

1 **Title:**

2 Unraveling the effects of spatial variability and relic DNA on the temporal dynamics of
3 soil microbial communities

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27
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29
30 **Abstract:**

31 Few studies have comprehensively investigated the temporal variability in soil microbial
32 communities despite widespread recognition that the belowground environment is
33 dynamic. In part, this stems from the challenges associated with the high degree of
34 spatial heterogeneity in soil microbial communities¹ and because the presence of relic
35 DNA² may mask temporal dynamics. Here we disentangle the relationships among
36 spatial, temporal, and relic DNA effects on microbial communities in soils collected from
37 contrasting hillslopes in Colorado, USA. These sites were chosen because they have
38 distinct soil microbial communities and experience strong seasonal changes in
39 precipitation and temperature regimes. We intensively sampled plots on each hillslope
40 over one year to discriminate between temporal variability, the intra-plot spatial
41 heterogeneity, and relic DNA effects on the soil prokaryotic and fungal communities. We
42 show that the intra-plot spatial variability in microbial community composition was strong
43 and independent of relic DNA effects and these spatial patterns persisted throughout
44 the study. When controlling for intra-plot spatial variability, we identified significant
45 temporal variability in both plots, particularly after relic DNA was removed, suggesting
46 that relic DNA hinders the detection of important temporal dynamics in soil microbial

47 communities. We also identified microbial taxa that exhibited shared temporal
48 responses and we show that these responses were often predictable from temporal
49 changes in soil conditions. These findings highlight approaches that can be used to
50 better characterize temporal shifts in soil microbial communities, information that is
51 critical for predicting the environmental preferences of individual soil microbial taxa and
52 identifying linkages between soil microbial community composition and belowground
53 dynamics.

54

55 **Introduction:**

56 Information on the temporal dynamics of microbial communities over different
57 time scales can be used to better understand the factors influencing the structure of
58 microbial communities and their contributions to ecosystem processes. We know that
59 the microbial communities found in the human gut³, leaf litter⁴, marine⁵, and freshwater⁶
60 habitats can exhibit a high degree of temporal variation. Although the magnitude and
61 timing of this temporal variation in community composition can vary depending on the
62 environment and taxon in question, such temporal variability is often predictable from
63 environmental factors⁷. For example, ocean microbial communities display predictable
64 periodic oscillations over time (seasonality) that has been linked to regular changes in
65 biotic and abiotic factors, including phytoplankton dynamics and physicochemical
66 factors (reviewed in refs^{5,8}). These changes in environmental conditions influence the
67 nature of biotic interactions within these ecosystems and can have important
68 ramifications for understanding the functional attributes of microbial communities and
69 the ecosystem services they provide⁹⁻¹¹.

70 Understanding how temporal changes in environmental conditions influence soil
71 microbial communities is necessary to accurately model how microbial communities
72 contribute to soil processes and for using microbes as bio-indicators of changes in
73 belowground conditions such as carbon and nutrient availability – parameters that are
74 often difficult to measure directly. However, results from previous studies of temporal
75 variability in soil microbial communities are idiosyncratic. While some studies show soil
76 microbial communities exhibit measurable temporal variation in response to
77 experimental warming^{12,13} and seasonal patterns in temperature and moisture¹⁴⁻¹⁸, other
78 studies show no or minimal variation over time, despite marked changes in
79 environmental conditions^{7,19,20}. One possible explanation for the discrepancies across
80 studies is that the spatial heterogeneity in soil microbial communities – even across
81 short distances – can be sufficiently large to obscure temporal patterns. This hypothesis
82 is supported by numerous studies demonstrating that the spatial variability in soil
83 microbial communities (even across locations only a few meters apart) can be large (for
84 example, ref. ¹). Another explanation is that relic DNA – legacy DNA from dead
85 microbes that can persist in soil – may dampen the observed temporal variability by
86 effectively hiding the true temporal dynamics of soil microbial communities. Relic DNA is
87 abundant in soil^{2,21}, and models suggest that during microbial community turnover relic
88 DNA can mask changes in community structure²¹.

89 We conducted a yearlong study aimed at disentangling the spatial and relic DNA
90 effects on temporal dynamics in belowground microbial communities. Our study sites
91 were soils on opposing hillslope aspects of a montane ecosystem within the Colorado
92 Front Range of the Rocky Mountains. We intensively sampled two 9 m x 9 m plots,

93 divided into 3 m × 3 m sub-plots, located in the Gordon Gulch subcatchment within the
94 Boulder Creek Critical Zone Observatory (BcCZO) every 40-55 days from November
95 2015 to November 2016 (Fig. 1; nine time points total). We chose these locations
96 because the soil microbial communities on the two hillslopes are compositionally
97 distinct², relic DNA is abundant (40-60% of the total soil DNA pool, ref. ²), and the two
98 sites undergo strong seasonal changes in moisture and temperature²². Moreover, the
99 temperature and moisture regimes are distinct across the two slopes²², providing us
100 with naturally contrasting systems in which to investigate temporal dynamics in
101 belowground microbial communities. We characterized the microbial communities at
102 each site using 16S rRNA gene and internal transcribed spacer 1 (ITS) marker
103 sequencing to profile the prokaryotic and fungal communities, respectively. Here, we
104 unravel the relationships between spatial and temporal variability in microbial
105 community composition, and show the effects of relic DNA on these apparent sources of
106 variability. Further, we use this information on temporal dynamics to identify groups of
107 microbes that share temporal patterns and similar responses to changes in
108 environmental conditions, information that provides novel insight into the ecologies of
109 understudied soil microbial taxa.

110

111 **Results & Discussion:**

112 **Spatial variation in soil microbial communities is unaffected by relic DNA and**
113 **stronger than temporal variation.** Consistent with previous studies conducted at these
114 sites², and other studies describing the spatial variability of soil microbial communities¹,
115 the prokaryotic and fungal communities on the south-facing hillslope (SFS) were distinct
116 from those on the north-facing hillslope (NFS), regardless of the time point sampled or
117 whether relic DNA was removed (Supplementary Fig. 1). Most notably, the SFS had
118 higher relative abundances of the archaeal phylum Crenarchaeota (all of which were
119 classified as probable ammonia-oxidizing '*Candidatus Nitrososphaera*'), and the
120 bacterial phyla Nitrospirae and Verrucomicrobia (Supplementary Fig. 2). Beyond these
121 expected slope-scale differences, we observed significant intra-plot spatial
122 heterogeneity in microbial community composition that persisted throughout the course
123 of the experiment, and this intra-plot heterogeneity was evident irrespective of whether
124 relic DNA was removed. Before removing relic DNA, there was significant spatial
125 variability across the sub-plots in both prokaryotic and fungal communities on the NFS
126 (Fig. 2 a,e; PERMANOVA $R^2=0.192$ and $R^2=0.328$; $P\leq 0.001$, respectively). Significant
127 spatial differences were still apparent on the NFS for both prokaryotes and fungi after
128 relic DNA was removed (Fig. 2 c,g; PERMANOVA $R^2=0.180$ and $R^2=0.287$; $P\leq 0.001$,
129 respectively). We also found significant spatial variability on the SFS in samples that
130 were not treated to remove relic DNA, but this spatial effect was much more
131 pronounced than the NFS, with a clear partitioning between sub-plots 5, 6, 8 and 9 (see
132 'Plot Design' in Fig. 1a for numbering) from the remainder of the sub-plots (Fig. 2 b,f;
133 PERMANOVA $R^2=0.511$, $P\leq 0.001$ for prokaryotes and $R^2=0.331$, $P\leq 0.001$ for fungi).
134 Similar to the NFS, these strong spatial patterns remained after relic DNA was removed
135 (Fig. 2 d,h; PERMANOVA $R^2=0.498$ for prokaryotes and $R^2=0.290$ for fungi; $P\leq 0.001$).
136 These data show that the spatial variability in soil microbial community composition on
137 the meter scale persists over time and that the presence of relic DNA does not affect
138 our ability to detect this persistent spatial variation.

139

140 **Removing relic DNA enhanced our ability to detect temporal changes in soil**
141 **microbial communities.** We investigated the temporal variability in belowground
142 microbial communities, and the effect of relic DNA on this temporal variability, on a sub-
143 plot basis to control for the aforementioned high degree of intra-plot spatial variability
144 and discriminate between temporal and spatial sources of variation in microbial
145 community structure. When limiting PERMANOVA permutations to within sub-plots over
146 time, we found significant temporal variability for both prokaryotes and fungi in both
147 untreated control soils (PERMANOVA $R^2=0.187$ $P\leq 0.001$ for prokaryotes and $R^2=0.147$
148 $P\leq 0.001$ for fungi on the NFS; and $R^2=0.123$ $P\leq 0.001$ for prokaryotes and $R^2=0.123$
149 $P\leq 0.001$ for fungi on the SFS) and soils that were treated to remove relic DNA
150 (PERMANOVA $R^2=0.177$ $P\leq 0.001$ for prokaryotes and $R^2=0.141$ $P\leq 0.001$ for fungi on
151 the NFS; and $R^2=0.108$ $P\leq 0.001$ for prokaryotes and $R^2=0.157$ $P\leq 0.001$ for fungi on
152 the SFS). However, on average, the fungal communities on both slopes, and prokaryotic
153 communities on the NFS were significantly more dissimilar over time after relic DNA
154 was removed, compared to untreated control soils that contained relic DNA (Fig. 3;
155 Kruskal-Wallis test $P\leq 0.05$). These results indicate that, while temporal signals in soil
156 microbial communities can be identified in the presence of relic DNA, the removal of
157 'legacy' DNA from dead microbes that can persist in soil significantly enhances the
158 ability to detect important temporal variation in the composition of soil microbial
159 communities.

160

161 **Temporal variability in distinct assemblages of prokaryotes and fungi are**
162 **predictable from soil variables.** Characterizing shifts in the relative abundances of
163 individual microbial taxa in temporally dynamic soil systems can give important insight
164 into the ecologies of individual taxa and, more generally, the environmental factors that
165 influence belowground communities. Thus, we next sought to identify specific groups of
166 taxa that exhibited correlated changes in relative abundances over time in soils after
167 relic DNA was removed. To do this, we used local similarity analysis (LSA)²³ to identify
168 strong (local similarity score ≥ 0.7) and significant (q-value ≤ 0.001) positive pairwise
169 microbe-microbe temporal correlations. We constructed and analyzed networks from
170 these correlations and extracted distinct groups (modules) of microbes from NFS and
171 SFS networks using modularity analysis²⁴ (Fig. 4). On the NFS, the mean normalized
172 relative abundances of 292 microbial taxa (184 bacteria and 108 fungi) were
173 significantly correlated with at least one other taxon over time (Fig. 4a). These
174 correlated taxa clustered into seven modules – the mean normalized relative
175 abundances of four of these modules changed significantly with time and displayed
176 distinct temporal trajectories (Fig. 4b). On the SFS, 291 taxa (1 archaeon, 191 bacteria
177 and 99 fungi) were included in the network, and clustered into six modules (Fig. 4c).
178 The relative abundances of three of these six SFS modules changed significantly with
179 time (Fig. 4d).

180 A large proportion of the temporal variation in the mean normalized relative
181 abundances of the modules that were found to change significantly over time could be
182 explained by temporal variation in measured soil or environmental characteristics. At
183 each time point, we measured a suite of soil and environmental parameters, including:
184 snow depth, soil temperature and moisture, extractable inorganic nitrogen ($\text{NO}_3^- +$

185 NH_4^+), salinity (electrical conductivity), extractable phosphorus (P), pH, and the
186 chromophoric properties of water-soluble organic matter (WSOM; a metric of organic
187 matter lability²⁵). These measured soil characteristics explained 12 to 76% of the
188 variance in the mean normalized relative abundance of a given module (Supplementary
189 Fig. 3). We identified two sets of modules that differed in the specific factors that
190 predicted temporal variation. The first set of modules, containing modules 0, 3, 7 and
191 12, were best predicted by climactic variables, most notably soil temperature and
192 moisture and snow depth (Supplementary Fig. 3). These results are in line with previous
193 studies demonstrating how changes in soil temperature^{12,16-18}, moisture²⁶ and snow
194 pack¹⁴ can influence belowground microbial communities. In contrast, modules 1, 2 and
195 11 were best explained by changes in inorganic nutrient concentrations (nitrogen and
196 phosphorus; Supplementary Fig. 3). While nitrogen and phosphorus inputs can have
197 predictable²⁷ and lasting⁴ effects on microbial community structure, we have a more
198 limited understanding of how short-term seasonal variation in the availability of these
199 nutrients can influence microbial community dynamics, despite evidence that
200 belowground microbial communities are important mediators of soil nutrient
201 dynamics^{28,29}. Our results show that a subset of soil microbes organize into modules
202 that are responsive to these subtle changes in nitrogen and phosphorus availability.
203 Variability in WSOM constituents did not contribute significantly to temporal variability in
204 environmental conditions (Supplementary Fig. 4) and thus, we excluded these
205 measures from the models describing the temporal variability of the modules. Given that
206 previous work at these sites showed a high degree of spatial variation in WSOM
207 distributions^{25,30}, we suspect that the pronounced spatial variability in WSOM
208 distributions may have obscured our ability to detect significant effects of WSOM
209 characteristics on the temporal dynamics of the soil microbial communities.

210 The construction of modules based on shared temporal patterns allowed us to
211 identify biotic or abiotic factors that are correlated with shifts in the relative abundances
212 of individual taxa. For example, similar to studies showing that ammonia-oxidizing
213 archaea are particularly sensitive to changes in temperature³¹ and pH^{32,33}, we found that
214 both temperature and pH were good predictors of the temporal distribution of module
215 12, which contained ammonia-oxidizing thaumarchaea (Fig. 4d and Supplementary Fig.
216 3). Because nitrification is often a coupled process – the oxidation of ammonium to
217 nitrite by ammonia oxidizers, and the subsequent oxidation of nitrite to nitrate by nitrite
218 oxidizers – we were surprised that probable nitrite-oxidizing Nitrospirae were not
219 temporally correlated with these thaumarchaea, but were instead a part of a distinct
220 module (module 8; Fig. 4d) that did not change significantly over time. As observed in
221 some marine systems^{34,35}, we suspect that nitrification in SFS soils may be periodically
222 uncoupled, though more work is necessary to test this hypothesis.

223 Our study also provides insight into the short-term temporal variation of
224 ectomycorrhizal communities, the environmental factors that influence these patterns
225 and other fungal and prokaryotic taxa that co-vary with ectomycorrhizal fungi.
226 Ectomycorrhizal fungi were found on both slopes and partitioned into several modules
227 that were significantly variable over time (modules 0, 1, 2, 3, 7, 11, and 12 in Fig. 4;
228 Supplementary Table 1). Interestingly, some of these modules were best predicted by
229 climactic variables (Supplementary Fig. 3; for example, those ectomycorrhizal fungi
230 found in modules 3 and 7). Modules 3 and 7 had peak abundances in the summer

231 months (Fig. 4), suggesting that the abundances of these ectomycorrhizal taxa were
232 elevated during months when plant productivity peaks. However, other ectomycorrhizal
233 fungi were found in modules best predicted by nutrient availability. These findings
234 indicate a degree of temporal niche partitioning in ectomycorrhizal fungal communities
235 on both slopes in response to distinct environmental conditions (Supplementary Fig. 3)

236

237 **Conclusions:**

238 This study provides new evidence that the temporal dynamics of groups of
239 prokaryotes and fungi are predictable in terrestrial ecosystems, and that a more detailed
240 characterization of the temporal variability in soil microbial communities is critical to
241 understanding the dynamic nature of the soil microbiome. The extensive spatial and
242 temporal sampling design of our study allowed us to disentangle the relationships
243 among spatial heterogeneity in microbial communities, temporal dynamics of these
244 communities, and the effect of relic DNA on these temporal patterns. Unsurprisingly,
245 spatial variation in community structure at both the hillslope scale, and the meter scale
246 (intra-plot) was the dominant source of variability in this study and relic DNA had no
247 significant effect on these patterns (Supplementary Fig. 1 and Fig. 2).

248 When controlling for this spatial variability, we were able to detect significant
249 temporal shifts in microbial community composition, regardless of whether relic DNA
250 was removed or not. We emphasize that the magnitude of the temporal variation in soil
251 microbial communities was consistently lower than the spatial variation, even between
252 sub-plots located only a few meters apart. This spatial variability in surface soil microbial
253 communities was relatively stable over time, suggesting that efforts to describe spatial
254 variation in overall community composition do not necessarily need to include samples
255 collected across multiple time points.

256 We also provide new evidence that the removal of relic DNA enhances our ability to
257 detect temporal patterns in the belowground communities. These findings support our
258 previous hypothesis², and predictions based on modeling²¹, that relic DNA can conceal
259 temporal patterns in soil microbial communities. The presence of relic DNA, even in high
260 amounts, does not automatically lead to relic DNA biases in other ecosystems²¹.
261 However, our data do suggest that relic DNA has important effects on studies of
262 temporal variation in soil microbial communities (and possibly in other ecosystems), and
263 that the consequences of failing to remove relic DNA would not be apparent from single
264 time point samples.

265 The belowground environment is one of the most complex and dynamic microbial
266 habitats on Earth. By controlling for spatial and relic DNA effects on temporal variability
267 in these soil microbial communities, we identified groups of microbes that have similar
268 temporal dynamics and the factors that predicted their temporal distributions. A deeper
269 understanding of relationships between soil microbiota can help resolve both the roles
270 of individual taxa and potential 'ecological clusters' with emergent function. For
271 example, taxa that covary may exhibit similar niche preferences and compete for growth
272 substrates. In contrast, taxa belonging to a given module may broadly cue in on similar
273 environmental signals but occupy distinct substrate niches³⁶. Alternatively, microbes that
274 are correlated over time may interact through cross-feeding of metabolic substrates or
275 co-utilization of leaky functions³⁷ - either directly or in a time-lagged manner.
276 Understanding the basis for shared temporal dynamics is important as microbial

277 interactions are crucial in shaping microbial communities³⁸ but difficult to measure
278 directly³⁹. Future investigations that combine cell culture, synthetic microbial
279 communities and genomics may help resolve the specific drivers of these co-occurrence
280 patterns^{36,40}.

281

282 **Methods:**

283 *Site description, plot design and sampling procedure:* The two plots were set up
284 on opposing slopes alongside an instrumented transect near the rain-snow transition at
285 ~2,530 meters elevation (approximately 40.01°N, 105.47°W), chosen on the expectation
286 that there would be a high level of temporal variability in soil microbial communities as a
287 result of intra-annual changes in soil moisture and temperature²². The north-facing slope
288 (NFS) and south-facing slope (SFS) have distinct soil and vegetation characteristics and
289 experience different water delivery patterns, particularly during snowmelt²² (Fig. 1). The
290 NFS and SFS soils are Ustic dystrocryept (Catamount series) and Lithic haplstoll,
291 respectively⁴¹. Soil moisture and temperature were variable over the course of the study
292 and followed expected seasonal trends (Fig. 1). In general, the NFS had a higher soil
293 moisture and a lower temperature than the SFS (Fig. 1). The NFS is vegetated with
294 moderately dense *Pinus contorta* (Lodgepole pines) and develops a snowpack during
295 the winter that melts in spring. In contrast, the SFS is much more sparsely vegetated
296 with *Pinus ponderosa* (Ponderosa pines), intervening grasses and *Arctostaphylos uva-*
297 *ursi* (kinnikinnick) shrubs and experiences pulses of snowmelt throughout the winter and
298 spring. We sampled ~10-15 random soil cores (0-5 cm, mineral soils only; 1" core
299 diameter) within each sub-plot at each of the nine time points. The soil cores from each
300 sub-plot were pooled, sieved to 2 mm and homogenized at each time point and
301 partitioned for microbial community and nutrient analyses. Sample dates are reported in
302 Supplementary Table 2. Sampling for the July 2016 sample was delayed by ~7 days
303 because a nearby wildfire prevented site access.

304

305 *Continuous environmental measurements:* Several automated measurements
306 were collected every 10 minutes at a meteorological station located near the sample
307 sites (see 'Data availability' for data source information). Each slope was instrumented
308 with a soil temperature sensor (Campbell Scientific T-107 temperature probe), and a
309 soil water content reflectometer (Campbell Scientific CS616) located 5 cm below
310 ground. The daily averages from these sensors on each slope are illustrated in Fig.
311 1b,c. When modelling the relative mean importance of temperature and volumetric
312 water content to module temporal distributions, we used the average of daily mean
313 values from these sensors between sample dates, except for the first time point, which
314 is the mean from the preceding 34 days. Snow depth was measured using digital
315 ultrasonic snow depth sensors (Judd Communications Inc.) fitted with CR1000
316 dataloggers (Campbell Scientific). Snow depth is reported as mean daily snow depth
317 between sampling points from three sensors on each slope (NFS at snow pole 3,
318 sensors 1-3 and SFS snow pole 10, sensors 9, 11 and 15).

319

320 *Discrete environmental measurements:* Inorganic N pools were measured for
321 each sub-plot at each time point except for the January 2016 sample on the NFS, sub-
322 plots 1 and 2 and SFS sub-plot 3, where insufficient soil was collected. Sieved soils for

323 inorganic N analyses were stored at 4°C for <72 h. Inorganic N pools were extracted
324 from 10 g field-moist soil in 100 mL 2M potassium chloride with periodic shaking for 18
325 h and filtered through cellulose Whatman 1 filters. Ammonium (NH₄⁺) was measured
326 from these extracts on a BioTek Synergy 2 with a detection limit of 0.009 mg N L⁻¹ and
327 nitrate (NO₃⁻) was measured on an OI Analytical FS-IV with a detection limit of 0.5603
328 µg N L⁻¹. Dissolved inorganic nitrogen (DIN) was calculated as the sum of NH₄⁺ and
329 NO₃⁻.

330 Water-soluble organic matter (WSOM) was analyzed for each sub-plot at each time
331 point except for the following plots, where insufficient sample was collected: NFS
332 February 2016 (all sub-plots), July 2016 sub-plot 1, August 2016 sub-plots 1-7,
333 November 2016 sub-plots 1, 2 and 5; and SFS February 2016 sub-plots 1, 8 and 9 and
334 April 2016 sub-plot 5. Sieved soils were stored at -20°C until WSOM extraction. WSOM
335 was extracted by leaching 10 g of soil with 50 ml 0.5 M K₂SO₄ following the methods
336 described in²⁵. The spectroscopically-active portion of the WSOM was characterized
337 with UV-Vis and fluorescence spectroscopy. Samples were diluted to minimize the inner
338 filter effect⁴² and the UV-Vis absorbance was measured from 200-800 nm in 1 nm
339 increments using an Agilent 8453 Spectrophotometer with a 1 cm path
340 length. Dissolved organic carbon (DOC) and total nitrogen were measured on a
341 Shimadzu TOC-V. SUVA₂₅₄, a proxy for the aromaticity of the WSOM, was calculated
342 as the absorbance at 254 nm normalized by the DOC concentration⁴³. Fluorescence
343 scans were collected on a Horiba Jobin Yvon Fluoromax-4 with a 1 cm quartz cuvette
344 and normalized to Raman units⁴⁴. The fluorescence index (FI)⁴⁵ and humification index
345 (HIX)⁴⁶ were calculated from the fluorescence scans using Parallel Factor Analysis
346 (PARAFAC) to further resolve discrete components representing different classes of
347 fluorophores²⁵.

348 Other standard soil characteristics were measured at each time point by pooling
349 equal masses of soil from each sub-plot plot on each slope. These measurements
350 included: pH, electrical conductivity (mmhos cm⁻¹) and P (ppm). Standard soil chemical
351 analyses were performed at the Colorado State University Soil Water and Plant Testing
352 Laboratory using their standard protocols.

353 *Relic DNA removal and DNA extraction:* Relic DNA was removed as described
354 previously². Briefly, 0.03 g of each soil from each sub-plot pool was sub-sampled,
355 resuspended in 3.0 mL phosphate buffered saline (PBS) (1% weight/vol slurry) and
356 either treated with 40 µM propidium monoazide (PMA) in the dark, or left untreated as a
357 control. Both treated and untreated samples were vortexed in the dark for 4 minutes and
358 exposed to a 650-watt light for 4 × 30 s light:30 s dark cycles to activate PMA in treated
359 samples. Light-exposed samples were frozen at -20°C until DNA extraction. DNA was
360 extracted from 800 µL of PMA treated and untreated soil slurries using a PowerSoil-htp
361 96 well soil DNA Isolation kit (MoBio) following the manufacturer's instructions, except
362 770 µL was used in the C2 step. All samples and 27 'no soil' negative controls were
363 randomized into these 96 well DNA extraction plates and extracted simultaneously.

364 *Amplicon sequencing and analytical methods:* For sequence-based analyses of
365 16S rRNA and ITS marker regions, we used the approaches described previously².
366 Briefly, we amplified each sample in duplicate in 25 µl PCR reactions containing: 12.5 µl
367 of Promega GoTaq Hot Start Colorless Master Mix; 0.5 µl of each barcoded primer
368 (bacterial 16S: 515F 5'-GTGCCAGCMGCCGCGGTAA-3' & 806R 5'-

369 GGACTACHVGGGTWTCTAAT-3'; fungal ITS: 5'-CTTGGTCATTTAGAGGAAGTAA-3' &
370 ITS2 5'-GCTGCGTTCTTCATCGATGC-3'); 10.5 µl water; 1 µl of template DNA.
371 Program: 94°C for 5 min, followed by 35 cycles of (94°C 45 s; 50°C 60 s; 72°C 90 s)
372 and a final extension 72°C 10 min. Duplicate PCR reactions for each sample were
373 pooled, cleaned and normalized using the ThermoFisher Scientific SequalPrep
374 Normalization Plate kit. Cleaned and normalized amplicons were pooled, spiked with
375 15% phiX and sequenced on an Illumina MiSeq using v2 500-cycle paired end kits. The
376 samples were sequenced in four batches total – two for prokaryotes and two for fungi.
377 The first two sequencing runs (one each for prokaryotes and fungi) contained all
378 treatments and control samples up to and including the May 2016 samples. The last two
379 sequencing runs (one each for prokaryotes and fungi) two contained samples collected
380 on July 2016 and thereafter, plus control samples. We analyzed the 'no soil' controls to
381 determine whether there were potential sequencing batch effects across the runs for
382 prokaryotes or for fungi that could be detected in the community composition of these
383 controls. We found no significant difference in the 'no soil' controls for prokaryotes
384 (rarefied to 89 reads to include all controls; PERMANOVA $R^2=0.028$; $P=0.677$) or fungi
385 (not rarefied to include all controls; PERMANOVA $R^2=0.028$ $P=0.613$) that would be
386 indicative of batch effects. Reads were processed as described in (ref. ²⁷). Briefly, raw
387 amplicon sequences were demultiplexed according to the raw barcodes and processed
388 with the UPARSE pipeline⁴⁷. A database of $\geq 97\%$ similar sequence clusters was
389 constructed in USEARCH (Version 8)⁴⁸ by merging paired end reads, using a "maxee"
390 value of 0.5 when quality filtering sequences, dereplicating identical sequences,
391 removing singleton sequences, clustering sequences after singleton removal, and
392 filtering out cluster representative sequences that were not $\geq 75\%$ similar to any
393 sequence in Greengenes (for prokaryotes; Version 13_8)⁴⁹ or UNITE (for fungi)⁵⁰
394 databases. Demultiplexed sequences were mapped against the *de novo* constructed
395 databases to generate counts of sequences matching clusters (i.e. taxa) for each
396 sample. Taxonomy was assigned to each taxon using the RDP classifier with a
397 threshold of 0.5⁵¹ and trained on the Greengenes or UNITE databases. To normalize the
398 sequencing depth across samples, samples were rarefied to 10,159 and 5,000
399 sequences per sample for the 16S rRNA and ITS analyses, respectively. Functional
400 predictions for fungal taxa were obtained using FUNGuild⁵².

401 *Statistical analyses:* Calculations of community dissimilarity and all other analyses
402 were conducted on a reduced dataset because of the spatial and temporal
403 heterogeneity. That is, we wanted to understand the temporal variation of microbes that
404 are consistently present across the sub-plots and over time. When comparing slope
405 differences, we included only taxa that were present on at least one slope *i*) with a
406 mean read abundance of greater than 81 or 40 reads after rarefaction, for prokaryotes
407 and fungi, respectively across all samples (an average of one (prokaryotes) or 0.5
408 (fungi) reads per sub-plot, per time point); and were *ii*) present in more than 27 samples
409 (1/3 of all samples). Second, we investigated only those taxa that were, on average,
410 $\geq 0.1\%$ of the community across all samples. When investigating within-plot differences,
411 we focused on only the taxa within that plot that met the above parameters. We
412 emphasize that these filtering steps were deliberately stringent to enable robust
413 temporal analyses of taxa that are consistently present both spatially and temporally.
414 Bray-Curtis distances were calculated on this subset using the mctoolsR R package.

415 Bray–Curtis dissimilarities were calculated on square root transformed taxon relative
416 abundances.

417 *Temporal analyses and network construction:* We identified significant temporal
418 correlations in the relative abundances of individual taxa derived from soils that were
419 treated to remove relic DNA using extended Local Similarity Analysis (eLSA)²³ with the
420 following parameters: `lsa_compute -s 9 -r 9 -p perm`. We defined significant
421 temporal associations as those with a local similarity (LS) score ≥ 0.7 (i.e.-strong to very
422 strong correlations) and a q value ≤ 0.001 . Pairs of significantly correlated taxa were
423 analyzed in Gephi (version 0.8.2). Network modularity was calculated by implementing
424 the ‘modularity’ function²⁴ built in within Gephi, with a resolution setting of 1.0 for both
425 slopes. Node IDs (individual taxa) belonging to the same module were extracted to
426 delineate temporal patterns. Normalized relative abundances for each node ID were
427 calculated using the tRank command in the multic R package.

428 *Random forest analysis:* For each slope, we used Random Forest⁵³ modeling to first
429 identify those measured environmental and soil variables that were significant ($P \leq 0.05$)
430 predictors of time, using time as a response variable (Supplementary Fig. 4). These
431 significant environmental factors are expected to predict changes in module abundance
432 over time (Supplementary Fig. 3). We then conducted a second round of Random
433 Forests analysis with the significant environmental predictors to identify the most
434 important environmental factors or soil characteristics that predicted the mean
435 normalized relative abundances of each module (see ref. ⁵⁴ for a similar approach). The
436 importance (increase in mean square error %) and significance of each predictor was
437 computed for each tree and averaged over the forest (9999 trees) using the rfPermute
438 R package. Significant predictors were defined as those with a P value ≤ 0.05 . Samples
439 for which environmental and soil characteristics were missing because of insufficient
440 sample were excluded from random forest and spearman correlation analysis.

441
442 **Data Availability:** Raw DNA sequence data, the corresponding mapfile and all soil and
443 environmental characteristics are available on figshare.com:

444 [10.6084/m9.figshare.6710087](https://figshare.com/figures/articles/10.6084/m9.figshare.6710087). Snow depth data are available through the Boulder
445 Creek Critical Zone Observatory website:

446 <http://criticalzone.org/boulder/data/dataset/2423/>. Temperature data for the NFS and
447 SFS are available through the Boulder Creek Critical Zone Observatory website
448 <http://criticalzone.org/boulder/data/dataset/2426/>.

449
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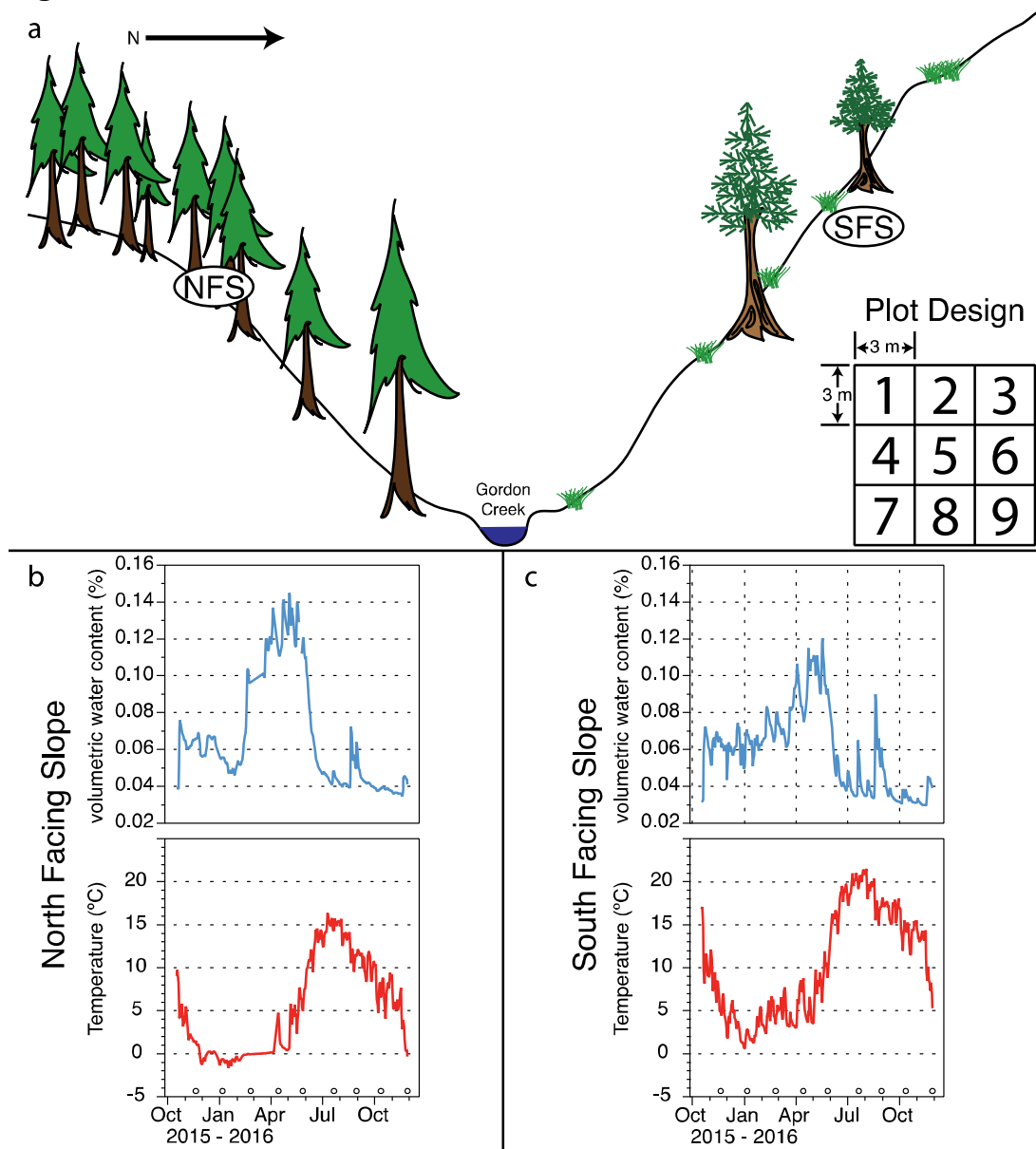
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606

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Figures:



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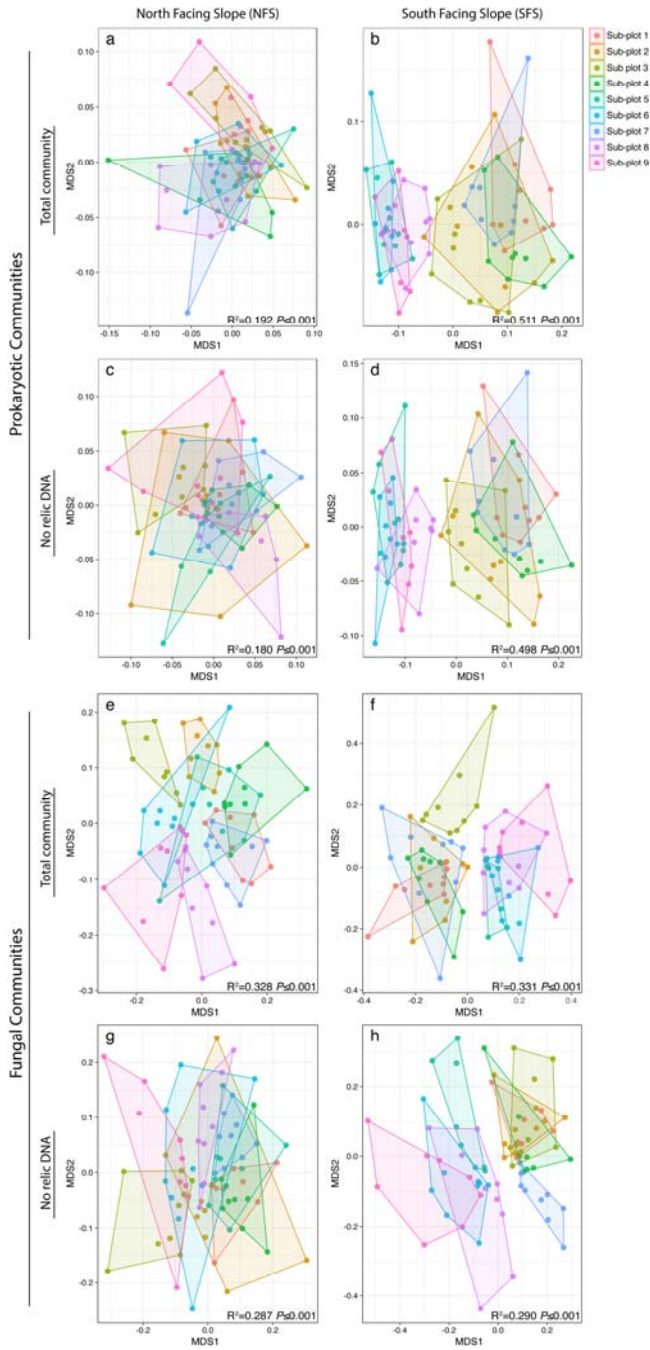
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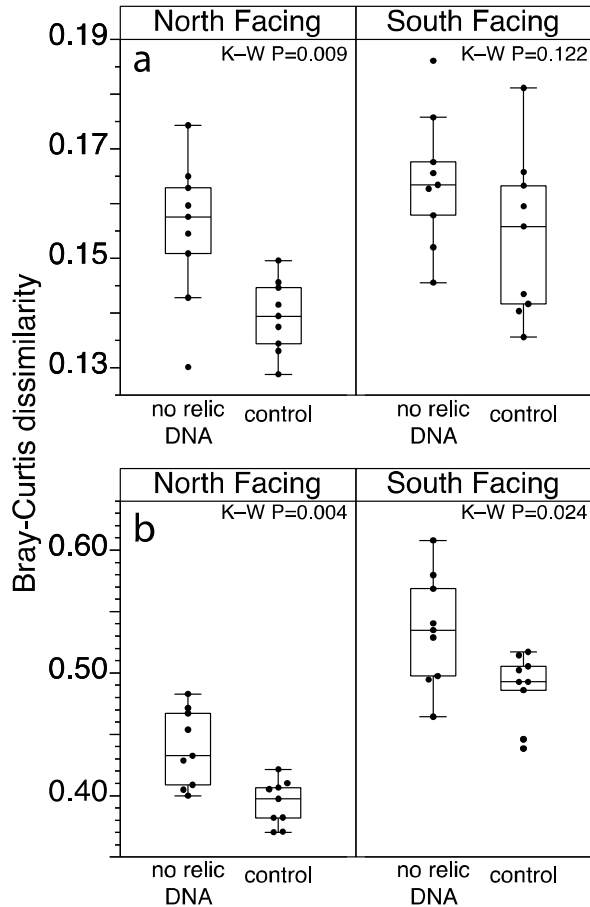
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Figure 1: Overview of the Gordon Gulch sampling sites and environmental conditions across the sampling sites. (a) Conceptual diagram of sampling site location and plot design, reproduced with modification from²⁹. The North facing slope (NFS) plot was centered at 40°0'44.759"N 105°28'9.123"W. The South facing slope (SFS) plot was centered at 40°0'48.551"N 105°28'8.355"W. Inset in (a) is an illustration of plot design. A single plot is comprised of nine 3 m x 3 m sub-plots. Numbers represent replicate sub-plots as described in the main text. Daily mean soil volumetric water content and soil temperature from *in situ* sensors at 5 cm depth for the NFS (b) and SFS (c) during the course of the experiment. Small circles on the temperature plots in (b) and (c) indicate sampling dates.



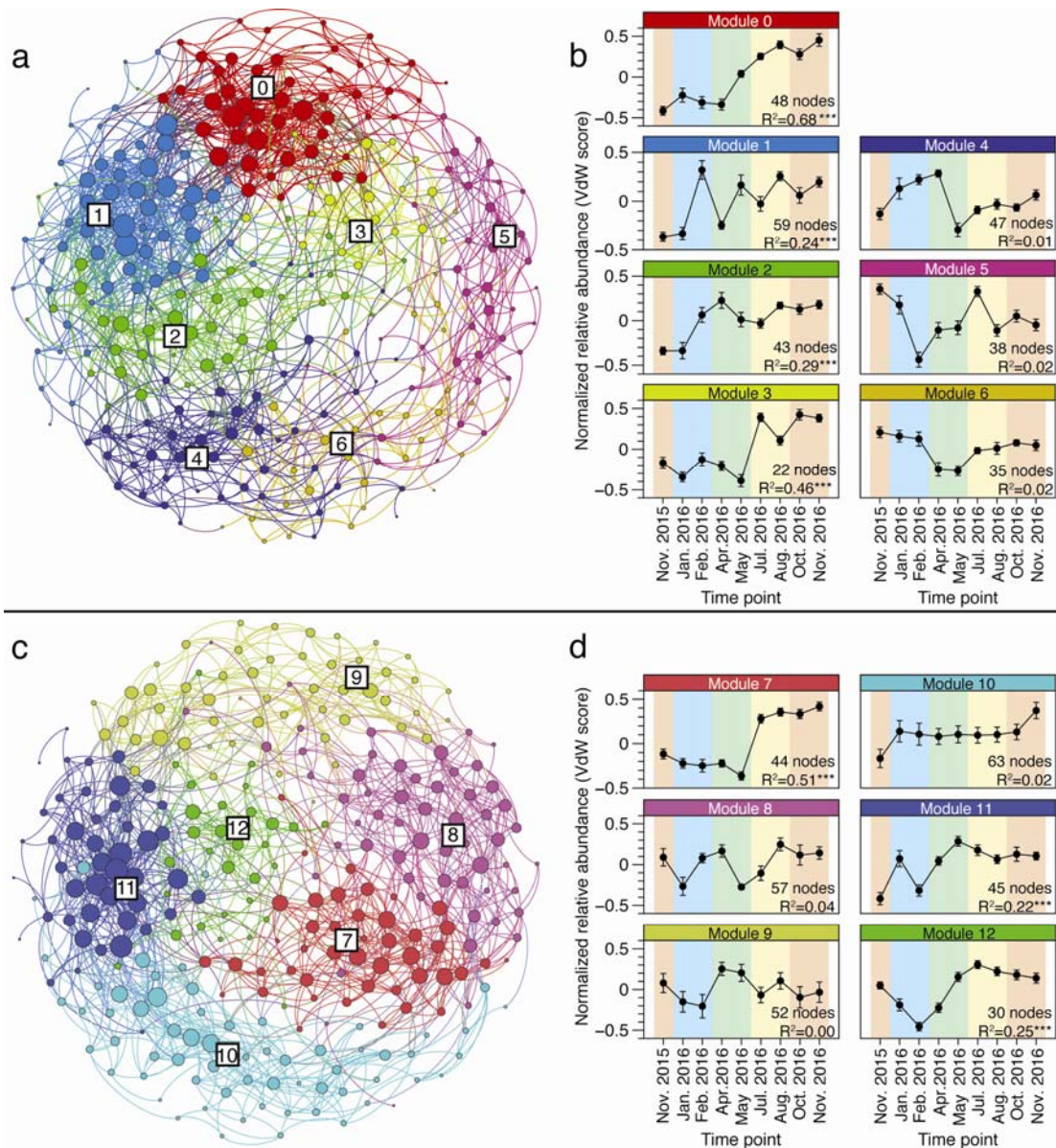
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Figure 2: Intra-plot spatial variability in soil microbial communities persists over time on both slopes regardless of whether relic DNA is removed. NMDS plots showing the prokaryotic (a-d) or fungal (e-h) communities on the north facing slope (a,c,e,g) and south facing slope (b,d,f,h). Points are colored by sub-plot number (plot layout is illustrated in Figure 1 in the main text). Hulls connect the outermost points on each slope. PERMANOVA statistics are listed on each panel.



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Figure 3: Soils without relic DNA were found to harbor microbial communities that are more dissimilar over time than in control soils containing relic DNA. (a) Prokaryotes (b) Fungi. Points are the mean community dissimilarity for a given sub-plot across all time points (n=9) for samples after relic DNA removal (no relic DNA) or untreated samples (control). Box plots illustrate interquartile range $\pm 1.5 \times$ interquartile range. The horizontal line in each box plot is the median. Outliers ($>1.5 \times$ interquartile range) are shown as points outside of whiskers. Kruskal-Wallis test (K-W) P values are shown.



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Figure 4: Cross-domain temporal dynamics in belowground microbial communities reveals temporal niche structure. Correlation networks based on significant microbe-microbe temporal correlations for the NFS (a) and SFS (c). Nodes in (a) & (c) are individual prokaryotic or fungal taxa. Lines between nodes represent significant (q value ≤ 0.001) and strong (local similarity score ≥ 0.7) positive temporal correlations. The sizes of nodes are proportional to the number of correlations to other nodes (the degree), whereby larger nodes have more connections. Colors represent distinct modules, as determined using the modularity algorithm described in ref. ²⁴. Boxed numbers in networks are arbitrary module numbers and match those in panels (b) and (d). Modularity analysis of each network revealed clusters of microbes that have similar temporal patterns. These temporal patterns were plotted for the NFS (b) and SFS (d). Points in (b) and (c) are the mean Van der Waerden (VdW) normalized relative abundance of all taxa in a given module. Error bars show \pm SEM. The number of nodes included in each module and the PERMANOVA P value describing the relationship of

663 the normalized relative abundances in relation to time are shown. P values marked with
664 asterisks are significant at $P \leq 0.001$. Background is shaded by season: orange=autumn;
665 blue=winter; green=spring; yellow=summer. See Supplementary Table 1 for taxonomic
666 module membership.