Coordinated Interannual Change in NEON Soil Bacterial Communities Across Continental North America

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Abstract

Soil bacterial communities change from year to year, but whether these changes are coordinated across distant ecosystems has not been well investigated. We leveraged publicly available soil data from the National Ecological Observatory Network (NEON), and applied statistical analyses to evaluate the temporal changes of soil bacterial communities across continental North America. Data were collected from 2020 to 2024, across 20 NEON sites spanning arctic tundra, tropical forest, grassland, and desert ecosystems. Bacterial composition, profiled by 16S rRNA gene amplicon sequencing, differed significantly among years in 16 of the 21 strata across the 1,253 cores with non-zero reads (between-year minus within-year Bray-Curtis dissimilarity, 999 within-stratum year-label permutations, pooled P = 0.001). PERMANOVA agreed in significance on 20 of the 21 strata. After applying a 50,000-read minimum depth floor and rarefaction, 1,212 cores remained across 21 strata. Fifteen of these strata met the temporal change criterion and formed the analytical set for synchrony analysis. Genus-level temporal trajectories were positively correlated among these strata (mean Spearman ρ = 0.167 across 105 pairs, rising to 0.218 when the single boundary stratum was excluded); no permutation of 999 reached the observed value, giving p = 0.001 and indicating coordinated interannual change across geographically separated sites. Among the prespecified predictors, more similar median soil pH was weakly associated with greater synchrony (ρ = -0.181, one-sided P = 0.043), while geographic distance was not (ρ = -0.143, P = 0.08, across separations of 110 to 9,193 km). None of the four declared climate-synchrony windows was associated with bacterial synchrony (ρ = +0.034 to +0.069, all P > 0.20 before correction for four tests). Overall, these results provide evidence for coordinated interannual dynamics of soil bacterial communities at continental scale, while the environmental drivers of that coordination remain undetermined and should be the focus of future work.

Keywords: soil microbiome; bacterial community; temporal synchrony; interannual variation; microbial ecology; soil pH; 16S rRNA gene sequencing

1. Introduction

Soil bacterial communities play central roles in biogeochemical processes, including carbon and nitrogen cycling [1]. These communities can change substantially over time within individual sites, and temporal turnover in soil microbial composition is already well established [2,3]. Microbial community composition also varies strongly across geographic locations and is associated with local soil properties [4-8]. However, these two observations do not establish whether geographically separated soil communities undergo similar temporal changes.

Evidence for coordinated microbial dynamics, or synchrony, has been reported in other systems. Bacterial communities in geographically separated lakes can exhibit synchronized temporal dynamics, potentially through shared climatic forcing or other regional environmental processes [9,10]. Synchrony has also been observed among microbial populations in wastewater-treatment reactors [11]. These studies demonstrate that microbial communities can respond coherently across separated locations, but aquatic and engineered systems may not represent natural soils distributed across continental-scale environmental gradients.

An important question therefore remains unresolved: do consistently detected soil bacterial genera exhibit concordant temporal trends across distant sites? Detecting such synchrony would indicate that broad-scale processes can organize microbial dynamics across geographically separated ecosystems, rather than temporal change being entirely site-specific. This could improve understanding of how soil microbial communities, and the biogeochemical processes they support, respond to environmental variation.

The objective of this study was to determine whether the multivariate direction of temporal bacterial-community change is synchronized across geographically separated soils. We further evaluated two potential environmental correlates of synchrony: climate and soil-chemistry. Climate represents broad-scale environmental variation that may expose distant ecosystems to similar temporal forcing, whereas soil chemistry represents a more proximal environmental filter that can shape microbial community composition and potentially influence how communities respond through time.

NEON provides standardized, repeated measurements of soil bacterial communities, climate, and soil properties across geographically distributed ecosystems [12]. These data allows us to quantify interannual bacterial-community change within sites, compare the direction of those changes among sites, and test whether temporal trajectories are more similar than expected by chance. They also allow us to evaluate whether greater similarity in climate history or soil chemistry is associated with stronger synchrony among sites.

2. Materials and Methods

2.1. Study sites and data acquisition

Soil 16S rRNA gene amplicon-sequencing data collected between 2019 and 2024 were obtained from the NEON soil microbial archive. Initial screening was conducted using a manifest containing 19,329 candidate soil cores from 47 NEON sites, representing 80 site-by-soil-horizon strata. Cores were retained if they met two criteria: (1) collection between 2020 and 2024, when the 515Y/926R primer pair remained consistent, and (2) collection between days 100 and 250 of the year, corresponding to the defined seasonal sampling window. A total of 5,004 cores across 70 strata satisfied both criteria.

To support trajectory estimation, a stratum was included only if it had been sampled in at least four distinct years, with a minimum of ten cores collected in each sampled year. Twenty-one strata from 20 sites met these requirements. Of the 49 excluded strata, 44 had been sampled in fewer than four of the five years because of NEON's rotating sampling schedule, while five had at least one year with fewer than ten cores. The largest excluded stratum, NIWO M, contained 146 cores spanning all five years but was excluded because only five cores were collected in 2022. The other four strata excluded because of insufficient annual sample size—OSBS O, WREF M, ORNL O, and WOOD O—only contained 26, 21, 12, and 10 cores in total, respectively.

Qualifying strata were subsequently subsampled to obtain an equal number of cores per stratum, yielding a final manifest of 1,255 cores. The dataset encompassed terrestrial sites representing major North American ecosystems, including Arctic tundra, temperate and tropical forests, grasslands, and deserts.

2.2. Sequence processing, normalization, and analytical selection

Amplicons targeted the 515Y/926R region of the 16S rRNA gene [13] and were denoised using DADA2 [14]. Forward and reverse reads were truncated to 250 and 240 bp, respectively. These lengths were selected by comparing denoising performance across five candidate truncation combinations applied to nine sequencing runs. Amplicon sequence variants were taxonomically classified at the genus level.

Two cores with zero reads were removed, leaving 1,253 cores for the initial year-effect analysis. Sixteen of the 21 strata exhibited significant temporal change in this dataset. Samples were subsequently required to contain at least 50,000 reads before rarefaction. An additional 41 cores failed this threshold, producing a synchrony-compatible dataset of 1,212 cores. Retained samples were rarefied to 50,000 reads to standardize sequencing effort. Although rarefaction has recognized limitations [15], using a common library size reduced variation attributable to sequencing depth.

The temporal-change criterion was reassessed using the synchrony-compatible dataset so that stratum selection and trajectory estimation were based on the same samples. Fifteen strata met the criterion. WOOD M was the only stratum whose eligibility changed after read-depth filtering: it lost 7 of 59 cores, and its year-effect test changed from (P=0.006) to (P=0.083). Analyses restricted to strata with demonstrated temporal change therefore included 15 strata and 105 unique between-stratum pairs. Annual coverage, sample retention, and stratum-level temporal-change statistics are reported in **Table 2**.

Following rarefaction, genus-level relative abundances were Hellinger-transformed [16]. Unless otherwise specified, subsequent community-composition and temporal-trajectory analyses used these depth-standardized, Hellinger-transformed data. These findings characterize synchrony among consistently detected genera in strata with detectable temporal change. Their broader applicability across other soils and taxa remains to be established.

2.3. Taxon Inclusion and Temporal Community Change

Analyses were restricted to 120 bacterial genera detected in at least 25% of samples within each of the 21 strata, ensuring that temporal trajectories were estimated from the same taxa across strata.

The first step was to evaluate the temporal variation in community composition within each stratum, prior to evaluation of the two-dimensional analysis for temporal-spatial synchrony. This was achieved by utilizing Bray-Curtis dissimilarity, calculated by:

$$BC_{ij} = 1 - \frac{2\sum_{k=1}^{K} min(x_{ik}, x_{jk})}{\sum_{k=1}^{K}(x_{ik} + x_{jk})}$$

Where i, j are indices for the two soil samples are being compared; k is the index for bacterial genus; x_{ik} is the abundance of genus k in sample; x_{jk} is the abundance of the same genus k in sample j; min(x_{ik}, x_{jk}) is the abundance of genus k shared by samples i and j for the Bray-Curtis calculation; BC_{ij} is the Bray-Curtis dissimilarity between samples i and j.

The year-effect statistics were calculated using:

$$\Delta BC = \overline{BC}_{between-year} - \overline{BC}_{within-year}$$

A positive ΔBC indicates that communities collected in different years were on average more dissimilar than communities collected within the same year. Statistical significance was assessed by permuting year labels 999 times within each stratum, and recomputing ΔBC to generate a null distribution under no year effect. The observed statistics were compared with this distribution to obtain the permutation P value; $P < 0.05$ was considered evidence of significant interannual variation.

For the pooled test, ΔBC was summed across strata and compared with the corresponding sums from 999 permutations of year labels within each stratum. PERMANOVA was further used as a validation of the interannual year effect. The PERMANOVA pseudo-F statistic, comparing multivariate variation attributable to year with residual variation within years, was calculated:

$$F* = \frac{SS_{year} \,/\, df_{year}}{SS_{res} \,/\, df_{res}}$$

$$df_{year} = g - 1, \quad df_{res} = N - g$$

Where N is the number of samples; g is the number of year groups; SS_{year} is the sum of squares for year; SS_{res} is the residual sum of squares; df_{year} is the Degrees of freedom for year; df_{res} is the residual degrees of freedom.

2.4. Temporal Trajectories and Between-Stratum Synchrony

We developed the following methodology to evaluate temporal synchrony (**Figure 1**). The primary analysis quantified synchrony in temporal bacterial-community change among soil strata. Within each site × soil-horizon stratum, we retained the same set of 120 consistently detected genera, applied a Hellinger transformation to relative abundances, and estimated a temporal slope for each genus across years. Slopes were estimated by ordinary least squares regression on sampling year, with each retained core contributing one equally weighted observation. These genus-level slopes were assembled into a community trajectory vector for each stratum, and pairwise synchrony was calculated as the Spearman correlation between trajectory vectors. Synchrony refers to agreement in the ranks of fitted temporal slopes across the 120 shared genera. Positive correlations indicated similar rankings, values near zero indicated little concordance, and negative correlations indicated opposing temporal trajectories. Overall synchrony was summarized as the mean pairwise correlation across strata and tested against a null distribution generated by 999 within-stratum permutations of year labels.

2.5. Soil chemistry, geographic distance, and climate as predictors

2.5.1. Soil pH and geographic distance as predictors

pH was selected as the chemical predictor because it was the most widely available soil-chemistry measurement, and is also a well-established determinant of bacterial community composition. Soil chemical dissimilarity was quantified as the absolute difference in median water-measured pH between strata (median pH range: 3.86-8.41). Geographic distance was included as a covariate to test whether synchrony persisted across widely separated sites and whether it declined with spatial separation. Great-circle distances calculated from site coordinates ranged from 110 to 9,193

km. Because the prespecified hypotheses predicted lower synchrony with increasing pH difference and geographic distance, associations were evaluated using one-sided tests.

Between-stratum bacterial synchrony is defined as:

$$S_{ij} = \rho_s(T_i, T_j)$$

S_{ij} is the synchrony between strata i and j; T_i and T_j are their 120-genus temporal trajectory vectors. ϱ_s denotes Spearman rank correlation.

Soil pH dissimilarity was defined by:

$$\Delta pH_{ij} = |\, median(pH_i) - median(pH_j) \,|$$

The stratum-level median pH values ranged from 3.86 to 8.41. A larger ΔpH_{ij} means greater soil-chemical dissimilarity.

Geographic great-circle distance was defined as:

$$D_{ij} = R\, arccos[\, sin(\varphi_i)\, sin(\varphi_j) + cos(\varphi_i)\, cos(\varphi_j)\, cos(\lambda_i - \lambda_j)\,]$$

$$R \approx 6371\ km$$

Where, φ is latitude, λ is longitude, and R is the Earth's mean radius. Pairwise distances ranged from 110 to 9,193 km.

Association with soil pH difference was tested by:

$$\rho_{pH} = \rho_s(S_{ij}, \Delta pH_{ij})$$

$$H_0: \rho_{pH} \geq 0 \quad versus \quad H_a: \rho_{pH} < 0$$

One-sided alternative: greater pH difference is associated with lower bacterial synchrony.

Association with geographic distance was calculated by:

$$\rho_D = \rho_s(S_{ij}, D_{ij})$$

$$H_0: \rho_D \geq 0 \quad versus \quad H_a: \rho_D < 0$$

For pH and geographic distance, P values were obtained from 999 permutations of year labels within strata. Each permutation recalculated genus slopes, pairwise synchrony, and environmental correlations while the selected strata and environmental distances remained fixed.

2.5.2. Climate as a predictor

Daily temperature and precipitation data were obtained from Daymet (NASA-supported research project managed by Oak Ridge National Laboratory) and aggregated into annual standardized anomalies relative to a 2010-2024 baseline. Four climate metrics were examined: namely temperature and precipitation over the full calendar year, and over a January-July preseason period. The January-July period was specified a priori as the primary window because NEON soil sampling generally occurred between approximately days 100 and 250, whereas full-year summaries may include climatic conditions occurring after sample collection.

Table 1. Climate metrics and time windows used to calculate annual quantities.

Metric	Window W	Annual quantity X
Temperature	Full year	Mean daily temperature, January-December
Precipitation	Full year	Total precipitation, January-December
Temperature (primary)	January-July	Mean daily temperature, January-July
Precipitation	January-July	Total precipitation, January-July

Standardized annual climate anomaly is defined as:

$$Z_{i,t}^{X,W} = \frac{X_{i,t}^{W} - \mu_i^{X,W}}{\sigma_i^{X,W}}$$

The standardized anomaly expresses whether a given year was warmer/cooler or wetter/drier than that site's own 2010-2024 baseline for the same metric and time window.

$$\mu_i^{X,W} = mean\{ X_{i,t}^{W} : t = 2010, \ldots, 2024 \}$$

$$\sigma_i^{X,W} = SD\{ X_{i,t}^{W} : t = 2010, \ldots, 2024 \}$$

Where $X_{i,t}^{W}$ is the mean temperature or total precipitation for site i, year t, time window W; $\mu_i^{X,W}$ is the site-specific baseline mean; $\sigma_i^{X,W}$ is the baseline standard deviation.

Climate synchrony between two sites is defined by:

$$C_{ij}^{X,W} = Corr(Z_i^{X,W}, Z_j^{X,W})$$

For sites i and j, climate synchrony is the correlation between their annual standardized-anomaly series for the same climate metric X and window W. Higher positive values mean that the two sites tended to have warm/cool (or wet/dry) years at the same times; values near zero indicated little temporal correspondence.

Relating climate synchrony to bacterial community synchrony is defined by:

$$\rho_{clim} = \rho_s(S_{ij}, C_{ij}^{X,W})$$

S_{ij} is the bacterial community synchrony for stratum pair (i,j), defined as the Spearman correlation between their genus-level temporal trajectory vectors. The association between climate synchrony and bacterial synchrony using Spearman ϱ, is assessed by:

$$H_0: \rho_{clim} \leq 0 \quad versus \quad H_a: \rho_{clim} > 0$$

The prespecified one-sided alternative was positive. Pairs of sites with more similar climate histories were hypothesized to have more similar bacterial temporal trajectories.

2.6. Data analysis platform

Raw sequence files were retrieved from the NEON REST API (soil microbe marker gene sequences, DP1.10108.001; RELEASE-2026) using a custom Python client and a NEON API token. Soil pH was taken from the NEON soil physical and chemical properties product (DP1.10086.001, RELEASE-2026), and daily climate data from Daymet. Denoising and taxonomic classification were performed in R on a Microsoft Azure Standard_D32as_v7 virtual machine (32 vCPU, Ubuntu 22.04

LTS) using the Bioconductor DADA2 package [14] with the SILVA nr99 v138.1 `assignTaxonomy` training set (Zenodo record 4587955), together with neonUtilities and neonMicrobe for sample resolution. Rarefaction, the Hellinger transformation, all statistical tests and all figures were produced in Python 3.13.3 using NumPy 2.3.0, pandas 2.3.0, SciPy 1.18.0 and Matplotlib 3.10.6. PERMANOVA and great-circle distance were implemented directly in this project's code rather than taken from a package, so that the permutation scheme and the distance formula are visible in the archived source.

3. Results

3.1. Study sites and analytical dataset

After year and sequence-depth filtering, the analytical dataset contained 1,212 soil cores from 20 NEON terrestrial sites, organized into 21 site × soil-horizon strata: 14 mineral and 7 organic. The 2020-2024 manifest initially contained 57-60 cores per stratum; after filtering, 51-60 cores remained. Strata represented 7-11 permanent plots and four or five sampling years. The sites encompassed broad geographic and ecological gradients, and median soil pH ranged from 3.86 in the Harvard Forest organic horizon (HARV O) to 8.41 in the Onaqui mineral horizon (ONAQ M; **Table 2**).

Baseline characterization confirmed substantial ecological differentiation among the 21 strata. Phylum-level relative abundance varied across strata ordered by median soil pH, and Shannon diversity appeared to differ visibly between mineral (n = 799 cores) and organic horizons (n = 413 cores) (**Figure 2A**). Shannon diversity appeared to differ between mineral (n = 799 cores) and organic soil horizons (n = 413 cores; Figure **2B**). Bray-Curtis ordination further separated communities by site and soil horizon; the first two principal-coordinate axes explained 42.3% and 8.7% of the variation, respectively. PERMANOVA attributed 64% of compositional variation to site and 26% to soil horizon (both P = 0.001) (**Figure 2C**). The site and horizon associations should be interpreted together as most sites contributed only one soil horizon.

The initial temporal-change analysis, conducted using 1,253 cores with nonzero reads, identified significant interannual change in 16 of the 21 strata. Reassessment after applying the 50,000-read threshold and rarefaction retained 15 strata. WOOD M was the only stratum whose eligibility changed: after losing 7 of 59 cores, its temporal-effect estimate decreased from +0.0126 (P = 0.006) to +0.0076 (P = 0.083). Because the synchrony analysis was intended to compare temporal trajectories among strata exhibiting detectable interannual change, the year-effect criterion was reassessed after depth filtering. Fifteen strata met this criterion and constituted the primary synchrony-analysis set. TOOL O met the inclusion criterion in the primary analysis (P = 0.043) but was sensitive to rarefaction, meeting the P < 0.05 criterion in 15 of 20 independent draws. Results influenced by its inclusion are therefore reported with sensitivity ranges.

Table 2. Sample retention and temporal-change eligibility by site × soil-horizon stratum

Stratum	Years	Median pH	Cores F / M	Filtered effect	Initial P	Filtered P	Included
BONA O	4	4.21	57 / 60	+0.0096	0.023	0.011	Yes
CLBJ M	4	5.92	52 / 60	+0.0203	0.001	0.004	Yes
CPER M	5	6.80	60 / 60	+0.0193	0.001	0.001	Yes

Stratum	Years	Median pH	Cores F / M	Filtered effect	Initial P	Filtered P	Included
GUAN M	5	7.71	56 / 57	+0.0094	0.001	0.003	Yes
HARV O	5	3.86	59 / 60	+0.0101	0.002	0.004	Yes
KONZ M	5	7.11	59 / 60	+0.0248	0.001	0.001	Yes
NIWO O	5	4.93	60 / 60	+0.0113	0.018	0.017	Yes
ONAQ M	5	8.41	56 / 60	+0.0214	0.001	0.001	Yes
ORNL M	5	4.91	59 / 60	+0.0200	0.003	0.004	Yes
OSBS M	5	5.42	60 / 60	+0.0178	0.001	0.001	Yes
PUUM O	5	4.76	60 / 60	+0.0127	0.007	0.009	Yes
SCBI M	5	6.28	60 / 60	+0.0247	0.001	0.001	Yes
SJER M	4	6.33	58 / 60	+0.0012	0.120	0.272	No
SRER M	4	7.80	51 / 60	-0.0016	0.113	0.547	No
TALL M	5	5.01	59 / 60	+0.0158	0.001	0.001	Yes
TOOL O†	4	4.97	59 / 60	+0.0105	0.027	0.043	Yes
UNDE M	5	5.16	59 / 60	+0.0016	0.234	0.302	No
UNDE O	5	4.46	58 / 59	-0.0043	0.631	0.666	No
WOOD M‡	5	7.42	52 / 59	+0.0076	0.006	0.083	No
WREF O	5	4.11	60 / 60	+0.0022	0.126	0.176	No
YELL M	5	6.23	58 / 60	+0.0068	0.011	0.029	Yes

Notes. Each row represents one site × soil-horizon stratum; M = mineral and O = organic. Cores are shown as F / M, where F is the number retained at the ≥50,000-read floor and M is the number in the 2020-2024 manifest. Initial P was calculated using 1,253 nonzero-read cores; filtered effect and filtered P were recalculated after depth filtering and rarefaction using 1,212 cores. Included = filtered $P < 0.05$. † TOOL O was sensitive to rarefaction draws. ‡ WOOD M changed from significant initially ($P = 0.006$) to nonsignificant after filtering ($P = 0.083$).

3.2. Interannual Variation in Soil Bacterial Communities

Bacterial community composition differed significantly among years in 16 of the 21 site × horizon strata based on Bray-Curtis dissimilarity ($p < 0.05$; pooled $p = 0.001$). Across strata, between-year dissimilarity exceeded within-year dissimilarity by a mean of 0.0124, with effects ranging from −0.0031 at the University of Notre Dame organic-soil stratum to 0.0265 at the Smithsonian Conservation Biology Institute mineral-soil stratum (**Figure 3**).

Because ΔBC is a measure of interannual turnover, we independently assessed the same biological question using PERMANOVA, with sampling year as the grouping factor within each stratum. PERMANOVA tests whether multivariate community composition differs among years relative to within-year variation. The strong agreement between the two statistics ($r = 0.92$; significance

classification agreement in 20 of 21 strata) indicates that the detected interannual signal was not specific to the ΔBC (**Figure 4**).

3.3. Shared Temporal Trajectories across Sites

Temporal trajectories showed significant network-wide synchrony across strata. Mean pairwise Spearman correlation across the analyzed stratum sets ranged from ϱ = 0.167 to 0.218. The strongest pairwise association occurred between Pu'u Maka'ala, Hawai'i, and Talladega, Alabama (ϱ = 0.751), despite a geographic separation of 6,823 km (**Figure 5**).

To evaluate whether this positive mean could arise under a null model with no shared temporal signal, year labels were independently permuted within each stratum, genus-specific temporal slopes were estimated, trajectory vectors were constructed, all pairwise correlations were estimated, and the network-wide mean was computed for each of 999 permutations. The permutation-derived mean synchrony values were centered near zero, and none of the 999 permuted means equaled or exceeded the corresponding observed value. The one-sided permutation p value was 0.001. Thus, the observed network-wide synchrony was greater than expected under the null model (**Figure 6**).

3.4. Environmental and Geographic Correlates of Synchrony

The primary environmental analysis used the 15 strata that satisfied the prespecified temporal-change eligibility criterion after depth filtering and rarefaction, giving 105 between-stratum pairs. Absolute difference in median soil pH showed the largest association with bacterial synchrony, although the effect was weak (Spearman ρ = -0.181, one-sided P = 0.043**; Figure 7A**): strata with more similar median pH tended to have more concordant genus-level trajectories. Geographic distance showed an association of the same sign and similar magnitude that did not reach significance (ρ = -0.143, one-sided P = 0.08, across separations of 110 to 9,193 km; **Figure 7B**). None of the four climate-synchrony metrics was associated with bacterial synchrony. Temperature synchrony over the annual and preseason windows resulted in ρ = +0.069 (P = 0.230) and +0.056 (P = 0.289), respectively; and precipitation synchrony over the same windows resulted in ρ = +0.050 (P = 0.276) and +0.034 (P = 0.343), respectively. The result of the primary preseason-temperature window is shown in **Figure 7C**. No clear association was detected between bacterial synchrony and the examined climate metrics.

4. Discussion

Across the NEON network, soil bacterial communities exhibited coordinated interannual changes, despite substantial geographic and environmental separation. The principal finding was not simply that community composition varied among years, but that genus-level changes were positively correlated among sites.

Spatial synchrony can arise when geographically separated communities respond to correlated environmental forcing, as described by the Moran effect [17,18]. In the present study, however, bacterial synchrony was not significantly associated with geographic distance or with synchrony in the measured climate variables in the primary analyses. Thus, the observed pattern does not provide support for a simple Moran mechanism, where geographically structured climatic variation would directly synchronize bacterial communities.

Overall, this interpretation should remain cautious. Only five annual observations were available, limiting the precision of pairwise climate correlations and the power to detect moderate climate-

community relationships. In addition, our analyses considered individual climate variables rather than multivariate or interactive environmental forcing. Ecological synchrony can arise through combinations of abiotic and biotic processes; for example, environmental forcing may act indirectly through biological intermediaries or interact with local environmental conditions [9,19]. The present results therefore do not exclude climatic forcing but rather indicate that the measured climate variables alone did not explain the observed bacterial synchrony.

Geographic distance and the primary climate metrics appeared not associated with bacterial synchrony, while bacterial synchrony appeared to be greater between strata with more similar median soil pH. The pH relationship should be considered as hypothesis-generating rather than concrete evidence of an established mechanism. Soil pH was observed, by other studies, as among the strongest environmental predictors of bacterial community composition across regional to global scales [5-7].

Experimental observations provide a plausible precedent for such context-dependent responses. Knight et al. [20] found that compositionally distinct soil microbiomes exposed to common heat, drought, flood, and freeze perturbations showed partly consistent responses, while local community properties influenced the magnitude and direction of those responses. Similarly, synchrony among freshwater bacterial communities has been observed even when the relationship with regional climate appeared to operate indirectly through phytoplankton dynamics [9]. Together, these studies illustrate how microbial communities can respond coherently to broad environmental forcing while their responses remain conditioned by local ecological context.

Several limitations should be considered when interpreting these findings. First, the study was based on five annual sampling periods, which limited the ability to distinguish sustained directional change from longer-term fluctuations and reduced power to identify relationships between bacterial synchrony and climate. Variation in within-year sampling dates among sites may have introduced additional temporal heterogeneity. Longer time series will therefore be necessary to determine the persistence and environmental drivers of the observed synchrony.

Second, the study is observational and cannot establish the mechanism underlying coordinated community change. Soil pH was represented by a median value for each stratum rather than contemporaneous measurements for every sequenced core, and the association between pH similarity and bacterial synchrony was sensitive to resampling and stratum selection.

Finally, the present analysis does not establish whether the observed synchrony is specific to bacterial communities. ITS data are available for many of the same NEON samples, but the current number of usable strata provides limited power for a comparable fungal analysis. Additional years and strata should enable stronger cross-domain comparisons, and help distinguish shared environmental forcing from other mechanisms, including convergent deterministic selection.

5. Conclusions

Using repeated soil microbiome observations across the NEON network, this study provides evidence that bacterial communities in geographically separated soils can undergo coordinated interannual change. By representing each community as a multivariate trajectory of genus-level temporal trends, we detected significant positive synchrony across sites despite substantial differences in geographic location, soil properties, and baseline community composition. The observed synchrony was not significantly associated with geographic distance or similarity in the

measured climate variables, while soil pH similarity showed a weak association with greater trajectory synchrony.

These findings suggest that temporal dynamics of soil bacterial communities are not entirely site-specific and that shared patterns of community change can emerge across widely separated ecosystems. The trajectory-based framework used here provides a way to quantify this temporal dimension of community similarity, complementing conventional approaches that primarily compare community composition at a given time. Longer time series and broader environmental measurements will help determine the processes responsible for this large-scale coordination and whether similar patterns occur across other microbial groups.

Figure Legends

Figure 1. Workflow for quantifying between-stratum synchrony in whole-community temporal trajectories and evaluating network-wide synchrony with within-stratum year-label permutations.

Figure 2. Baseline composition and diversity of 16S rRNA soil bacterial communities across 21 NEON site × soil-horizon strata. (A) Mean phylum-level relative abundance by stratum, with strata ordered by median soil pH; taxa not shown individually were grouped as "Other." (B) Sample-level Shannon diversity in mineral and organic horizons; horizontal lines indicate group medians. (C) Principal-coordinate analysis of Bray-Curtis dissimilarities, with points shaped by soil horizon and colored by stratum median pH. Percentages on the axes indicate the variation explained. PERMANOVA results summarize the contributions of site and soil horizon to community composition. Sample sizes were 799 mineral and 413 organic soil cores.

Figure 3. The between-year minus within-year Bray-Curtis difference for each of the 21 site × horizon strata, sorted from strongest to weakest. Orange bars are the 16 strata significant at $p < 0.05$; gray bars are the 5 that are not, including the single group (University of Notre Dame, organic horizon) with a negative effect. The dashed line marks the mean across all 21 strata (+0.0124).

Figure 4. Validation of the interannual effect using PERMANOVA R^2. The primary between-year minus within-year Bray-Curtis statistic was strongly correlated with PERMANOVA R^2 across the 21 strata, with significance classifications agreeing for 20 of 21 strata. The Toolik Lake tundra organic-soil stratum was the sole disagreement.

Figure 5. Example of cross-site trajectory agreement. Per-genus temporal slopes at Pu'u Maka'ala, Hawai'i, are plotted against slopes for the same genera at Talladega, Alabama. This was the most synchronized pair in the dataset ($\varrho = 0.751$) despite a separation of 6,823 km. Blue points indicate genera changing in the same direction at both sites; gray points indicate opposite directions. Selected genera with large same-direction changes are labeled.

Figure 6. Permutation assessment of network-wide temporal synchrony among soil bacterial communities. Histograms show network-wide mean pairwise Spearman correlations obtained from 999 permutations in which year labels were independently shuffled within each stratum. Orange vertical lines indicate the observed mean synchrony. (A) Primary analysis including 15 strata and 105 unique pairs, including TOOL O. (B) Sensitivity analysis including 14 strata and 91 unique pairs after excluding TOOL O. No permuted mean equaled or exceeded the

corresponding observed value; the corrected one-sided permutation value was (P=0.001) for both analyses.

Figure 7. Site-pair synchrony (Spearman ϱ) plotted against the three candidate variables. A: |Δ median pH|, on the prespecified 15-group selection. B: great-circle distance, across all 15 groups at any distance from 110 to 9,193 km. C: climate synchrony (temperature, preseason window)

Supplementary Materials

No supplementary materials

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Data Availability Statement

All sequence, soil chemistry, and metadata are pre-existing public NEON data products, requiring no new accession numbers. This analysis used the NEON soil microbe marker gene sequences data product (DP1.10108.001) and the NEON soil physical and chemical properties data product (DP1.10086.001), RELEASE-2026, both publicly available with no access fee through the NEON data portal (https://data.neonscience.org).

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Conflicts of Interest

The authors declare no conflict of interest.

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