247 ± 0.169	0.143
	Simpson	-0.003 ± 0.009	0.736	-0.012 ± 0.013	0.353	-0.01 ± 0.007	0.124
	Pielou	-0.008 ± 0.022	0.711	-0.034 ± 0.033	0.297	-0.012 ± 0.015	0.465

Table S2 Statistical details of the canonical correlation analysis (CCA) of bacterial 16S sequence data across three sampling points: end of Year 2 (October 2021), mid-Year 3 (March 2022), and end of Year 3 (October 2022). The analysis was performed on normalized bacterial genus-level relative abundance data, constrained by soil physicochemical characteristics. The table reports model components, degrees of freedom (df), chi-square values, F-values, and P-values for each axis and the overall model. Significant soil physicochemical variables are indicated (*P < 0.05, **P < 0.01, ***P < 0.001).

BACTERIAL GENUS	Model component	df	ChiSquare	F	p-value	
2021 October	Model	10	0.140	1.229	0.0009	***
Axes	CCA1	1	0.025	2.227	0.004	**
	CCA2	1	0.020	1.915	0.033	*
Variables	EC	1	0.020	1.745	0.0004	***
	Δ POM-C	1	0.015	1.348	0.042	*
	MBC	1	0.015	1.302	0.045	*
	Δ POM-N	1	0.014	1.257	0.061	.
	Ca	1	0.015	1.287	0.061	.
	WEOC	1	0.014	1.222	0.089	.
	Mg	1	0.014	1.188	0.123	
	pH	1	0.012	1.093	0.252	
	Δ MAOM-N	1	0.011	0.979	0.502	
	Δ MAOM-C	1	0.010	0.866	0.748	
2022 March	Model	10	0.204	1.216	0.041	*
Axes	CCA1	1	0.050	2.973	0.215	
	CCA2	1	0.039	2.486	0.215	
Variables	pH	1	0.029	1.730	0.020	*
	Δ MAOM-N	1	0.029	1.725	0.025	*
	WEOC	1	0.022	1.329	0.100	.
	MBC	1	0.022	1.315	0.155	
	EC	1	0.019	1.159	0.230	
	Δ POM-N	1	0.019	1.146	0.240	
	Ca	1	0.017	1.020	0.407	
	Mg	1	0.016	0.973	0.448	
	Δ POM-C	1	0.016	0.947	0.537	
	Δ MAOM-C	1	0.014	0.812	0.771	
2022 October	Model	10	0.211	1.202	0.007	**
Axes	CCA1	1	0.046	2.612	0.029	*
	CCA2	1	0.030	1.850	0.193	
Variables	EC	1	0.031	1.759	0.003	**
	pH	1	0.027	1.564	0.012	*
	Δ MAOM-C	1	0.020	1.158	0.165	
	Ca	1	0.020	1.132	0.201	

MBC	1	0.020	1.148	0.214
Mg	1	0.019	1.108	0.230
Δ POM-C	1	0.019	1.106	0.233
WEOC	1	0.019	1.071	0.298
Δ POM-N	1	0.018	1.027	0.372
Δ MAOM-N	1	0.017	0.945	0.558

Table S3 Statistical details of the canonical correlation analysis (CCA) of fungal ITS sequence data across three sampling points: end of Year 2 (October 2021), mid-Year 3 (March 2022), and end of Year 3 (October 2022). The analysis was performed on normalized fungal genus-level relative abundance data, constrained by soil physicochemical characteristics. The table reports model components, degrees of freedom (df), chi-square values, F-values, and P-values for each axis and the overall model. Significant soil physicochemical variables are indicated (*P < 0.05, **P < 0.01, ***P < 0.001).

FUNGAL GENUS	Model component	df	ChiSquare	F	p-value	
2021 October	Model	10	0.642	1.179	0.056	.
Axes	CCA1	1	0.155	2.855	0.076	.
	CCA2	1	0.097	1.921	0.636	
Variables	Δ MAOM-N	1	0.101	1.865	0.030	*
	WEOC	1	0.075	1.371	0.078	.
	Mg	1	0.069	1.274	0.124	
	pH	1	0.062	1.147	0.231	
	EC	1	0.060	1.100	0.309	
	MBC	1	0.059	1.084	0.331	
	Δ POM-N	1	0.058	1.058	0.352	
	Δ POM-C	1	0.055	1.002	0.445	
	Δ MAOM-C	1	0.051	0.938	0.476	
	Ca	1	0.052	0.952	0.545	
2022 March	Model	10	0.675	1.042	0.309	
Axes	CCA1	1	0.115	1.778	0.489	
	CCA2	1	0.097	1.614	0.721	
Variables	Δ POM-C	1	0.079	1.214	0.091	.
	MBC	1	0.079	1.220	0.155	
	pH	1	0.076	1.179	0.189	
	WEOC	1	0.073	1.126	0.205	
	Δ MAOM-N	1	0.074	1.138	0.227	
	EC	1	0.070	1.084	0.305	
	Mg	1	0.060	0.919	0.660	
	Ca	1	0.058	0.894	0.744	
	Δ POM-N	1	0.058	0.893	0.773	
	Δ MAOM-C	1	0.049	0.758	0.945	
2022 October	Model	10	0.659	1.156	0.054	.
Axes	CCA1	1	0.159	2.785	0.004	**
	CCA2	1	0.089	1.687	0.691	
Variables	pH	1	0.104	1.826	0.002	**
	Δ MAOM-C	1	0.068	1.196	0.142	
	Ca	1	0.069	1.203	0.164	
	MBC	1	0.071	1.251	0.180	

Δ POM-C	1	0.064	1.131	0.232
Mg	1	0.063	1.104	0.279
Δ MAOM-N	1	0.061	1.064	0.320
EC	1	0.058	1.023	0.403
WEOC	1	0.051	0.886	0.676
Δ POM-N	1	0.050	0.872	0.700

Table S4. Differential abundance results for bacterial genera identified by DESeq2 across amendment contrasts and sampling times: end of Year 2 (October 2021), mid-Year 3 (March 2022), and end of Year 3 (October 2022). The table reports phylum, genus, log2 fold change, standard error (LFC SE), and Benjamini–Hochberg adjusted *P*-values. Only taxa meeting the adjusted significance threshold ($P < 0.05$) are included.

Year	Treatment Comparison (No rock - With rock)	Phylum	Genus	log2Fold Change	LFC SE	Adjusted P-value
End of Year 2	control vs. rock	Actinobacteriota	Streptomyces	**1.38**	0.33	0.007
		Actinobacteriota	Aeromicrobium	-22.11	3.39	3.27E-08
Mid-Year 3	control vs. rock	Bacteroidota	WCHB1-32	**21.89**	5.89	0.025
		Desulfobacterota	Citrifermentans	**9.40**	2.42	0.022
		Proteobacteria	Dongia	-1.28	0.36	0.041
		Bacteroidota	Ohtaekwangia	-1.36	0.36	0.025
		Proteobacteria	Hyphomicrobiaceae_Unclassified	-6.38	1.65	0.022
		Proteobacteria	Rhodomicrobium	-9.02	1.99	0.004
	compost vs. rock+compost	Bacteroidota	37-13_Unclassified	-6.56	1.52	0.010
	biochar vs. rock+biochar	Actinobacteriota	Angustibacter	**7.48**	1.63	0.003
		Bacteroidota	Fluviicola	-7.50	1.69	0.003
End of Year 3	control vs. rock	Firmicutes	Clostridium_sensu_stricto_10	**43.56**	5.89	7.25E-11
		Myxococcota	Anaeromyxobacter	**37.04**	5.90	8.41E-08
		Actinobacteriota	Intrasporangium	**26.66**	5.89	0.001
		Actinobacteriota	Microbacteriaceae_Unclassified	-22.76	5.89	0.014
	compost vs. rock+compost	Proteobacteria	Rhizobiaceae_Unclassified	**8.44**	1.61	0.000
		Firmicutes	Clostridium_sensu_stricto_10	-43.38	5.89	9.1E-11
	compost+biochar vs. rock+compost+biochar	Proteobacteria	Caulobacter	**35.11**	5.89	0.000
		Myxococcota	Anaeromyxobacter	-36.72	5.90	0.000

Table S5. Differential abundance results for fungal genera identified by DESeq2 for end of Year 2 (October 2021). The table reports phylum, genus, log2 fold change, standard error (LFC SE), and Benjamini–Hochberg adjusted *P*-values. Only taxa meeting the adjusted significance threshold ($P < 0.01$) are included.

Treatment Comparison (No rock - With rock)	Phylum	Genus	log2Fold Change	LFC SE	Adjusted P-value
control vs. rock	Ascomycota	Humicola	**42.80**	5.77	6.66E-12
	Ascomycota	Pseudeurotium	**31.22**	5.89	2.94E-06
	Ascomycota	Chaetomium	**25.45**	4.57	1.01E-06
	Ascomycota	Arachnomycetaceae_gen_Incertae_sedis	**23.53**	4.45	2.94E-06
	Ascomycota	Microascaceae_Unclassified	**22.84**	5.57	5.88E-04
	Ascomycota	Peziza	-21.47	5.57	0.001
	Ascomycota	Sordariales Unclassified	-21.66	5.13	3.97E-04
	Ascomycota	Arachnomyces	-22.32	5.25	3.97E-04
	Basidiomycota	Agaricomycetes Unclassified	-48.52	5.37	1.82E-17
compost vs. rock+compost	Ascomycota	Pseudeurotium	**30.83**	5.89	8.31E-06
	Ascomycota	Sporidesmiella	**23.17**	5.60	7.95E-04
	Ascomycota	Arachnomyces	**22.35**	5.25	5.90E-04
	Ascomycota	Microascaceae_Unclassified	-22.26	5.57	1.05E-03
	Ascomycota	Peziza	-22.63	5.57	9.33E-04
	Ascomycota	Arachnomycetaceae_gen_Incertae_sedis	-23.10	4.46	8.31E-06
	Basidiomycota	Lyomyces	-32.88	5.90	2.80E-06
biochar vs. rock+biochar	Ascomycota	Chaetomium	**21.93**	4.57	9.42E-05
	Ascomycota	Trichosporiella	-19.48	4.67	0.001
	Basidiomycota	Conocybe	-19.60	3.89	5.49E-05
	Ascomycota	Pseudeurotium	-22.10	5.89	0.005
compost+biochar vs. rock+compost+biochar	Ascomycota	Trichosporiella	**49.02**	4.67	5.46E-24
	Basidiomycota	Conocybe	**47.01**	3.89	1.61E-31
	Ascomycota	Pseudeurotium	**31.84**	5.89	1.49E-06
	Ascomycota	Arachnomyces	**23.04**	5.25	1.18E-04
	Ascomycota	Sordariales Unclassified	**20.94**	5.13	4.30E-04
	Ascomycota	Arachnomycetaceae_gen_Incertae_sedis	-21.23	4.46	2.19E-05
	Ascomycota	Cladorrhinum	-22.78	4.54	7.41E-06
	Basidiomycota	Psathyrella	-23.12	4.42	3.28E-06
	Ascomycota	Thelebolaceae_gen_Incertae_sedis	-23.14	4.70	1.07E-05
	Ascomycota	Hormiactis	-30.62	5.89	3.29E-06
	Ascomycota	Humicola	-41.72	5.77	1.34E-11
	Ascomycota	Lecythophora	-49.65	5.39	1.17E-18

Table S6. Differential abundance results for fungal genera identified by DESeq2 for mid-Year 3 (March 2022). The table reports phylum, genus, log2 fold change, standard error (LFC SE), and Benjamini–Hochberg adjusted *P*-values. Only taxa meeting the adjusted significance threshold ($P < 0.01$) are included.

Treatment Comparison (No rock - With rock)	Phylum	Genus	log2Fold Change	LFC SE	Adjusted P-value
control vs. rock	Basidiomycota	Oliveonia	**23.25**	5.43	3.17E-04
	Ascomycota	Pseudogymnoascus	**22.61**	3.31	5.41E-10
	Ascomycota	Lasiosphaeriaceae Unclassified	**21.23**	5.47	0.001
	Basidiomycota	Tubaria	**20.98**	5.39	0.001
	Ascomycota	Aspergillus	**20.32**	4.27	3.96E-05
	Ascomycota	Didymellaceae Unclassified	-22.68	5.53	6.23E-04
	Ascomycota	Podospora	-25.66	4.54	4.88E-07
	Ascomycota	Marquandomyces	-29.18	5.89	1.76E-05
	Basidiomycota	Entoloma	-33.78	5.33	9.43E-09
	Basidiomycota	Cyathus	-40.24	5.56	5.52E-11
compost vs. rock+compost	Mortierellomycota	Mortierellaceae Unclassified	**26.15**	5.91	1.65E-04
	Basidiomycota	Waitea	**23.33**	5.27	1.65E-04
	Ascomycota	Edenia	-22.68	4.25	2.87E-06
	Ascomycota	Pezizales Unclassified	-23.50	3.45	6.21E-10
	Ascomycota	Lasiosphaeriaceae Unclassified	-23.54	5.47	2.56E-04
	Ascomycota	Marquandomyces	-28.23	5.89	4.02E-05
	Basidiomycota	Entoloma	-31.32	5.33	1.74E-07
	Basidiomycota	Conocybe	-37.85	4.15	8.39E-18
biochar vs. rock+biochar	Basidiomycota	Agrocybe	**25.48**	5.47	9.54E-05
	Basidiomycota	Sistotrema	**25.06**	4.33	8.34E-07
	Ascomycota	Helicoma	**23.16**	4.90	9.09E-05
	Ascomycota	Didymellaceae Unclassified	**21.97**	5.53	0.001
	Basidiomycota	Tubaria	**21.74**	5.39	0.001
	Ascomycota	Coniochaeta	**21.68**	4.96	2.94E-04
	Chytridiomycota	Chytridiomycota Unclassified	-20.56	5.89	0.007
	Basidiomycota	Conocybe	-21.51	4.14	1.27E-05
compost+biochar vs. rock+compost+biochar	Ascomycota	Marquandomyces	**27.74**	5.89	6.06E-05
	Ascomycota	Didymellaceae Unclassified	**20.13**	5.53	0.004
	Ascomycota	Helicoma	-24.36	4.90	1.98E-05
	Mortierellomycota	Mortierellaceae Unclassified	-24.67	5.94	5.65E-04
	Basidiomycota	Psathyrella	-24.95	3.41	3.29E-11
	Basidiomycota	Oliveonia	-25.17	5.43	7.13E-05
	Ascomycota	Hormiactis	-29.71	5.57	3.89E-06
	Chytridiomycota	Chytridiomycota Unclassified	-35.70	5.89	8.20E-08

Table S7. Differential abundance results for fungal genera identified by DESeq2 for end of Year 3 (October 2022). The table reports phylum, genus, log2 fold change, standard error (LFC SE), and Benjamini–Hochberg adjusted *P*-values. Only taxa meeting the adjusted significance threshold ($P < 0.01$) are included.

Treatment Comparison (No rock - With rock)	Phylum	Genus	log2Fold Change	LFC SE	Adjusted P-value
control vs. rock	Basidiomycota	Entoloma	**30.13**	5.89	1.70E-05
	Ascomycota	Chaetomium	**24.60**	5.59	4.32E-04
	Ascomycota	Lophiotrema	**21.48**	5.35	0.002
	Ascomycota	Thelonectria	**20.73**	5.52	0.004
	Ascomycota	Plectosphaerella	-23.72	5.89	0.002
	Ascomycota	Podospora	-31.84	5.89	5.20E-06
	Ascomycota	Scytalidium	-34.52	5.89	7.42E-07
compost vs. rock+compost	Ascomycota	Naviculispora	**40.58**	4.71	1.20E-15
	Ascomycota	Schizothecium	**33.10**	4.03	1.59E-14
	Ascomycota	Scytalidium	**29.29**	5.89	1.54E-05
	Ascomycota	Plectosphaerella	**25.56**	5.89	2.56E-04
	Basidiomycota	Psathyrella	**23.02**	3.85	9.57E-08
	Ascomycota	Pezizales Unclassified	**21.67**	5.41	9.10E-04
	Ascomycota	Thelonectria	-23.33	5.51	3.78E-04
	Ascomycota	Onygenales Unclassified	-28.04	5.89	3.91E-05
	Ascomycota	Ophioceras	-29.33	5.89	1.54E-05
	Basidiomycota	Entoloma	-30.52	5.89	7.14E-06
	Ascomycota	Didymellaceae Unclassified	-31.13	4.24	1.18E-11
biochar vs. rock+biochar	Ascomycota	Chordomyces	**24.13**	5.89	0.002
	Ascomycota	Didymellaceae Unclassified	-18.88	4.24	6.94E-04
	Ascomycota	Naviculispora	-19.87	4.71	0.001
	Ascomycota	Schizothecium	-20.33	4.03	7.18E-05
compost+biochar vs. rock+compost+biochar	Ascomycota	Naviculispora	**41.16**	4.71	4.04E-16
	Ascomycota	Pseudeurotium	**24.01**	4.18	4.94E-07
	Basidiomycota	Filobasidium	**21.68**	4.15	7.26E-06
	Ascomycota	Plectosphaerella	**20.55**	5.89	0.010
	Ascomycota	Sporormiella	-23.30	3.75	4.08E-08
	Ascomycota	Chordomyces	-23.68	5.89	0.001
	Ascomycota	Ophioceras	-27.41	5.89	8.91E-05
	Ascomycota	Hormiactis	-29.72	5.89	1.46E-05

Table S8. Mean and standard error (1SE) for soil pH, electrical conductivity (EC), and exchangeable magnesium (Mg^{2+}) and calcium (Ca^{2+}) across the eight treatments, reported for baseline (October 2019) and the three post-amendment sampling times used for the microbial community analyses: end of Year 2 (October 2021), mid-Year 3 (March 2022), and end of Year 3 (October 2022) (N=3 per treatment). For each sampling time, Tukey's Honestly Significant Difference (HSD) pairwise comparisons were used as the post-hoc test. Treatments sharing the same letter do not differ significantly, while different letters indicate significant differences. F-values with numerator and denominator degrees of freedom, along with P-values are reported for the overall model at each timepoint. Data are reproduced from Sohng et al. (2025), Table S5, distributed under a CC BY 4.0 license.

Measurements	Time	Descriptive statistics (Mean ± SE, letter display for HSD summary)								F-value	P-value
		Control	Rock	Compost	Rock + Compost	Biochar	Rock + Biochar	Compost + Biochar	Rock + Compost-Biochar		
pH	2019-10-15	6.85 ± 0.08	6.81 ± 0.05	6.8 ± 0.03	6.86 ± 0.08	6.85 ± 0.01	6.81 ± 0.13	6.84 ± 0.03	6.79 ± 0.02	0.2001 (7, 14)	0.9801
	2021-10-15	6.9 ± 0.06 ab	7.13 ± 0.09 cd	7.03 ± 0.03 bd	7.1 ± 0.06 acd	6.87 ± 0.03 b	7.03 ± 0.09 bd	6.93 ± 0.03 bc	7.2 ± 0 d	4.8276 (7, 14)	0.006
	2022-03-15	7.27 ± 0.09 a	7.67 ± 0.09 b	7.53 ± 0.03 bc	7.6 ± 0.06 bc	7.37 ± 0.09 ac	7.57 ± 0.09 bc	7.4 ± 0.06 ac	7.7 ± 0.06 b	6.76 (7, 14)	0.0013
	2022-10-15	7.4 ± 0.12 a	7.67 ± 0.09 bc	7.67 ± 0.03 bc	7.63 ± 0.15 ac	7.63 ± 0.09 ac	7.67 ± 0.12 bc	7.53 ± 0.07 ab	7.8 ± 0.06 c	3.8644 (7, 14)	0.0151
EC ($\mu S\ cm^{-1}$)	2021-10-15	626 ± 66.73	540.33 ± 115.48	441 ± 64.65	687.33 ± 96.91	711.33 ± 40.55	577.67 ± 58.95	560.67 ± 49.13	517 ± 85.68	1.6769 (7, 14)	0.1943
	2022-03-15	124.67 ± 12.44 ab	102 ± 7.81 a	128.33 ± 8.45 ab	129 ± 6.81 ab	154.67 ± 17.94 b	114.67 ± 4.7 ab	118 ± 11.53 ab	142 ± 16.37 ab	1.9699 (7, 16)	0.1238
	2022-10-15	243.33 ± 32.94	326.67 ± 39.22	240.33 ± 32.63	259.33 ± 44.08	241.33 ± 19.89	266 ± 58.05	270 ± 16.52	246.67 ± 54.46	0.7812 (7, 14)	0.6136
Mg (mg kg soil-1)	2021-10-15	914.87 ± 38.28 a	826.18 ± 13.34 ab	864.2 ± 25.24 ab	833.07 ± 23.87 ab	898 ± 20.15 ab	847.5 ± 32.28 ab	864.9 ± 5.62 ab	813.57 ± 44.81 b	2.5207 (7, 14)	0.0668
	2022-03-15	774.53 ± 38.29	711.03 ± 29.03	765.83 ± 24.1	742.63 ± 38.65	799.97 ± 16.6	737.87 ± 36.44	795.07 ± 14.38	750.3 ± 38.68	0.9497 (7, 14)	0.5012
	2022-10-15	825.57 ± 47.87	727.63 ± 23.76	812.4 ± 26.36	769.8 ± 56.1	837.67 ± 9.77	733.83 ± 34.34	822.43 ± 28.96	769.77 ± 24.88	1.6417 (7, 14)	0.2034
Ca (mg kg soil-1)	2021-10-15	1173.47 ± 50.29	1153.01 ± 74.74	1140.07 ± 78.4	1170.63 ± 38.61	1238.23 ± 51.02	1278.67 ± 17.52	1141.67 ± 40.01	1144.67 ± 97.48	1.0446 (7, 14)	0.4448
	2022-03-15	1022.5 ± 67.52	1040.43 ± 22.23	1065.57 ± 48.74	1113.03 ± 27.19	1094.27 ± 31.03	1073.63 ± 61.34	1124.57 ± 62.34	1153.77 ± 38.69	0.8478 (7, 16)	0.5652
	2022-10-15	1184.63 ± 39.52	1195.53 ± 31.43	1291.27 ± 91.65	1261.27 ± 21.28	1290.3 ± 76.37	1185.6 ± 48.9	1360.97 ± 132.21	1254.93 ± 14.1	0.9609 (7, 14)	0.4942

Table S9. Mean and standard error (1SE) for microbial biomass carbon (MBC), water-extractable organic carbon (WEOC), and C and N concentration in particulate organic matter (POM) and mineral-associated organic matter (MAOM) across the eight treatments, reported for baseline (October 2018(and the three post-amendment sampling times used for the microbial community analyses: end of Year 2 (October 2021), mid-Year 3 (March 2022, and end of Year 3 (October 2022) (N=3 per treatment). For each sampling time, Tukey's Honestly Significant Difference (HSD) pairwise comparisons were used as the post-hoc test. Treatments sharing the same letter do not differ significantly, while different letters indicate significant differences. F-values with numerator and denominator degrees of freedom, along with P-values are reported for the overall model at each timepoint. Data are reproduced from Sohng et al., (2025), Table S7, distributed under a CC BY 4.0 license.

Measurements	Time	Descriptive statistics (Mean ± SE, letter display for HSD summary)								F-value	P-value
		Control	Rock	Compost	Rock + Compost	Biochar	Rock + Biochar	Compost + Biochar	Rock + Compost-Biochar		
MBC (µg g soil-1)	2021-10-15	211.93 ± 11.6 ab	186.1 ± 7.98 ab	269.8 ± 27.97 a	209.07 ± 13.03 ab	170.63 ± 46.69 b	244.47 ± 19.18 ab	203.03 ± 12.1 ab	245.77 ± 14.5 ab	2.1861 (7, 16)	0.0925
	2022-03-15	246.47 ± 23.77	156.17 ± 51.52	278.13 ± 23.73	277.73 ± 4.96	280.47 ± 24.44	276.13 ± 13.35	249.97 ± 87.53	291.4 ± 5.97	1.2381 (7, 16)	0.3392
	2022-10-15	263.83 ± 28.07	256.77 ± 40.18	310.17 ± 33.3	243.93 ± 16.85	253.77 ± 2.33	285.3 ± 45.25	293.57 ± 34.77	299.17 ± 63.35	0.5918 (7, 14)	0.7526
WEOC (µg g soil-1)	2021-10-15	276.13 ± 20.8 ab	286.93 ± 14.06 ab	296.43 ± 28.94 b	287.23 ± 24.27 ab	245.37 ± 12.39 ab	224.3 ± 10.98 a	302.13 ± 26.31 b	290.33 ± 13.27 ab	2.7321 (7, 14)	0.0519
	2022-03-15	231.23 ± 4.1 a	207.9 ± 0.25 a	277.97 ± 11.38 bc	297.6 ± 10.69 b	218.6 ± 4.27 a	243.97 ± 22.83 ac	243.07 ± 17.3 ac	306.1 ± 4.71 b	12.8441 (7, 14)	< 0.0001
	2022-10-15	204.57 ± 1.47 a	215.13 ± 8.44 a	286.97 ± 27.41 b	223.53 ± 8.75 a	212.23 ± 3.59 a	217.53 ± 11.58 a	258.53 ± 20.41 ab	222.23 ± 22.89 a	4.8329 (7, 14)	0.006
POM-C (mg C g soil-1)	2019-10-15	2.16 ± 0.35	1.86 ± 0.14	1.87 ± 0.09	1.81 ± 0.15	2.03 ± 0.26	1.95 ± 0.15	1.9 ± 0.07	1.8 ± 0.12	0.4166 (7, 16)	0.878
	2021-10-15	2.2 ± 0.15 a	2.22 ± 0.1 a	3.19 ± 0.33 ab	2.63 ± 0.18 ac	4.21 ± 0.44 bd	3.6 ± 0.05 bcd	4.5 ± 0.24 d	4.58 ± 0.47 d	13.498 (7, 14)	< 0.0001
	2022-03-15	2.33 ± 0.31 ab	1.74 ± 0.23 a	3.15 ± 0.55 bc	2.66 ± 0.13 ab	4.09 ± 0.33 cd	4.11 ± 0.24 cd	4.02 ± 0.32 cd	4.63 ± 0.22 d	10.6947 (7, 16)	0.0001
	2022-10-15	1.9 ± 0.12 a	2.61 ± 0.15 ab	3.23 ± 0.41 ac	2.51 ± 0.21 ad	4.3 ± 0.35 bc	3.93 ± 0.66 bcd	4.92 ± 0.06 c	4.75 ± 0.86 c	7.9505 (7, 14)	0.0006
POM-N (mg N g soil-1)	2019-10-15	0.15 ± 0.02	0.14 ± 0.01	0.14 ± 0.001	0.15 ± 0.01	0.16 ± 0.02	0.16 ± 0.01	0.13 ± 0.001	0.15 ± 0.01	0.8568 (7, 14)	0.5614
	2021-10-15	0.18 ± 0.01 a	0.18 ± 0.001 a	0.23 ± 0.01 b	0.21 ± 0.01 ab	0.21 ± 0.01 bc	0.19 ± 0.001 ac	0.22 ± 0.01 b	0.22 ± 0.01 b	7.66 (7, 14)	0.0007
	2022-03-15	0.19 ± 0.01 ab	0.15 ± 0.02 a	0.22 ± 0.02 b	0.19 ± 0.01 ab	0.2 ± 0.01 b	0.19 ± 0.01 ab	0.21 ± 0.02 b	0.21 ± 0.001 b	2.9854 (7, 16)	0.0332
	2022-10-15	0.13 ± 0.03	0.16 ± 0.04	0.2 ± 0.05	0.15 ± 0.02	0.18 ± 0.04	0.15 ± 0.05	0.17 ± 0.04	0.19 ± 0.06	1.3578 (7, 14)	0.2959
MAOM-C (mg C g soil-1)	2019-10-15	7.32 ± 0.36	6.11 ± 0.34	6.35 ± 0.29	6.67 ± 0.24	6.82 ± 0.36	6.88 ± 0.55	6.36 ± 0.53	6.88 ± 0.23	1.0334 (7, 16)	0.4464
	2021-10-15	7.93 ± 0.16 ab	6.84 ± 0.18 a	7.7 ± 0.35 ac	7.54 ± 0.11 ad	9.33 ± 0.62 b	8.81 ± 0.11 bcd	9.1 ± 0.6 bcd	9.22 ± 0.48 bc	6.1662 (7, 14)	0.002
	2022-03-15	8.18 ± 0.29 ab	6.77 ± 0.34 a	8.17 ± 0.15 ab	7.76 ± 0.33 ac	9.39 ± 0.33 bc	9.31 ± 0.7 bc	9.42 ± 0.65 bc	9.82 ± 0.31 b	6.1093 (7, 16)	0.0013

	2022-10-15	8.12 ± 0.22 ab	7.29 ± 0.21 b	8.42 ± 0.14 bc	7.92 ± 0.18 ab	9.55 ± 0.37 ac	9.12 ± 0.72 bc	10.06 ± 0.61 c	9.48 ± 0.59 ac	4.781 (7, 16)	0.0046
MAOM-N (mg N g soil-1)	2019-10-15	0.82 ± 0.06	0.69 ± 0.03	0.73 ± 0.02	0.73 ± 0.03	0.77 ± 0.04	0.77 ± 0.06	0.72 ± 0.04	0.79 ± 0.04	0.9575 (7, 16)	0.4925
	2021-10-15	0.99 ± 0.04	0.86 ± 0.03	0.93 ± 0.03	0.93 ± 0.02	0.96 ± 0.04	0.92 ± 0.03	0.95 ± 0.05	0.94 ± 0.05	1.0165 (7, 14)	0.4609
	2022-03-15	0.95 ± 0.04	0.77 ± 0.04	0.93 ± 0.02	0.88 ± 0.04	0.93 ± 0.03	0.89 ± 0.08	0.94 ± 0.07	0.96 ± 0.04	1.6037 (7, 16)	0.2049
	2022-10-15	0.87 ± 0.02	0.78 ± 0.02	0.9 ± 0.01	0.85 ± 0.02	0.87 ± 0.03	0.82 ± 0.06	0.92 ± 0.05	0.86 ± 0.04	1.538 (7, 16)	0.2244