Drivers of temporal beta diversity and ecological resilience of plant communities across the United States

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Abstract

Long-term monitoring surveys offer more reliable assessments of biodiversity responses to climate change. In this study, we evaluated community shifts in 1105 1 m^2 plots across 20 NEON sites, representing each of the 20 eco-climatic domains across the U.S. and Puerto Rico, using long-term plant surveys (5-10 years). We computed alpha, spatial and temporal beta, and gamma diversity for the plots in both the earliest surveys (T_1) and those conducted in 2023 (T_2). We used permutational analysis of variance (PERMANOVA) and permutational multivariate analysis of dispersion (PERMDISP) to test for statistical significance in compositional variance between the two surveys, and investigated the effects of T_1 alpha diversity, elevation, soil, and climatic variables on temporal beta diversity using generalized linear mixed effect models (GLMM). We also checked if temporal beta diversity was significantly structured along geographical gradients and among different vegetation classes. The results showed increases in alpha and gamma diversity, comprising mostly native species; therefore, temporal beta diversity was high for most of the NEON sites, driven mainly by turnover and species gains between T_1 and T_2 periods. We observed latitudinal and longitudinal but not elevational, gradients in temporal beta diversity; herbaceous vegetation classes experienced the lowest temporal community changes, while forest ecosystems and pasture hay showed the highest shifts. PERMANOVA and PERMDISP tests showed significant community shifts in 16 NEON sites that is explained by compositional variance within or between survey years. GLMM showed significant effects of three soil (soil pH, silt content, fine organic carbon) and climatic (monthly Tmax standard deviation, monthly Tmean standard deviation, and AET coefficient of variation) variables on temporal beta diversity, while T_1 alpha diversity has no effect. Given the land use history in many of the NEON sites, observed temporal beta diversity may indicate post-disturbance succession or climate-induced reshuffling of regional species pool.

Keywords: temporal beta diversity, ecological resilience, NEON, United States, alpha diversity, gamma diversity, community ecology, continental scale biology

1. Introduction

Climate change, evidenced by increasing temperature, aridification, and altered precipitation, is expected to have profound impacts on biodiversity, and ecosystem health and functioning (Masson-Delmotte et al. 2023). This is to be expected since species distributions and community assemblages are mainly driven by climatic factors (Keddy 1992; Woodward et al. 2004). Biogeography theory predicts that under climate change, species will either migrate, adapt, or go into extinction (Feeley et al. 2012). Global climatic events are predicted to result in species range shifts to higher latitudes and elevations (Chen et al. 2011; Lenoir et al. 2008; Becker-Scarpitta et al. 2022), range expansions of heat-adapted species (Rumpf et al. 2018), and the movement of marine species into lower depths (Brown and Thatje 2015). However, species' responses to climate change vary in different taxonomic groups and ecosystems, which makes generalizing the effects of local and global changes on complex biological communities nontrivial (Abella et al. 2019; Antão et al. 2022; Rubenstein et al. 2023). Moreover, several anthropogenic activities such as grazing, biological invasions, species overharvesting, fire, and land use changes interact with climate change (Abella et al. 2019). Ecosystem services are linked to biodiversity functioning, and such services could be lost or reduced under climate-driven extinctions; therefore, climate change research is pivotal to national and global conservation policy, which necessitates accurate assessments and predictions of biodiversity responses and climate vulnerabilities of various ecological regions (Keeley et al. 2018).

The effect of climate change on biodiversity is studied broadly in two ways: ecological modeling and long-term biological monitoring. The former includes the use of species distribution models (SDMs) to explain species-climate relationships which are then mapped in geographical space as the species' potential niche (Booth et al. 2014). Extrapolating the model predictions into future time based on projected climate models are used to predict loss or gains of habitat suitability and the direction and magnitude of species range shifts (Aitken et al. 2008). However, most SDMs do not account for adaptive phenological shift, trait variability, and genetic diversity within species and symbiotic relationships among plants and soil micro-organisms that can mitigate climate stress (Maestre et al. 2009; Catullo et al. 2015). Genetic and functional diversity are important since natural selection and evolution are best assessed at the individual and population levels, respectively (Mayr and Provine, 1980). Therefore, the effects of climate change, an evolutionary process, can be best evaluated at below-species level. Additionally, SDMs are fitted using occurrence records which represents a small portion of the areas and abiotic niche associated with a species physiological tolerance and persistence (that is, realized niche), and they assume species equilibrium with environmental conditions; both of these statistical assumptions result in an underestimation of a species ability to adapt to novel climates and habitats (Booth 2017; Soberón and Arroyo-Peña, 2017). Finally, predicted future range shifts do not explicitly model dispersal (Travis et al. 2013) or landscape permeability and resistance; thus, it is not guaranteed that a species can effectively track future suitable habitats (Riva et al. 2023). Consequently, studies are equivocal on the popular hypotheses of latitudinal and elevational species range shifts due to climate change (Van Der Wal et al. 2012; Rubenstein et al. 2023).

Long-term biological monitoring offers more reliable assessment of biodiversity response to environmental stressors (Antão et al. 2022). Statistical analysis of repeated surveys across different ecological regions can be used to investigate the ecological integrity and resilience of biological communities and ecosystem functions under disturbance and climate change (Buckley et al. 2021). Despite the importance of long-term datasets in understanding community dynamics, temporal ecology is yet to be fully developed, and it was only recently that statistical tools to analyze temporal beta diversity have begun to be developed (Buckley et al. 2021). Many examples of biological monitoring include National Ecological Observatory Network (NEON; Keller et al. 2008; Barnett et al. 2019), Long-Term Ecological Research (LTERs; Vanderbilt and Gaiser, 2017), ForestGEO dynamics plots (Anderson-Teixeira et al. 2015), periodic surveys in protected areas and public lands (Fancy et al. 2009; Tinkham et al. 2018; Kachergis et al. 2022) and field biological stations (Chytrý et al. 2014; Becker-Scarpitta et al. 2022).

This study investigated temporal beta diversity in 1105 1 m^2 plots representing 20 NEON sites across the U.S. and Puerto Rico. The broad scale of this study across the entire U.S. and Puerto Rico exemplifies continental-scale biology, an emerging field, that highlights the importance and interconnectedness of biodiversity-environment relationships across multiple spatial and temporal scales through integrative and hierarchical analyses of biological communities and driving abiotic factors (Heffernan et al. 2014; National Academies of Sciences, Engineering, and Medicine, 2025). We accessed repeated surveys of plant communities from the oldest possible surveys in each of the 20 NEON sites and their corresponding 2023 sampling to answer the following questions: (a) How has alpha diversity changed over time? (b) Is there a significant temporal beta diversity across the 20 NEON sites? (c) What are the relative contributions and effects of climate variability and local site conditions on plant community temporal beta diversity? (d) what is the effect of invasive species on temporal beta diversity? (e) Are there latitudinal, longitudinal, and elevational patterns in community shifts across the 20 NEON sites?

2. Materials and Methods

2.1 Study sites and community data

We selected 20 National Ecological Observatory Network (NEON) sites, representing each of the 20 ecoclimatic domains in the U.S. and Puerto Rico (Table 1). NEON was funded by the U.S. National

Science Foundation (NSF) for 30 years to conduct daily to annual surveys collecting ecological, edaphic, hydrological, biogeochemical, geospatial, and climatic data in both terrestrial and freshwater aquatic environments that can be used to investigate biodiversity changes and responses to climate and land use changes (Keller et al. 2008; Barnett et al. 2019). For each of the 20 NEON sites, we downloaded plant presence and percent cover data (1 m^2) between the oldest survey year (T_1) and 2023 (T_2) surveys ensuring similarity in the seasons when surveys were conducted in the two time periods for temporal comparisons (NEON 2024). In the SRER site, however, lack of matching data with the T_1 surveys necessitated the use of the 2024, instead of 2023 survey data. From the downloaded data, we retained information on surveyed plants, percent cover, their native status, plot coordinates, recorded in WGS 1984, the National Land Cover Database (NLCD) vegetative class, elevation, plot ID, and subplot ID. Because the 1 m^2 surveys were nested within several corresponding 10 × 10 m plots, we used subplotID and coordinates to separate the data into respective 1 m^2 subplots (hereafter, plots). We did not include ancillary data such as bare ground, litter, and moss in the analysis. For fully identified species with unknown native status, we used Kew's Plants of the World Online (https://powo.science.kew.org/) to determine the species' native distribution. The final dataset contained 1105 1 m^2 plots across the 20 NEON sites.

2.2 Alpha, spatial beta, and gamma diversity

We assessed several alpha diversity measures for the two survey periods in all 1 m^2 plots, including species richness, total plant cover, Shannon-Weiner *H*' diversity index, and *H'* index-based effective number of species. We assessed gamma diversity for the two survey periods, based on species richness and effective number of species, representing the total species pool in each NEON site. This was conducted using functions in vegan R package version 2.7-2 (Oksanen et al. 2025). We calculated two spatial beta diversity measures: total variation, expressed as total sum of squares (SS_{total}), and total beta diversity, expressed as the total variance or dissimilarity (BD_{total}) for the T_1 surveys. We also decomposed spatial beta diversity into two components: local contributions to beta diversity (LCBD) and species contributions to beta diversity (SCBD) (Legendre and De Cáceres 2013). While SCBD indicates the relative contribution of each species in each NEON site, LCBD represents the uniqueness of each 1 m^2 plot and the effects of microsite conditions to the overall floristic variation in the dataset; therefore, sites with high LCBD may indicate unique environmental conditions or sites under ecological stress or disturbance (Legendre and De Cáceres 2013; Hill et al. 2021). We computed site LCBD and species SCBD along with 999 permutations and Holm p value correction, using beta.div() function in adespatial R package 0.3-28 (Dray et al. 2025).

2.3 Temporal beta diversity

We investigated temporal beta diversity using three complementary methods. Sorensen-based temporal beta diversity was decomposed into species replacement or turnover between T_1 and T_2 survey periods, and the difference in species richness between two time periods (i.e., nestedness) indicating that low richness is nested within high species richness. Both species replacement and nestedness are summed to obtain the overall temporal beta diversity (Baselga and Orme 2012). We generated 1000 random species dissimilarities for T_1 and T_2 survey periods for all 20 NEON sites to assess statistical significance of the Sorensen temporal beta diversity. The analyses were conducted in betapart R package version 1.6.1 (Baselga et al. 2025).

Temporal beta-diversity indices (TBI) between T_1 and T_2 periods for the 1 m^2 plots was calculated based on the percentage difference index (that is, Bray-Curtis dissimilarity) with 999 permutations for each 1 m^2 plot in adespatial R package version 0.3-28 (Dray et al. 2025). The TBI measures were calculated using species cover data, thus they provide additional insight into community dynamics and allow for comparison with incidence-based Sorensen temporal beta diversity (Avolio et al.

2019; Komatsu et al. 2019). Statistical significance of the TBI was assessed with 999 permutations with adjusted p values based on Holm correction to account for multiple species testing across all sites (Legendre and Condit 2019; Legendre 2019). The TBI indices describe losses (B index) and gains (C index) in species composition or abundance between two periods, while the D index is the overall temporal beta diversity for the site between two periods (Legendre and Condit 2019; Legendre 2019). Because the D index is nondirectional, we computed the difference between B and C indices to indicate if the plot experienced an overall gain or loss in species richness or abundance between two survey periods, and this was shown in B-C plots for all 1 m^2 plots in each NEON site (Legendre and Condit 2019; Legendre 2019). The three indices were also calculated for all 20 NEON sites to explain community shifts at regional pool scale.

We calculated community stability, which describes the magnitude of overall floristic changes for each plot between the two surveys (Appendix S1). However, this measure was not used for downstream analyses. Complete data of temporal beta diversity measures for all 1105 1 m^2 plots were deposited in Knowledge for Network Biocomplexity (Borokini 2026).

2.4 Climatic and soil variables

We downloaded daily values of precipitation, minimum, mean, and maximum temperature, between January 1, 2013 and December 31, 2023 for all NEON sites from the PRISM daily climatic normal at 800 m resolution (PRISM Climate Group, 2025). For NEON sites not represented on PRISM, we downloaded climatic variables from the time series climatic data obtained from the nearest weather stations and managed by the Western Region Climate Center (https://wrcc.dri.edu/) of the Desert Research Institute: GUAN Puerto Rico (Guanica weather station), HEAL, Alaska (Eielson Visitor Center weather station, Denali National Park), BARR (Inigok Airfield weather station), and PUUM (Keaumo, Hawaii weather station). We also extracted values from the global monthly actual evapotranspiration (AET) model version 6.1 via the USGS data portal [https://earlywarning.usgs.gov/fews/product/460/] and the global aridity aridity index (version 3; Trabucco and Zomer 2019). Global AET, available in 1 km resolution, was computed using the Operational Simplified Surface Energy Balance (SSEBop) model (Senay et al. 2013; 2022) from 2012 to present and parametrized using the novel Forcing and Normalizing Operation (FANO) algorithm (Senay et al. 2023). Aridity index (AI), accessed in 1 km resolution, is calculated as the ratio of total annual precipitation to potential evapotranspiration, and used to explain water deficiency (Zomer et al. 2022). We then calculated the mean, standard deviation, and coefficient of variation for AET, precipitation, minimum, mean, and maximum temperatures corresponding to periods between the T_1 and T_2 surveys. Additionally, we downloaded and extracted values for several edaphic factors including soil nitrogen, organic carbon density, bulk density, fine organic carbon, coarse fragment content, sand content, silt content, and pH at 5 cm depth (1 km resolution) for all survey plots using geodata R package version 0.6-8 (Hijmans et al. 2024). The soil variables were modeled using the quantile regression forest, and accessed from the SoilGRIDS database (Poggio et al. 2021).

2.5 Statistical tests

We investigated latitudinal, longitudinal, and elevational gradients in both the Sorensen-based temporal beta diversity and the TBI indices across all plots. We used student's t-test to determine if turnover was significantly higher than nestedness and their relative contributions to the overall Sorensen-based temporal beta diversity. Similarly, we investigated the relative contributions of B metric and C metric to the D metric. Furthermore, we used paired t-tests to assess significant differences in total plant cover and effective number of species of T_1 and T_2 across all 1 m^2 plots. We checked if local contributions to beta diversity (LCBD) of T_1 surveys have significant relationship with temporal beta diversity. As an indicator of human disturbance, species rarity, or other unique ecological features, LCBD could elucidate on the effects of site conditions on community resilience. We used separate analysis of variance (ANOVA) and post-hoc Tukey HSD test to investigate (a) whether native or invasive species have higher SCBD across the 1105 plots, (b) whether the total plant cover of native vs invasive species increased or decreased over the two survey periods, and (c) how significantly different temporal beta diversity measures are across different vegetative classes across all surveyed plots. We used Pearson correlation to test the predictions of the biodiversity-stability hypothesis to investigate if higher alpha diversity indices (i.e., species richness and effective number of species) across the 1 m^2 plots in T_1 surveys would result in lower temporal beta diversity. If species diversity plays a significant role in community resilience, then we would observe negative linear relationships between the T_1 floristic richness and effective number of species and the two temporal beta diversity indices, consistent with the biodiversity-stability hypothesis (Wagg et al. 2022; Eisenhauer et al. 2024). Additionally, we conducted correlation between total variance (SS_{total}) and spatial beta diversity (BD_{total}) in the community data of the 20 NEON sites at T_1 survey and temporal beta diversity measures.

We conducted permutational analysis of variance (PERMANOVA) to investigate if the compositional variance in each of the 20 NEON sites was significant. PERMANOVA is a non-parametric distance-based multivariate analysis suitable for complex and zero-inflated data (Anderson et al. 2008). We performed separate PERMANOVA analysis for each of the 20 NEON sites using the Bray-Curtis distance measure of the community data in both survey years as response variable, except for DELA, GUAN, ONAQ, and PUUM where Gower distance was used to eliminate negative dissimilarity values, and survey years as the explanatory variable. The PERMANOVA, with 999 permutations, were conducted using adonis2() function in the vegan R package. While PERMANOVA test decomposes compositional variance by survey years, it does not account for significant variation within each survey year, therefore, we conducted Permutational Multivariate Analysis of Dispersion (PERMDISP) tests. If

PERMDISP test is nonsignificant, it indicates equal variances explained by the explanatory variable and significant PERMANOVA for the NEON site could be attributed to difference in survey years only. However, if PERMDISP is significant, then variance is explained by both group centroid means and dispersion, representing between and within survey years, respectively. Both the PERMANOVA and PERMDISP analyses were conducted with 999 permutations to assess statistical significance. Additionally, we conducted post-hoc ANOVA and TukeyHSD on the PERMDISP results. We also conducted indicator species analysis for the surveys in both T_1 and T_2 periods to investigate the effects of local environmental changes and track temporal shifts in community structure (Dufrêne and Legendre 1997; Appendix S2, Table S2). Finally, we conducted Bray-Curtis based non-metric multidimensional scaling (NMDS) ordination and plotted a biplot of the first two axes to visualize the shift in community composition and abundance between the two survey years for each of the 20 NEON sites. For DELA, GUAN, ONAQ, and PUUM sites, Gower distance measure was used in the NMDS ordination. The ordination analysis was conducted using the metaMDS() function in vegan R package, and biplots were plotted using ggplot2.

We fitted separate generalized linear mixed models (GLMMs), accounting for the NEON survey nestedness (six 1 m^2 plots in 10 x 10 m plots) and the 20 sites as random effects, to investigate the effects of climatic variability, edaphic factors, elevation, and T_1 alpha diversity on the temporal beta diversity measures. We calculated means, standard deviation, and coefficient of variation of minimum, maximum, and mean monthly temperature and precipitation and actual evapotranspiration, between January 2013 and December 2023 for all plots, and the extracted values for aridity index, all soil variables, T_1 alpha diversity, LCBD, and total plant cover. We conducted z-score transformation on the predictor variables prior to modeling to reduce bias. GLMMs were fitted in lme4 R package version 1.1-38 (Bates et al. 2015), and p values were estimated using the approximate degrees of freedom in lmertest

R package (Kuznetsova et al. 2025). We conducted an ANOVA post-hoc test on the GLMM, and determined the residuals explained by the fixed effects (marginal R^2) and both fixed and random effects (conditional R^2). The best GLMM model among different response variables was selected using the Akaike Information Criterion (AIC). Additionally, we investigated the relative contributions of grouped predictors: climatic variability, site conditions (elevation and LCBD), soil variables, and T_1 alpha diversity (total plant cover and Hill's numbers) on temporal beta diversity using variance partitioning (Borcard et al. 1992). Variance partitioning analysis was conducted in vegan R package version 2.7-2 (Oksanen et al. 2025).

3. Results

3.1 Alpha, spatial and temporal beta, and gamma diversity

We observed significant increases in the plot-level effective number of species (mean of T_1=3.72, mean of T_2=4.21, t=-4.64, df=2155.8, $p<0.01$) and percentage cover (mean of T_1=26.77, mean of T_2=30.69, t=-3.14, df=2200.3, $p<0.01$) between the two survey periods. NEON site-level spatial beta diversity (BD_{total}) ranged from 0.33 in SCBI to 0.87 in SRER, and compositional total sum of squares (SS_{total}) ranged from 1.64 in SCBI to 111.33 in HARV sites (Table 2), while LCBD was low (median = 0.01; min=0.001, max=0.39) across all 1105 plots. Sorensen-based overall temporal beta diversity was considerably high across all plots and is significantly driven by species replacement than by nestedness (Mean of replacement=0.47, mean of nestedness=0.11, t=34.37, df=1477.5, $p<0.01$, n=1088 plots). Complementary to this, species gains (C metric) were significantly higher than species loss (B metric) between time surveys (Mean of B metric=0.31, mean of C metric=0.39, t=-6.70, df=2181.1, $p<0.01$, n=1105 plots; Figure 1). The significant linear relationship between turnover metric and Sorensen temporal beta diversity and between the C metric and D metric were confirmed by correlation tests (Appendix S3, Table S3). Across all 20 NEON sites, 13 sites experienced more floristic gains than loss between the two survey periods (Table 2; Figure 1), which is attributed to new native and invasive plants surveyed in 2023 (Figure 2). Therefore, it is not surprising that gamma diversity increased in 14 of the 20 NEON sites between T_1 and T_2 survey periods (Table 2). Community stability ranged from 0.7 to 217.1 (n=1070 plots) while several plots had infinity values (Appendix S1).

3.2 Patterns of temporal beta across NEON sites

We observed latitudinal gradient in temporal beta diversity, with moderate inverse relationship between temporal beta diversity and latitude (Sorensen: $r=-0.49$, $p<0.01$; TBI: $r=-0.44$, $p<0.01$), which could be explained by moderate overall species gains in NEON sites at lower latitudes ($r=-0.40$, $p<0.01$), while species loss has no latitudinal gradient. We also observed a longitudinal pattern in temporal beta diversity, which increases towards the eastern U.S., primarily driven by higher species gains in the eastern locations of NEON sites (Sorensen: $r=0.47$, $p<0.01$; TBI: $r=0.46$, $p<0.01$; B metric: $r=0.07$, $p=0.02$; C metric: $r=0.37$, $p<0.01$). However, there was no elevational gradient for the measured temporal beta diversity. Among all spatial-temporal beta diversity correlation tests, we observed significant relationship only between BD_{total} and mean species gains ($r=0.58$, $p=0.01$), while LCBD at T_1 had no relationship with any of the temporal beta diversity measures.

Sorensen temporal beta diversity was significantly different across the 10 vegetation classes spread across the 20 NEON sites (ANOVA F=38.75, df=9, $p<0.01$), while TBI was also significantly different across the vegetative classes (ANOVA F=41.44, df=9, $p<0.01$). Post-hoc Tukey multiple comparison of means on the two temporal beta diversity measures showed the highest frequencies of pairwise significant difference among emergent herbaceous wetlands, sedge herbaceous, evergreen forest, sedge herbaceous, and woody wetlands (Table 3). Among all vegetation classes, mixed forests, pasture hay, woody wetlands, deciduous forests, and emergent forests have high values for both temporal beta diversity measures, while emergent herbaceous wetlands have the lowest temporal beta diversity (Appendix S4, Figure S2a-b). Species gains were noticeably high in emergent forests, mixed forests, woody wetlands, and evergreen forests, and low in emergent herbaceous wetlands, grassland herbaceous, and sedge herbaceous wetlands (Appendix S4, Figure S2c). In contrast, species loss was the highest in pasture hay vegetation (Appendix S4, Figure S2d). Stability values were generally small across all sites, thus post-hoc tests revealed very few pairwise significant differences among the vegetation classes (Appendix S1, Figure S1, Table S1).

3.3 Indicators of community shifts across NEON sites

Permutational analysis of variance (PERMANOVA) showed that compositional variance between the two survey years was significantly different in all NEON sites except GUAN, PUUM, RMNP, SCBI, and WREF (Table 4). However, we observed nonsignificant results for the PERMDISP analysis in 11 NEON sites (CLBJ, DELA, HARV, MOAB, NOGP, ORNL, SJER, UKFS, UNDE, WREF, and YELL), indicating that floristic variances in the dataset was equally explained by the two surveys. For NEON sites with significant PERMANOVA and PERMDISP results (e.g., BARR, DSNY, HEAL, ONAQ, and SRER), variance in the community data is explained by both variations between and within survey years (Table 4). Results of Tukey HSD post-hoc test and the median distances of explained variances to the group centroids for both T_1 and T_2 surveys support the PERMDISP results in decomposing the sources of variations in the species composition and abundance data for all NEON sites (Table 4; Figure 3). For example, visual inspection of the dispersion ordination plots shows differences in the perimeter sizes of the T_1 and T_2 group hulls in CLBJ, DSNY, HEAL, SCBI, SJER, SRER, and UNDE (Fig. 3). The indicator species analysis showed an overall increase in indicator species richness between T_1 and T_2 surveys in most of the nested plots, most of which are native plants, but only a few of the floristic indicators in T_1 were retained in T_2 surveys indicating major community shifts in the plots (Table 5, Appendix S2, Table S2).

The non-metric multidimensional scaling (NMDS) biplots (Figure 4) illustrate the low levels of overlap in species composition and abundance between the two surveys across most of the 20 NEON sites. In some sites such as DELA, DSNY, GUAN, and NOGP, biplots indicate that species diversity in T_1 were nearly or completely nested within the T_2 floristic data, but in contrast, biplots for ONAQ, ORNL, SJER, UKFS, and WREF sites showed that T_2 survey data were mostly nested within T_1 community data (Figure 4). Floristic divergence between the two surveys were observed in CLBJ, HEAL, YELL, and PUUM, while high species overlap was observed in UNDE and SCBI (Figure 4).

3.4 Species effects on temporal beta diversity

A total of 1576 species, (n=1302 unique species) representing 629 genera, were surveyed across all 20 NEON sites. This 1576 species comprises 123 invasive species, 1366 native plants, 14 native and introduced infraspecific taxa (NI), and 73 unknown taxa and morphospecies. The effects of invasive species vary widely across the 20 NEON sites. For example, species contributions to beta diversity (SCBD) were significantly different among plants with different native status (ANOVA $F=6.55$, $p<0.01$). Overall, native plants had the highest SCBD values in all NEON sites except in ONAQ, SCBI, and SRER, however, SCBD values had the greatest dispersion for invasive species (Figure 5). Tukey post-hoc test shows significant difference in SCBD values between invasive and native plants ($p<0.01$) and between native and NI plants ($p=0.04$). On one hand, no invasive species were recorded at BARR, HEAL, and WREF sites in both surveys, while the SCBD values for invasive species were very low in YELL and RMNP, indicating minimal effects on plant community structure and dynamics. Additionally, invasive *Poa compressa* and *Lactuca serriola* were extirpated at RMNP, and invasive *Morus alba* was completely undetected in T_2 at UKFS. On the other hand, some NEON sites were impacted by invasive species. ONAQ is severely invaded by nonnative weedy species such as *Alyssum* sp., *Ceratocephala testiculata, Sanguisorba minor*, *Malcomia africana*, *Bromus tectorum,* and *Alyssum alyssoides*. These six invasive plants have relatively high SCBD values, were identified as indicator species, and their total cover increased greatly between surveys. SJER was also noted as one of the most invaded NEON sites, with 20 invasive species recorded between the two surveys. This includes *Bromus diadrus, Erodium botrys,* and *B. hordeaceus* which were noted for relatively high SCBD values. High SCBD values and indicator species status were reported for seven invasive species at ORNL. Invasive species with high SCBD values or indicator species status were noted in SCBI, SRER, HARV, and NOGP sites. We did not observe a significant difference in the total plant cover among the plant species of different native status between the two survey periods (ANOVA F=1.76, p=0.15), however, some invasive species were noted with exceptionally high total plant cover or increases in abundance between the two surveys. This includes *Eragrostis lehmanniana* at SRER having the highest SCBD value and its total cover nearly doubled between T_1 and T_2 survey, *Elaeagnus umbellata, Albizia julibrissin*, and *Rosa multiflora* at ORNL, *B. hordeaceus, Hypochaeris glabra*, *Avena barbata*, and *Carduus pycnocephalus* at SJER, *Berberis thunbergii* and *Celastrus orbiculatus* at HARV, *Triadica sebifera* at DELA, and *Alliaria petiolata* at UKFS. Several sites recorded new invasive species in the T_2 surveys that warrants monitoring and treatments. This includes four invasive species at GUAN, including *Leucaena leucocephala*, which had become an indicator species, three new invasives each at PUUM and UKFS, *Cirsium arvense* at RMNP, *Myosotis scorpioides* at UNDE with relatively large total cover in T_2 survey, and several invasive arrivals at MOAB and NOGP. See Borokini (2026) for the SCBD values of all species across all 20 NEON sites.

3.5 Drivers of temporal beta diversity

Our GLMM analysis with Sorensen temporal beta diversity has the lowest AIC (AIC = -543.9) among the five separate GLMM analyses for different temporal beta diversity measures. This model showed significant effects of three climatic (standard deviation of monthly Tmax, standard deviation of monthly Tmean, and the AET coefficient of variation) and soil (soil pH, soil silt content, fine organic carbon) variables on temporal beta diversity, while several other climatic and soil variables were marginally significant (Table 6). Elevation, and diversity measures of T_1 period did not have significant effects on temporal beta diversity. For the random effects, plot nestedness, but not site differences, contributed to the variance (variance = 0.02, standard deviation = 0.14) in the model. The fixed effects components explained 39.4% of the residuals in the model, while both random and fixed effects jointly explained 64.7% of the model residuals. A separate GLMM with TBI as a response variable (AIC = -429.5) had similar results, however, only three soil variables had significant effects on temporal beta diversity (Table 6). Correlation tests between each of the predictor variables and the three temporal beta diversity measures reveal contrasting relationships (Table S4). Many of the climatic predictors and only two edaphic variables showed significant positive or negative correlations with both Sorensen temporal beta diversity and TBI (Table S4). Additionally, both species richness and diversity of the T_1 surveys have no relationship with the three temporal beta diversity measures.

Variance partitioning decomposed the variation explained by each predictor groups showed that soil and climate variables jointly contribute 25.9% of the variance, while the diversity, soil and climate predictor groups individually contributed 0.2%, 3.5%, and 8.7%, respectively. Site conditions, capturing LCBD and elevation, have no contribution to the variance in temporal beta diversity. Soil components and site conditions jointly contribute another 2.7%, while climate and site conditions explain 0.4% variance, and another 0.3% variance shared by all predictor groups. However, unexplained variance in the model was 62.2% (Figure 6).

Discussion

Our study was the first to investigate temporal beta diversity of plant communities at continental scale across the United States, made possible by the long-term ecological monitoring program of NEON. This study computed several temporal beta diversity measures for 1105 1 m^2 plant communities across 20 NEON long-term monitoring sites representing different eco-climatic domains in the United States and Puerto Rico. Overall, plot-level species diversity and total cover were significantly higher in T_2 surveys, while site-level gamma diversity was higher in the majority of the studied NEON sites. Consequently, we observed high temporal beta diversity across most of the plots, primarily driven by turnover and species gains rather than by nestedness and species losses, respectively. Observed floristic turnover and species gains were characterized by new native plant entries and a few invasive species. We observed latitudinal and longitudinal, but not elevational gradients in floristic temporal beta diversity measures and species gains across all NEON sites. Furthermore, beta diversity was higher for pasture hay, woody wetlands, and mixed forest habitats, but lower in emergent herbaceous wetlands and scrub shrub ecosystems. While invasive species have had severe effects in some NEON sites, we do not find strong association between invasions and temporal beta diversity in most of the sites. GLMM and variance partitioning analyses showed that temporal beta diversity is jointly influenced by soil and climatic variables.

The high temporal beta diversity observed across the 1105 1 m^2 plots is an evidence of community shifts between the two surveys across the 20 NEON sites, and this was corroborated by higher plot-level alpha diversity (that is, Hill’s numbers), total plant percentage cover, beta diversity-based species gains, and site-level gamma diversity measures. This indicates increases in local species richness and total plant covers in these NEON-monitored plots shape temporal beta diversity and community shifts across the U.S. and Puerto Rico (Gotelli et al. 2017). While we anticipate surveyors' bias or errors and limited taxonomic capacities in the earlier surveys, this alone cannot explain the significance of the floristic differences in 1105 1 m^2 plots. Our results are consistent with previous studies that have reported significant temporal beta diversity in different ecosystems (Li et al. 2020; Midolo et al. 2026), increases in species richness or abundances (McCune and Vellend 2013; Steinbauer et al. 2018; Legendre and Condit 2019; Midolo et al. 2026), and stability or increase in the density or cover of native populations over time (Daniëls et al. 2010; Kapfer and Grythnes 2017). PERMANOVA and PERMDISP analyses (Figures 3-4) strongly support the observed community shifts in most of the 20 sites, while the NMDS plots showed low overlap in variance between T1 and Ts surveys, attributing this to significant compositional variance. The effect of repeated surveys (that is, year effect) on species temporal dissimilarities is reported in previous studies (Savage and Vellend 2015; Zorio et al. 2016; Palaj and Kollár 2026). Furthermore, the Indicator species analysis, which identifies plants with relatively high frequency and abundance in specific plots, provides supporting evidence for community shifts. While most of the grouped plots have no indicator species, richness of indicator species increased between T_1 and T_2, and most of the indicator species identified in T_1 were not retained in T_2. This change in indicator species between time periods supports community abundance dynamics and compositional turnover. Shifts in indicator species across plots are indicative of community changes and have been reported in numerous studies (Klinkovská et al. 2025).

Temporal beta diversity is uneven across plant communities in the United States. We observed moderate latitudinal and longitudinal pattern of floristic dissimilarity between surveys, suggesting higher temporal beta diversity in NEON sites in the eastern and southern U.S. and Puerto Rico. This pattern may be driven by community shifts observed in GUAN, HARV, and CLBJ sites. For example, HARV, one of the richest NEON sites, and GUAN experienced major floristic gains, while CLBJ lost almost two-thirds of the 112 species recorded in T_1 in T_2. Additionally, temporal beta diversity was higher in pasture hay, woody wetlands, and mixed forest habitats, but lower in emergent herbaceous wetlands and scrub shrub ecosystems. This may be driven by greater species loss in pasture hay found exclusively in DSNY, while floristic gains were the highest in evergreen forest and mixed forests, associated with the WREF site which also recorded the lowest temporal beta diversity, jointly shared with BARR site. Lack of support for elevational gradient in temporal beta diversity in these 1105 plots is inconsistent with the prediction that species will migrate towards higher altitudes (Savage and Vellend 2015), but is consistent with previous studies where a large number of monitored alpine species exhibited either upward or downward range shifts, or expanded their thermal niches (Chen et al. 2011; 2025; Abella et al. 2019; Downing et al. 2024; Palaj and Kollár 2026). In the remaining five highly invaded NEON sites, historical and current human activities (Table 1), rather than invasive species, are the likely key drivers of higher temporal beta diversity (McCune and Vellend 2013; Abella et al. 2019).

Species contributions to beta diversity (SCBD) were significantly higher for native plants than invasive species, indicating minimal effects of invasive species in most of the NEON sites. Invasive species were not recorded in BARR, WREF, and HEAL sites, which may be due to their remoteness thus reducing human-assisted invasions (Abella et al. 2019). In other cases, such as in UKFS, RMNP, and ONAQ, several invasive species recorded in T_1 were undetected in T_2, and invasive species total cover reduced at CLBJ, these could be attributed to constant monitoring and treatments in these sites. Nonetheless, we observed outlier SCBD values or increased plant cover for invasive species in ONAQ, SJER, ORNL, SRER, SCBI, and NOGP sites. The impact of invasive species is particularly high in ONAQ where many nonnative plants have relatively high SCBD values and were shown as indicator species. Additionally, we observed major increases in total cover of invasive species such as *Eragrostis lehmanniana* (SRER), *Elaeagnus umbellata*, *Albizia julibrissin*, and *Rosa multiflora* (ORNL), *Bromus* *hordeaceus, Hypochaeris glabra, Avena barbata*, and *Carduus pycnocephalus* (SJER), and *Berberis thunbergii* and *Celastrus orbiculatus* (HARV). Moreover, several invasive species were noted among indicator species or those with relatively high SCBD values: *Alyssum* sp., *Ceratocephala testiculata, Bromus tectorum, Alyssum alyssoides, Sanguisorba minor*, and *Malcomia africana* (ONAQ), *Bromus diadrus, Erodium botrys*, and *B. hordeaceus* (SJER), *Cirsium arvense* and *Elymus repens* (NOGP), and *Rubus phoenicolasius* and *Microstegium vimineum* (SCBI). These invasive species should be monitored closely and managed effectively to maintain the biological integrity of these protected sites and prevent biotic homogenization (Li and Waller 2015; Abella et al. 2019). Some of these invasive species have been reported elsewhere to outcompete native plants, slow restoration activities, alter soil chemistry, and impact wildfire frequencies (Rockwell-Postel et al. 2020; Borokini et al. 2021). Broadly, invasive species were linked to temporal beta diversity in Vancouver Island, Canada (McCune and Vellend 2013), and about 25% loss of native flora at Acadia National Park (MacKenzie et al. 2019), but minimal impacts on temporal turnover in New Zealand (Day and Buckley 2013). In our study, only two – CLBJ and ONAQ – of the seven sites with major non-native invasion challenges had reduced gamma diversity, high temporal beta diversity, and higher species losses than gains, thus suggesting limited overall impacts of invasive species in the temporal beta diversity across the 20 NEON sites.

Most of the edaphic variables have minimal effects on plot-level temporal beta diversity, with only soil pH, silt content, and fine organic carbon having statistical effects. Temporal beta diversity has a moderate positive relationship with soil pH, but inverse association with fine organic carbon and soil silt content. Soil pH can be affected by climate extremes, anthropogenic soil acidification, and increased organic matter decomposition (Philippot et al. 2023), which can result in higher shifts in microbial communities (Pu et al. 2023) and cascading effects on plant communities and overall ecosystem functioning (Feng and Deng 2025). Increases in species richness, which drive temporal beta diversity in our study, has been reported to be associated with higher soil organic carbon in literature (Spohn et al. 2023); therefore, an inverse relationship between temporal beta diversity and soil organic carbon in our study highlights its strong association with the aboveground plant communities (Yang et al. 2019; Weiskopf et al. 2024). This relationship is crucial for soil carbon sequestration, an important driver of climate change mitigation (Anderies et al. 2013). Soil silt content determines water retention capacity and nutrient availability, which can affect microbial communities and plant assemblages (Wang et al. 2018), therefore higher temporal beta diversity associated with sites with low silt content suggest greater species gains in well-drained soils (Wang et al. 2025). We expected soil nitrogen to have significant effect on community shifts, due to its influence on primary net productivity, which is expected to increase as human activities produce and fix more atmospheric nitrogen in the soil (Palaj and Kollár 2026); however, our study did not find a statistical association between soil nitrogen and temporal beta diversity. Though these soil variables were measured at one time period and they do not represent changes over time, the result highlights the role of edaphic factors in community assemblages and dynamics (Xue et al. 2019).

Between 2013 and 2023, North America experienced extreme weather events, increased aridity, record-high temperatures, and intense heatwaves (Overpeck and Udall 2020), therefore, we expected strong climatic signal in temporal beta diversity of the studied plant communities. However, only three – standard deviation of monthly Tmax, standard deviation of monthly Tmean, and the AET coefficient of variation – of the 15 climatic variables included in this study had significant effect on temporal beta diversity. Surprisingly, we observed higher temporal beta diversity in sites with lower variability of maximum temperature, actual evapotranspiration, mean temperature, and relative floristic stability in sites that experienced climatic extremes. Habitats with minimal perturbations and stable abiotic conditions may experience post-disturbance succession or competition-driven resource partitioning

(Seddon et al. 2016; Liu et al. 2024), both of which can result in gains in native species, as we have observed in this study. These results are inconsistent with the environmental refugia hypothesis which identifies climate or environmental refugia as sites with relatively low temporal climatic variability and predicts low temporal beta diversity in these locations (ERH; Ashcroft 2010; Keppel et al. 2012; Chen et al. 2025). The ERH prediction is based on empirical evidence that extreme climatic variability significantly reduced alpha diversity in plant communities due to local extirpations (Reyer et al. 2013; Vasseur et al. 2014). However, inverse relationship between climatic stability and temporal beta diversity is not uncommon, as it has been reported for terrestrial vertebrates in the Mediterranean ecosystem of Spain (Martin and Ferrer 2015). The GLMM result was partly corroborated by the variance partitioning analysis which showed that about 38% of the variance in the temporal beta diversity was explained by individual and joint contributions of edaphic, site, species diversity measures, and climatic variables. The low variance explained by these variables may be attributed to the coarse resolution (~ 1 km) of the remotely obtained interpolated climatic and soil data which do not capture microclimatic conditions. While this study did not show strong mechanistic effects of climate change on temporal beta diversity, we caution on dismissing climate effects because ecoregions dominated by long-lived plants such as forest ecosystems, arctic tundra, and alpine habitats, experience lags in climate change effects and may inhabit plants that are resilient to extreme climatic conditions (Daniëls et al. 2010; Bertrand et al. 2011).

In this study, we have shown that most of the 1105 NEON-managed plots exhibit high temporal beta diversity and are experiencing minor to major floristic shifts. In all statistical analyses, plot-level alpha diversity at T_1 has no significant effect on temporal beta diversity, which is incongruent with the predictions of low ecological stability and high temporal beta diversity due to high turnover in sites with low alpha diversity (biodiversity-stability hypothesis; McCann, 2000; Tilman et al. 2006). This could be explained by the fact that temporal beta diversity was higher in NEON sites in eastern U.S. and subtropical ecosystems with greater alpha and gamma diversity. We do not interpret high temporal beta diversity as low ecological resilience in these study systems. Previous studies have shown that changes in ecological communities are driven by higher temperatures especially in alpine sites, anthropogenic-driven soil nitrogen enrichment, biological invasions, and historical and current land use (McCune and Vellend 2013; Stevens et al. 2018; Becker-Scarpitta et al. 2022). Since temporal beta diversity is driven by native species gains and floristic turnover, and minimally affected by invasive species and abiotic factors, we attribute these floristic changes to post-disturbance succession, effects of active restoration projects, or local to regional floristic reshuffling and substitutions of native species. In our study, 10 of the 20 NEON sites were or are under cattle grazing regimes, and another two sites that have experienced historical intensive logging (see Table 1), which can promote invasive species or significant loss in native plant cover (Li and Waller 2015; Abella et al. 2019). In NEON sites where active grazing has ceased, coupled with active management, plant communities may undergo rapid compositional shifts and post-disturbance recovery towards native assemblage (Abella et al. 2019; Palaj and Kollár 2026), resulting in higher temporal beta diversity driven by native species gains and turnover, as we have reported in this study. Sites such as HARV, RMNP, WREF that have high temporal beta diversity despite minimal human degradations and prolonged protection may be experiencing native plant reshuffling of native species, a pattern predicted in neutral theory (Hubbell 2001; Dornelas et al. 2014) and dynamic equilibrium models (Diamond et al. 2016). The two NEON sites in Alaska produced contrasting results – while HEAL exhibited high temporal beta diversity driven by species gains, BARR (the northern most NEON site), experienced one of the lowest community shifts. We anticipate lag effects of climate change on plant communities in BARR, while increased warming may be creating new niches for increased floristic cover and richness. This underscores the importance of historical vegetation records and legacy sites with minimal disturbance for historical floristic reference and comparison (Balaguer et al. 2014) and long-term management for post-disturbance recovery and restoration (Bagchi et al. 2017).

In this study, we compared community assemblages between two time periods ranging from five to 10 years across 20 NEON sites. Long-term ecological surveys can help us identify which species and communities are resilient to environmental changes. While survey data over a few years may capture shifts in communities dominated by annuals, decadal datasets are necessary for communities characterized by long-lived plants and can reliably detect long-term trends and eliminate noise in the data due to interannual variabilities (Zhang et al. 2026). Previous studies in New Zealand tussock grasslands showed reduced species richness in one decade, followed by increased richness the subsequent decade (Day and Buckley 2013). Given the experimental designs used for the NEON plot surveys, we recommend that future studies use species total or relative abundances for the nested (10 m x 10 m plots) rather than the 1 m^2 subplots used in this paper. Previous studies have shown that different taxonomic groups respond to environmental changes differently (Chen et al. 2011; Becker-Scarpitta et al. 2022), therefore, we encourage running similar temporal beta diversity analyses on insect and bird species recorded in these NEON sites concurrently with plant data for cross-taxonomic comparisons (Jarzyna et al. 2022). Species-based analyses may have limited use unless they are paired with trait data and phylogenies to capture the functional diversity and evolutionary history underpinning community resilience vs vulnerability (Jarzyna et al. 2022). For example, species turnover may not be associated with functional turnover and ecosystem functioning shifts, if the replacements are functionally similar to the extirpated taxa (Jarzyna et al. 2022); moreover, biotic homogenization resulting in species gains can be detected when functional identities of the species are included (McCune and Vellend 2013). As long-term ecological datasets continue to increase in spatial and temporal resolution, statistical analyses of these datasets should refine our understanding of biodiversity responses to environmental changes

(Hallet et al. 2016). We also see opportunities for integrating long-term community datasets with remote sensing to detect floristic changes (Schimel et al. 2013).

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Supporting information for

Drivers of temporal beta diversity and ecological resilience of plant communities across the United States

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Appendix S1. Community stability measure

We calculated community stability, which describes the magnitude of overall floristic changes for each plot between the two surveys (Appendix S1). However, this measure was not used for downstream analyses. This is computed as the temporal mean divided by the temporal standard deviation of aggregate species abundances (Hallett et al. 2016; Avolio et al. 2019). This metric is expressed as an inverse of the coefficient of variation for each plot based on factor analysis (Lehman and Tilman 2000; She et al. 2023); higher values indicate less change and more ecological resilience. We calculated community stability values for each survey plot between T_1 and T_2 surveys for all 1 m^2 plots and an overall stability value for each NEON site. Community stability metric was calculated using functions in the codyn R package version 2.0.5 (Hallett et al. 2016; 2020).

Community stability ranged from 0.7 to 217.1 (n=1070 plots), with 35 plots having infinity values and were discarded from downstream analyses. We did not observe latitudinal gradient (r=0.04, p=0.22, n=1070 plots) nor longitudinal structure (r=-0.03, p=0.28, n=1070 plots) in community stability measure across the NEON plots. Across the 1070 1 m^2 plots, community stability was significantly different (ANOVA: F=3.18, df=9, p<0.01) among the 10 vegetation classes (Figure S1). However, pasture hay was the only vegetation class that was significantly different from other vegetation classes (Table S1).

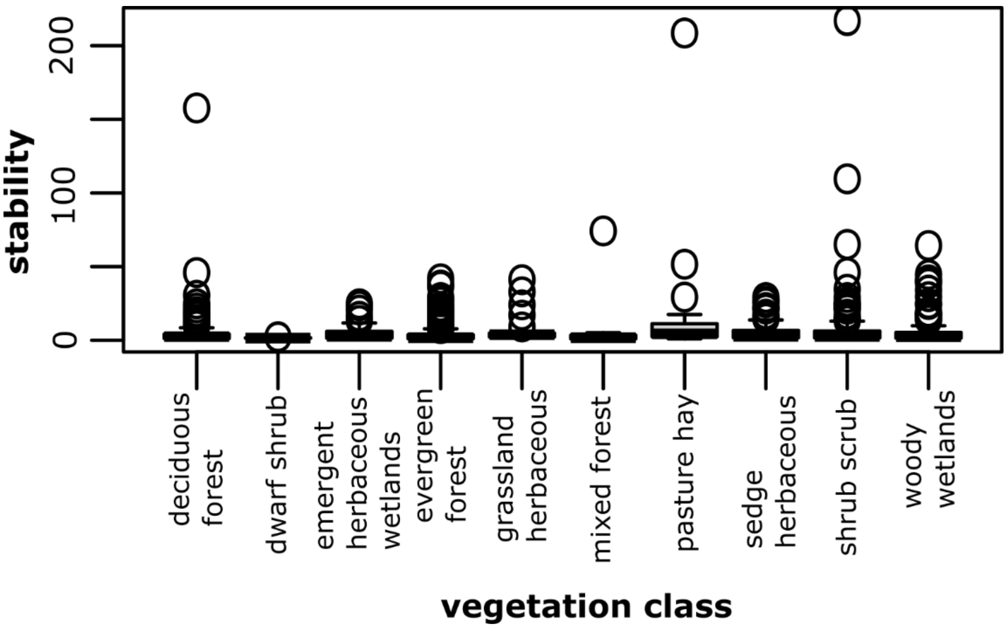

Figure Sup. Methods 1. Boxplots illustrating the spread of community stability measure across the 10 vegetation classes across NEON 1 m^2 (n=1070) plots

Table S1. Adjusted p-values from Tukey post-hoc test on ANOVA between community stability and vegetation classes of the 1 m^2 (n=1070) NEON plots. *Bold texts indicate p values below α=0.05*

Vegetation class	Deciduous forest	Dwarf scrub	Emergent herbaceous wetland	Evergreen forest	Grassland herbaceous	Mixed forest	Pasture hay	Sedge herbaceous	Shrub scrub	Woody wetlands
Deciduous forest	-	0.99	0.99	0.97	0.99	0.99	**<0.01**	1.00	0.99	0.99
Dwarf scrub		-	0.99	0.99	0.99	0.99	0.22	0.99	0.99	0.99
Emergent herbaceous wetland			-	0.99	0.99	1.00	**<0.01**	1.00	0.97	0.99
Evergreen forest				-	0.93	0.99	**<0.01**	0.99	0.16	0.75
Grassland herbaceous					-	0.99	0.23	0.99	1.00	0.99
Mixed forest						-	**0.02**	1.00	0.99	0.99
Pasture hay							-	**<0.01**	**0.02**	**0.01**
Sedge herbaceous								-	0.97	0.99
Shrub scrub									-	0.99

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Appendix S2. Indicator species analysis

We conducted indicator species analysis for the surveys in both T_1 and T_2 periods. Indicator species analysis uses species relative occurrence frequency and abundance to identify species association and affinity with a site and can be used to predict the diversity of the site, investigate the effects of local environmental changes, and track temporal shifts in community structure (Dufrêne and Legendre 1997). Difference in indicator species between surveys could be indicative of changes in the community structure and reduced species evenness in a site. In this study, we grouped the six 1 m^2 plots into their corresponding 10 × 10 m plots and used a permutation test with 999 iterations to assess the statistical significance of site-species affinity. Indicator species analysis was conducted in indicspecies R package version 1.8.0 (De Cáceres and Legendre 2009; De Cáceres et al., 2010).

Indicator species varied widely between the two surveys across most of the NEON sites. Of the 14 indicator species recorded in T_1, only three were retained among the 19 indicator species observed in T_2. At CLBJ, only one of the 10 indicators noted in T_1 was retained in T_2 which had four indicator species. Seven indicator species were recorded in T_1 at DELA, which increased to 11 in T_2 of which only one species was retained. At DSNY, 32 indicator species recorded in 2017 increased to 56 in 2023, which included none of those observed in the 2017 survey. Similar trend was observed in GUAN where none of the 14 indicator species recorded in T_1 was observed among the 13 indicators computed in T_2. Indicator species richness increased from 40 to 52 in HARV of which only two species were retained in both surveys. None of the 15 indicators detected in T_1 were observed among the 10 floristic indicators in T_2 at HEAL. Fifteen indicators detected in T_1 for MOAB slightly reduced to 14 in T_2 including one species that was observed in both surveys. Eight indicator species were calculated in both T_1 and T_2 at NOGP, but only one of the species was common to both surveys. A total of 32 indicator species across 16 nested plots were observed in 2016 at ONAQ, which reduced to 17 indicator species across 11 nested plots in 2023, of which only one indicator species was common to both surveys. In PUUM, one species was retained among the five indicator species, each detected in T_1 and T_2 surveys. RMNP has three indicator species in 2017, all of which were lost in the 2023 survey having no indicator species. The 2015 survey at SJER produced three indicators which were replaced by two different floristic indicators in 2023. Seven of the 12 indicator species in 2016 were retained in the 2023 survey which had 26 indicators. Two different sets of three indicators species each were detected in both surveys at UKFS. The 2014 survey at UNDE produced 10 indicator species which increased to 21 in 2023 including seven that were common to both surveys. Two of the four indicator species recorded at WREF in T_1 survey were among the five indicators detected in T_2. ORNL produced 31 indicator species in 2014, 11 of which were noted among the 37 floristic indicators detected in the 2023 survey. SCBI and YELL did not record any indicator species in both surveys. Additionally, many of the NEON sites, the nested plots that produced indicator species in T_1 were replaced by other nested plots in T_2 (Table S3).

Table S2. List of indicator species identified across nested 10 × 10 m plots in each of the 20 NEON sites surveyed in two time periods. ***Asterisk on a species in T_1 indicates it was an indicator species in both T_1 and T_2 periods. SCBI and YELL NEON sites were not shown because no indicator species was computed from them.***

Site name	Plot ID	T_1 year	Indicator species	Indicator value	p value	T_2 year	Indicator species	Indicator value	p value
BARR	p1-6	2017	-	-	-	2023	*Salix pulchra*	0.46	0.02
	p19-24		-	-	-		*Calamagrostis holmii*	0.47	0.03
	p37-42		*Saxifraga cernua*	0.34	0.01		-	-	-
			Ranunculus nivalis	0.32	0.04		-	-	-
	p43-48		-	-	-		*Saxifraga foliolosa*	0.34	0.01
	p49-54		-	-	-		*Caltha palustris*	0.39	0.04
	p55-60		-	-	-		*Saxifraga nelsoniana*	0.57	<0.01
			-	-	-		*Saxifraga hieraciifolia*	0.43	0.03
			-	-	-		*Stellaria longipes*	0.42	<0.01
			-	-	-		*Ranunculus nivalis*	0.41	<0.01
	p73-78		-	-	-		*Potentilla nana*	0.66	<0.01

			-	-	-		*Luzula confusa*	0.43	0.03
			-	-	-		*Petasites frigidus*	0.42	0.01
			-	-	-		*Saxifraga cernua*	0.37	<0.01
	p85-89		-	-	-		*Carex aquatilis*	0.36	0.01
	p102-107		*Cardamine pratensis*	0.61	<0.01		-	-	-
			Saxifraga hirculus	0.59	<0.01		-	-	-
			Polygonum viviparum	0.53	<0.01		-	-	-
			Saxifraga hieraciifolia	0.39	0.05		-	-	-
	p108-113		*Luzula arctica**	0.53	<0.01		*Luzula arctica*	0.56	<0.01
	p120-125		*Arctophila fulva**	0.54	<0.01		*Arctophila fulva*	0.54	<0.01
	p150-155		-	-	-		*Polygonum viviparum*	0.39	0.05
	p156-161		*Ranunculus pallasii**	0.54	0.02		*Ranunculus pallasii*	0.57	0.01
	p168-173		*Eriophorum angustifolium*	0.58	0.03		-	-	-
			Dupontia fisheri	0.51	<0.01		-	-	-
			Petasites frigidus	0.44	0.01		-	-	-
			Cerastium beeringianum	0.44	0.03		-	-	-
			Saxifraga foliolosa	0.31	<0.01		-	-	-
			-	-	-		*Arctagrostis latifolia*	0.81	<0.01
	p174-179		-	-	-		*Hierochloe pauciflora*	0.52	<0.01
CLBJ	p1-6	2016	*Symphyotrichum patens* var. *patens*	0.91	<0.01	2023	-	-	-
			Bulbostylis sp.	0.91	<0.01		-	-	-
			Helianthemum georgianum	0.91	<0.01		-	-	-
			Dichanthelium sphaerocarpon	0.9	<0.01		-	-	-
			Bouteloua curtipendula	0.9	<0.01		-	-	-
			Scleria ciliata	0.9	<0.01		-	-	-
			Liatris sp.	0.82	0.01		-	-	-
			Dalea purpurea	0.82	0.01		-	-	-
			-	-	-		*Acalypha monococca*	0.81	0.03
	p7-12		*Parthenocissus quinquefolia**	0.82	0.02		*Schizachyrium scoparium*	0.82	0.02
			-	-	-		*Croton glandulosus*	0.82	0.02

			-	-	-		*Parthenocissus quinquefolia*	0.79	0.02
	p13-18		*Oxalis dillenii*	0.82	0.02		-	-	-
DELA	p1-6	2015	-	-	-	2023	*Pinus taeda*	1	<0.01
			-	-	-		*Parthenocissus quinquefolia*	0.74	0.03
			-	-	-		*Boehmeria cylindrica*	0.71	0.04
			-	-	-		*Melothria pendula*	0.71	0.03
	p7-12		*Trachelospermum difforme**	0.91	<0.01		*Proserpinaca palustris*	0.82	<0.01
			Rudbeckia sp.	0.77	0.01		*Trachelospermum difforme*	0.82	0.01
			-	-	-		*Justicia ovata*	0.71	0.03
	p13-18		*Ageratina altissima*	1	<0.01		*Elephantopus carolinianus*	0.82	<0.01
			Panicum sp.	0.82	0.01		*Equisetum hyemale*	0.82	0.01
			Pluchea sp.	0.71	0.04		*Arundinaria gigantea*	0.77	<0.01
			Parthenocissus quinquefolia	0.69	0.02		-	-	-
	p19-24		*Polygonum virginianum**	0.71	0.05		*Polygonum virginianum*	0.71	0.04
DSNY	p1-6	2017	*Quercus myrtifolia*	0.58	0.04	2023	-	-	-
	p7-12		*Polypremum procumbens*	0.78	<0.01		*Paspalum notatum*	0.82	<0.01
			-	-	-		*Cyperus retrorsus*	0.64	<0.01
			-	-	-		*Paspalum setaceum*	0.59	<0.01
	p13-18		*Paspalum sp.*	0.66	<0.01		-	-	-
			Paspalum setaceum	0.57	0.01		-	-	-
			Desmodium triflorum	0.56	0.02		-	-	-
	p19-24		*Pityopsis sp.*	0.46	0.05		*Euthamia caroliniana*	0.76	<0.01
			-	-	-		*Panicum anceps*	0.69	<0.01
			-	-	-		*Chamaecrista nictitans*	0.61	0.02
			-	-	-		*Eleocharis* sp.	0.59	<0.01
			-	-	-		*Desmodium triflorum*	0.58	0.04
			-	-	-		*Amphicarpum muehlenbergianum*	0.54	0.05
	p25-30		*Vaccinium myrsinites*	0.76	<0.01		*Houstonia procumbens*	0.66	0.01
			Viola lanceolata	0.578	0.05		*Oldenlandia uniflora*	0.63	<0.01
			Galactia elliottii	0.48	0.04		-	-	-
	p31-36		*Xyris caroliniana*	0.72	<0.01		*Rubus cuneifolius*	0.81	<0.01
			Centella asiatica	0.71	<0.01		*Pinus elliottii* var. *densa*	0.60	0.03

	Eleocharis baldwinii	0.6	0.01	-	-	-
	Euthamia caroliniana	0.57	<0.01	-	-	-
	Schizachyrium scoparium	0.53	0.03	-	-	-
p37-42	*Paspalum notatum*	0.61	<0.01	-	-	-
p43-48	-	-	-	*Licania michauxii*	0.68	<0.01
	-	-	-	*Pityopsis* sp.	0.58	<0.01
	-	-	-	*Lechea* sp.	0.53	0.03
	-	-	-	*Gratiola hispida*	0.47	0.03
	-	-	-	*Xyris caroliniana* var. *platylepis*	0.45	0.03
p49-54	*Elephantopus elatus*	0.53	0.05	*Chapmannia floridana*	0.76	<0.01
	-	-	-	*Bulbostylis ciliatifolia*	0.72	<0.01
	-	-	-	*Quercus minima*	0.63	<0.01
	-	-	-	*Galactia elliottii*	0.47	0.04
p55-60	*Gaylussacia dumosa*	0.71	<0.01	*Rhexia nuttallii*	0.85	<0.01
	Chamaecrista nictitans	0.58	0.01	*Gaylussacia nana*	0.71	<0.01
	Asimina reticulata	0.46	0.02	*Ilex glabra*	0.61	<0.01
	-	-	-	*Oclemena reticulata*	0.52	0.02
	-	-	-	*Serenoa repens*	0.45	0.01
p61-66	*Licania michauxii*	0.71	<0.01	*Rhexia mariana*	0.86	<0.01
	Lechea sp.	0.68	<0.01	*Drosera capillaris*	0.82	<0.01
	-	-	-	*Rhynchospora* sp.	0.74	<0.01
	-	-	-	*Rhynchospora ciliaris*	0.71	<0.01
	-	-	-	*Oxypolis filiformis*	0.71	<0.01
	-	-	-	*Andropogon glaucopsis*	0.63	<0.01
	-	-	-	*Xyris* sp.	0.56	0.01
	-	-	-	*Centella asiatica*	0.55	0.01
	-	-	-	*Scleria muehlenbergii*	0.54	0.02
	-	-	-	*Hypericum* sp.	0.52	0.05
	-	-	-	*Hypericum cistifolium*	0.46	0.02
p67-72	*Scleria* sp.	0.81	<0.01	*Drosera brevifolia*	0.6	0.02
	Panicum anceps	0.71	<0.01	*Galactia regularis*	0.6	0.01
	Asteraceae	0.69	0.01	*Asimina reticulata*	0.51	0.01
	Rhexia sp.	0.65	<0.01	-	-	-

			Aristida beyrichiana	0.62	<0.01		-	-	-
	p73-78		*Chapmannia floridana*	0.62	<0.01		*Symphyotrichum subulatum*	0.91	<0.01
			Gratiola hispida	0.48	0.05		*Solidago* sp.	0.76	<0.01
			Quercus minima	0.44	0.04		*Viola lanceolata*	0.75	<0.01
			-	-	-		*Ludwigia suffruticosa*	0.71	<0.01
			-	-	-		*Mikania scandens*	0.71	<0.01
			-	-	-		*Rhexia* sp.	0.65	0.01
			-	-	-		*Woodwardia virginica*	0.63	0.01
			-	-	-		*Fuirena breviseta*	0.58	0.05
			-	-	-		*Hyptis alata*	0.55	0.05
			-	-	-		*Sacciolepis striata*	0.58	0.05
			-	-	-		*Cyperaceae*	0.52	0.03
			-	-	-		*Proserpinaca pectinata*	0.52	0.05
	p85-90		*Oclemena reticulata*	0.82	<0.01		*Axonopus fissifolius*	0.79	<0.01
			Rhexia nuttallii	0.59	0.01		*Eragrostis* sp.	0.65	<0.01
			Ilex glabra	0.57	0.01		*Dichanthelium* sp.	0.52	<0.01
GUAN	p1-6	2015	-	-	-	2023	*Turnera diffusa*	0.58	0.04
	p7-12		*Cissus trifoliata*	0.71	<0.01		*Croton discolor*	0.68	<0.01
			Teramnus labialis	0.58	<0.01		*Sida abutifolia*	0.58	0.04
			Comocladia dodonaea	0.51	0.05		-	-	-
	p13-18		*Lasiacis divaricata*	0.67	0.01		-	-	-
			Plumeria alba	0.57	0.04		-	-	-
	p31-36		-	-	-		*Hibiscus phoeniceus*	0.6	0.03
	p37-42		Cactaceae	0.55	0.01		-	-	-
			Pithecellobium unguis-cati	0.5	0.02		-	-	-
	p73-78		-	-	-		*Leucaena leucocephala*	0.6	<0.01
			-	-	-		*Distictis lactiflora*	0.59	<0.01
			-	-	-		*Serjania polyphylla*	0.58	0.03
			-	-	-		*Capparis hastata*	0.58	0.03
			-	-	-		*Swietenia mahagoni*	0.58	0.05
			-	-	-		*Argythamnia candicans*	0.51	0.01
	p79-84		*Capparis sp.*	0.56	0.04		-	-	-
	p91-96		*Schaefferia frutescens*	0.58	0.04		-	-	-
			Eugenia rhombea	0.54	0.01		-	-	-

	p103-108		-	-	-		*Capparis indica*	0.58	0.04
	p109-114		*Guaiacum officinale*	0.78	<0.01		-	-	-
			Amyris elemifera	0.48	0.01		-	-	-
	p115-120		*Serjania polyphylla*	1	<0.01		*Schaefferia frutescens*	0.58	0.03
			Capparis hastata	0.58	0.04		*Eugenia rhombea*	0.54	0.01
HARV	p1-6	2015	*Prunus serotina*	0.46	0.03	2023	*Mitchella repens*	0.6	<0.01
			Pinus strobus	0.42	0.01		*Prunus pensylvanica* var. *pensylvanica*	0.5	0.01
	p13-18		*Lycopodium obscurum*	0.72	<0.01		*Betula papyrifera*	0.58	0.04
			Vaccinium corymbosum	0.54	<0.01		-	-	-
	p19-24		*Rubus idaeus* ssp. *strigosus*	0.76	<0.01		*Celastrus orbiculatus*	0.58	0.04
			Galium triflorum	0.58	0.04		-	-	-
			*Celastrus orbiculatus**	0.58	0.05		-	-	-
	p31-36		-	-	-		*Vaccinium pallidum*	0.42	0.04
			-	-	-		*Carex* sp.	0.41	0.01
	p37-42		-	-	-		*Uvularia sessilifolia*	0.56	0.01
			-	-	-		*Corylus cornuta* var. *cornuta*	0.56	0.04
			-	-	-		*Quercus alba*	0.49	0.04
			-	-	-		*Dennstaedtia punctilobula*	0.43	0.04
	p49-54		*Lyonia ligustrina* var. *ligustrina*	0.59	0.01		*Parthenocissus quinquefolia*	0.71	<0.01
			Gaylussacia baccata	0.46	0.01		*Impatiens capensis*	0.65	<0.01
			-	-	-		*Arisaema triphyllum ssp. triphyllum*	0.58	<0.01
			-	-	-		*Hydrocotyle americana*	0.58	0.05
			-	-	-		*Geranium maculatum*	0.58	0.04
			-	-	-		*Oxalis stricta*	0.58	0.04
			-	-	-		*Toxicodendron radicans*	0.58	0.05
			-	-	-		*Brachyelytrum erectum*	0.53	0.02
			-	-	-		*Ilex mucronata*	0.46	0.03
	p55-60		*Rubus pubescens* var. *pubescens*	0.7	<0.01		*Maianthemum canadense*	0.42	0.01

	Arisaema triphyllum ssp. *triphyllum*	0.69	<0.01	-	-	-
	Parthenocissus quinquefolia	0.55	0.04	-	-	-
p61-66	*Quercus alba*	0.53	<0.01	*Prenanthes* sp.	0.91	<0.01
	Uvularia sessilifolia	0.39	0.04	*Viola* sp.	0.84	<0.01
	-	-	-	*Viola labradorica*	0.82	<0.01
	-	-	-	*Fraxinus americana*	0.8	<0.01
	-	-	-	*Athyrium filix femina ssp. angustum*	0.8	<0.01
	-	-	-	*Betula alleghaniensis*	0.74	<0.01
	-	-	-	*Panax trifolius*	0.71	<0.01
	-	-	-	*Acer nigrum*	0.71	<0.01
	-	-	-	*Carex blanda*	0.63	0.01
	-	-	-	*Polygonatum pubescens*	0.58	0.03
	-	-	-	*Amphicarpaea bracteata*	0.58	0.03
	-	-	-	*Tiarella cordifolia*	0.58	0.04
p67-72	*Medeola virginiana*	0.45	0.01	-	-	-
	Dennstaedtia punctilobula	0.43	0.02	-	-	-
p73-78	*Dryopteris intermedia*	0.46	0.05	*Gaultheria procumbens*	0.65	<0.01
p79-84	*Prenanthes* sp.	1	<0.01	*Thelypteris noveboracensis*	0.92	<0.01
	Viola sp.	0.99	<0.01	-	-	-
	Fraxinus americana	0.83	<0.01	-	-	-
	Athyrium filix-femina ssp. *angustum*	0.75	<0.01	-	-	-
	Polygonatum pubescens	0.58	0.04	-	-	-
	Deparia acrostichoides	0.58	0.04	-	-	-
	Amphicarpaea bracteata	0.58	0.04	-	-	-
	Tiarella cordifolia var. *cordifolia*	0.58	0.03	-	-	-
	Anemone quinquefolia var. *quinquefolia*	0.5	0.01	-	-	-
p85-90	-	-	-	*Betula populifolia*	0.71	<0.01
	-	-	-	*Ilex verticillata*	0.58	0.04
	-	-	-	*Onoclea sensibilis*	0.43	0.04

	p91-96		-	-	-		*Thelypteris simulata*	0.82	<0.01
			-	-	-		*Lysimachia terrestris*	0.82	<0.01
			-	-	-		*Toxicodendron vernix*	0.71	<0.01
			-	-	-		*Spiraea alba* var. *latifolia*	0.71	<0.01
			-	-	-		*Phragmites australis* ssp. *australis*	0.71	<0.01
			-	-	-		*Symphyotrichum lanceolatum*	0.69	<0.01
			-	-	-		*Carex echinata*	0.58	0.04
			-	-	-		*Frangula alnus*	0.58	0.04
			-	-	-		*Thelypteris palustris* var. *pubescens*	0.56	0.04
			-	-	-		*Rubus hispidus*	0.49	0.01
	p97-102		*Vaccinium angustifolium**	0.49	<0.01		*Vaccinium angustifolium*	0.51	<0.01
	p103-108		*Thelypteris noveboracensis*	0.85	<0.01		*Acer saccharum* var. *saccharum*	0.43	0.04
	p109-114		*Fraxinus* sp.	0.58	0.04		*Hamamelis virginiana*	0.75	<0.01
			Betula sp.	0.58	0.04		*Osmunda claytoniana*	0.65	0.01
			Osmunda cinnamomea var. *cinnamomea*	0.47	0.01		-	-	-
	p115-120		*Thelypteris simulata*	1	<0.01		-	-	-
			Phragmites australis ssp. *australis*	0.91	<0.01		-	-	-
			Carex sp.	0.85	<0.01		-	-	-
			Spiraea alba var. *latifolia*	0.82	<0.01		-	-	-
			Lysimachia terrestris	0.82	<0.01		-	-	-
			Spiraea tomentosa	0.71	<0.01		-	-	-
			Rubus hispidus	0.7	<0.01		-	-	-
			Symphoricarpos sp.	0.69	<0.01		-	-	-
			Drosera rotundifolia var. *rotundifolia*	0.58	0.04		-	-	-
	p127-132		-	-	-		*Aralia nudicaulis*	0.65	<0.01
HEAL	p31-36	2015	*Mertensia paniculata*	0.91	<0.01	2023	-	-	-
			Equisetum sylvaticum	0.82	<0.01		-	-	-
			Cornus canadensis	0.82	<0.01		-	-	-

			Arctagrostis latifolia	0.76	<0.01		-	-	-
			Ranunculaceae	0.71	<0.01		-	-	-
			Chamerion angustifolium	0.71	0.01		-	-	-
	p13-18		*Eriophorum vaginatum*	0.86	<0.01		*Ledum palustre*	0.5	0.01
	p7-12		*Arctostaphylos alpina*	0.66	0.02		*Alnus viridis*	0.82	<0.01
			-	-	-		*Petasites frigidus*	0.79	<0.01
	p19-24		*Spiraea stevenii*	0.71	0.01		*Salix pulchra*	0.96	<0.01
			Comarum palustre	0.71	0.01		*Calamagrostis canadensis*	0.76	<0.01
			Picea mariana	0.71	0.01		*Equisetum arvense*	0.62	0.02
			*Equisetum arvense**	0.66	0.02		-	-	-
	p37-42		*Calamagrostis stricta*	0.65	<0.01		*Empetrum nigrum*	0.55	0.05
			Vaccinium uliginosum	0.48	0.02		-	-	-
	p1-6		-	-	-		*Spiraea stevenii*	0.77	0.01
			-	-	-		*Calamagrostis lapponica*	0.71	<0.01
			-	-	-		*Rubus chamaemorus*	0.57	0.01
	p25-30		*Ledum palustre*	0.63	<0.01		-	-	-
MOAB	p1-6	2015	*Hesperostipa comata*	0.82	<0.01	2023	*Ephedra viridis*	0.73	<0.01
			*Ephedra viridis**	0.67	<0.01		-	-	-
	p7-12		*Chaetopappa ericoides**	0.63	0.01		*Chaetopappa ericoides*	0.83	<0.01
			-	-	-		*Vulpia octoflora*	0.49	<0.01
	p13-18		*Vulpia octoflora*	0.43	0.01		*Bouteloua gracilis*	0.5	0.05
	p19-24		*Chaenactis stevioides*	0.73	<0.01		*Hesperostipa comata*	1	<0.01
			Ipomopsis longiflora	0.71	0.01		*Oenothera albicaulis*	0.47	0.04
	p25-30		*Krascheninnikovia lanata*	0.71	0.01		*Cryptantha* sp.	0.66	<0.01
	p31-36		*Gutierrezia sarothrae**	0.75	<0.01		*Gutierrezia sarothrae*	0.71	<0.01
			Aristida purpurea	0.58	0.01		-	-	-
			Sphaeralcea coccinea	0.55	0.01		-	-	-
	p37-42		*Plantago patagonica**	0.47	0.01		*Androstephium breviflorum*	0.82	<0.01
			-	-	-		*Achnatherum hymenoides*	0.52	0.03
			-	-	-		*Plantago patagonica*	0.45	0.01

	p43-46		*Pinus edulis**	0.7	0.02		*Pinus edulis*	0.71	0.01
			Draba sp.*	0.6	0.01		*Draba* sp.	0.67	<0.01
	p47-52		*Arabis* sp.	0.91	<0.01		*Arabis pulchra*	0.71	0.01
	p53-58		*Aliciella hutchinsifolia*	0.58	0.02		-	-	-
NOGP	p1-6	2016	*Anemone cylindrica*	1	<0.01	2023	*Cirsium flodmanii*	0.82	0.01
			*Cirsium flodmanii**	1	<0.01		*Anemone canadensis*	0.82	0.01
			Nassella viridula	0.88	<0.01		*Symphoricarpos occidentalis*	0.77	0.02
			Melilotus officinalis	0.79	<0.01		*Amorpha canescens*	0.71	0.04
			Lactuca tatarica	0.69	0.04		-	-	-
	p13-18		*Euphorbia esula*	0.83	0.01		-	-	-
	p16-21		*Astragalus agrestis*	0.82	0.01		*Bromus inermis*	0.67	<0.01
			Cirsium arvense	0.77	0.01		-	-	-
	p22-26		-	-	-		*Asclepias verticillata*	0.78	0.02
			-	-	-		*Gypsophila paniculata*	0.63	0.04
			-	-	-		*Lygodesmia juncea*	0.63	0.05
ONAQ	p1-6	2016	*Bromus* sp.	0.91	<0.01	2023	*Lactuca serriola*	0.91	<0.01
			*Lactuca serriola**	0.83	<0.01		*Sanguisorba minor*	0.82	<0.01
			*Sanguisorba minor**	0.82	<0.01		*Pascopyrum smithii*	0.71	<0.01
			Onobrychis viciifolia	0.82	<0.01		*Tragopogon dubius*	0.55	0.02
			Vicia americana	0.59	<0.01		-	-	-
			*Tragopogon dubius**	0.58	0.05		-	-	-
			Alyssum alyssoides	0.51	<0.01		-	-	-
	p7-12		*Artemisia tridentata*	0.46	0.04		*Sarcobatus vermiculatus*	0.58	0.04
			-	-	-		*Ceratocephala testiculata*	0.41	<0.01
	p19-24		*Agropyron cristatum*	1	<0.01		-	-	-
	p25-30		*Allium* sp.	0.58	0.05		*Physaria chambersii*	0.71	<0.01
	p31-36		*Halogeton glomeratus*	0.82	<0.01		-	-	-
			Elymus elymoides	0.76	<0.01		-	-	-
			Sarcobatus vermiculatus	0.71	0.01		-	-	-
	p37-42		*Ceratocephala testiculata*	0.39	<0.01		*Allium* sp.	0.58	0.05
	p49-54		*Sphaeralcea parvifolia*	0.71	<0.01		-	-	-
			Descurainia pinnata	0.47	<0.01		-	-	-

	p55-60		*Sisymbrium altissimum*	0.5	0.03		-	-	-
	p61-66		*Bromus tectorum*	0.59	0.03		*Purshia tridentata*	0.58	0.04
	p67-72		*Chrysothamnus viscidiflorus*	0.6	<0.01		*Phlox hoodii*	0.53	0.01
			Atriplex confertifolia	0.55	<0.01		-	-	-
	p79-84		*Malcolmia africana*	0.67	<0.01		*Chrysothamnus viscidiflorus*	0.58	<0.01
			Alyssum sp.	0.35	0.02		-	-	-
	p85-90		*Crepis intermedia*	0.61	<0.01		-	-	-
			Symphoricarpos oreophilus	0.58	0.04		-	-	-
			Elymus sp.	0.51	0.01		-	-	-
	p91-96		*Purshia tridentata*	0.58	0.04		*Gilia inconspicua*	0.58	0.01
	p97-102		*Poa fendleriana*	0.71	0.04		*Malcolmia africana*	0.55	0.01
			Calochortus nuttallii	0.54	0.01		-	-	-
			Poa secunda	0.46	<0.01		-	-	-
			Astragalus sp.	0.45	0.02		-	-	-
	p103-108		*Artemisia nova*	0.71	<0.01		-	-	-
			-	-	-		*Sphaeralcea coccinea*	0.87	<0.01
			-	-	-		*Bromus tectorum*	0.5	<0.01
			-	-	-		*Alyssum* sp.	0.37	<0.01
	p109-114		*Pseudoroegneria spicata*	0.66	<0.01		*Pseudoroegneria spicata*	0.64	<0.01
PUUM	p1-6	2018	*Hedyotis terminalis*	0.71	0.05	2023	*Cibotium menziesii*	0.71	0.04
			Broussaisia arguta	0.71	0.04		-	-	-
	p7-12		*Uncinia uncinata**	0.82	0.01		*Vaccinium calycinum*	0.91	<0.01
			-	-	-		*Uncinia uncinata*	0.82	0.02
	p19-24		*Dryopteris wallichiana**	0.82	<0.01		*Dryopteris wallichiana*	0.85	<0.01
			*Elaphoglossum wawrae**	0.71	0.05		*Elaphoglossum wawrae*	0.71	0.05
RMNP	p1-6	2017	*Androsace septentrionalis*	0.8	0.04	2023	-	-	-
			Achillea millefoium	0.78	0.04		-	-	-
	p13-18		*Gayophytum diffusum*	0.85	<0.01		-	-	-
SJER	p1-6	2015	-	-	-	2023	*Lupinus bicolor*	0.78	0.04
			-	-	-		*Carduus pycnocephalus*	0.76	0.04
	p7-12		*Avena barbata*	0.95	0.01		-	-	-
			Bromus hordeaceus	0.92	0.02		-	-	-

	p13-18		*Amsinckia menziesii*	0.77	0.02		-	-	-
SRER	p1-6	2016	*Eragrostis lehmanniana**	0.76	<0.01	2023	*Gilia flavocincta*	1	<0.01
			-	-	-		*Eragrostis lehmanniana*	0.75	<0.01
	p7-12		-	-	-		*Evolvulus alsinoides*	1	<0.01
			-	-	-		*Gomphrena sonorae*	0.82	<0.01
			-	-	-		*Lepidium densiflorum*	0.82	<0.01
			-	-	-		*Ayenia filiformis*	0.69	0.01
	p13-18		*Chenopodium incanum*	0.83	<0.01		*Evolvulus arizonicus*	0.91	<0.01
			Plantago patagonica	0.82	<0.01		*Spermolepis echinata*	0.82	<0.01
			*Calliandra eriophylla**	0.65	<0.01		*Androsace occidentalis*	0.76	<0.01
			-	-	-		*Daucus pusillus*	0.71	<0.01
			-	-	-		*Mimosa dysocarpa*	0.71	0.01
			-	-	-		*Bouteloua curtipendula*	0.71	<0.01
			-	-	-		*Calliandra eriophylla*	0.64	<0.01
	p31-36		*Digitaria californica**	0.71	<0.01		*Allionia incarnata*	0.71	<0.01
			*Allionia incarnata**	0.63	0.02		*Digitaria californica*	0.59	0.04
			Sida abutifolia	0.58	0.02		-	-	-
	p43-48		*Erodium texanum*	0.86	<0.01		*Isocoma tenuisecta*	0.64	0.02
			Anthemis cotula	0.82	<0.01		-	-	-
	p49-54		-	-	-		*Senecio flaccidus*	0.61	0.02
			-	-	-		*Argythamnia neomexicana*	0.49	0.04
	p61-66		*Zinnia acerosa**	0.97	<0.01		*Sporobolus contractus*	0.89	<0.01
			*Bahia absinthifolia**	0.82	<0.01		*Bahia absinthifolia*	0.82	<0.01
			*Dasyochloa pulchella**	0.82	<0.01		*Allionia choisyi*	0.71	<0.01
			-	-	-		*Acourtia nana*	0.71	0.01
			-	-	-		*Dasyochloa pulchella*	0.71	0.01
			-	-	-		*Lepidium lasiocarpum*	0.71	0.01
			-	-	-		*Zinnia acerosa*	0.67	0.01
			-	-	-		*Pectocarya recurvata*	0.58	0.02
UKFS	p1-6	2015	*Carya ovata*	0.91	0.01		*Ulmus americana*	0.91	0.02
			-	-	-		*Alliaria petiolata*	0.91	0.01
	p7-12		*Celtis occidentalis*	1	<0.01		*Carex* sp.	0.86	0.01

			Phryma leptostachya	0.91	0.01	-	-	-
UNDE	p1-6	2014	*Galium tinctorium*	0.73	0.012	*Orthilia secunda*	0.71	0.03
			-	-	-	*Triadenum fraseri*	0.71	0.03
	p7-12		*Equisetum sylvaticum*	0.82	0.004	*Carex brunnescens*	0.77	0.01
	p13-18		*Lycopodium annotinum**	1	<0.01	*Carex communis*	0.82	<0.01
			*Lycopodium dendroideum**	0.91	<0.01	*Lycopodium annotinum*	0.82	0.01
			-	-	-	*Oryzopsis asperifolia*	0.82	0.01
			-	-	-	*Carex pedunculata*	0.73	0.01
			-	-	-	*Maianthemum canadense*	0.73	0.01
			-	-	-	*Lycopodium dendroideum*	0.71	0.03
	p19-24		*Ledum groenlandicum**	1	<0.01	*Ledum groenlandicum*	1	<0.01
			Gaultheria hispidula	0.91	<0.01	*Menyanthes trifoliata*	0.91	<0.01
			*Menyanthes trifoliata**	0.91	<0.01	*Vaccinium oxycoccos*	0.91	<0.01
			*Vaccinium oxycoccos**	0.82	0.01	*Chamaedaphne calyculata*	0.82	<0.01
			Carex trisperma	0.75	0.02	*Kalmia polifolia*	0.82	<0.01
			*Chamaedaphne calyculata**	0.71	0.03	*Picea mariana*	0.77	0.01
			-	-	-	*Carex chordorrhiza*	0.71	0.03
			-	-	-	*Gaultheria hispidula*	0.71	0.03
			-	-	-	*Maianthemum trifolium*	0.7	0.02
			-	-	-	*Ilex mucronata*	0.68	0.03
	p25-30		-	-	-	*Acer spicatum*	0.71	0.03
			-	-	-	*Luzula acuminata*	0.71	0.02
WREF	p1-6	2016	*Tsuga heterophylla**	0.98	<0.01	*Tsuga heterophylla*	0.93	<0.01
			Pteridium aquilinum	0.9	<0.01	*Gaultheria shallon*	0.85	<0.01
			*Gaultheria shallon**	0.87	0.01	*Linnaea borealis*	0.84	0.01
	p7-12		*Mahonia nervosa**	0.82	0.02	*Mahonia nervosa*	0.75	0.05
	p13-18		-	-	-	*Symphoricarpos albus*	0.82	0.01
ORNL	p1-6	2014	-	-	-	*Sassafras albidum*	0.55	0.04
	p7-12		*Ligustrum sinense**	0.82	<0.01	*Celastrus orbiculatus*	0.83	<0.01
			Lonicera japonica	0.59	0.02	*Microstegium vimineum*	0.83	<0.01

	Toxicodendron radicans	0.54	0.01	*Galium triflorum*	0.82	<0.01
	*Asplenium platyneuron**	0.54	0.03	*Ligustrum sinense*	0.76	<0.01
	-	-	-	*Asplenium platyneuron*	0.73	<0.01
	-	-	-	*Cynoglossum virginianum*	0.71	0.04
	-	-	-	*Ophioglossum vulgatum*	0.61	0.01
p13-18	*Botrychium virginianum**	0.69	<0.01	*Fraxinus pennsylvanica*	0.75	<0.01
	Vitis rotundifolia	0.6	0.01	*Botrychium virginianum*	0.58	<0.01
	Carya tomentosa	0.5	0.05	*Aristolochia serpentaria*	0.52	0.04
p19-24	*Liquidambar styraciflua*	0.58	0.04	-	-	-
p25-30	*Quercus montana**	0.74	<0.01	*Quercus montana*	0.78	<0.01
	Nyssa sylvatica	0.5	0.04	*Acer rubrum*	0.59	0.03
	-	-	-	*Smilax glauca*	0.43	0.05
p31-36	*Polystichum acrostichoides**	0.89	<0.01	*Polystichum acrostichoides*	0.78	<0.01
p37-42	*Euonymus fortunei**	0.82	<0.01	*Fraxinus americana*	0.79	<0.01
	Fraxinus pennsylvanica	0.68	0.01	*Euonymus fortunei*	0.71	<0.01
	Cercis canadensis	0.64	<0.01	*Hexastylis arifolia*	0.5	0.03
	Parthenocissus quinquefolia	0.55	<0.01	-	-	-
p43-48	-	-	-	*Albizia julibrissin*	0.71	0.01
p49-53	*Duchesnea indica*	0.78	<0.01	*Asimina triloba*	0.53	0.02
	*Asimina triloba**	0.61	0.01	-	-	-
p54-59	*Quercus rubra**	0.7	<0.01	*Quercus rubra*	0.52	0.03
	Quercus alba	0.51	0.04	-	-	-
p60-65	*Elymus hystrix*	0.71	0.01	*Euonymus americanus*	0.62	<0.01
	Ulmus alata	0.69	0.01	*Ranunculus recurvatus*	0.61	0.02
	*Euonymus americanus**	0.56	<0.01	*Brachyelytrum erectum*	0.55	0.03
	Frangula caroliniana	0.56	0.02	-	-	-
	Pinus virginiana	0.55	0.05	-	-	-
	Juniperus virginiana	0.49	0.04	-	-	-
p66-71	*Verbesina virginica*	0.82	<0.01	*Elaeagnus umbellata*	0.91	<0.01

	*Elaeagnus umbellata**	0.62	<0.01	*Toxicodendron radicans*	0.67	0.01
p72-74	*Polygonatum biflorum*	0.97	<0.01	*Uvularia perfoliata*	0.85	<0.01
	*Uvularia perfoliata**	0.79	<0.01	*Bignonia capreolata*	0.59	0.01
	-	-	-	*Scutellaria elliptica*	0.58	0.04
	-	-	-	*Ruellia caroliniensis*	0.58	0.04
	-	-	-	*Acer saccharum*	0.56	0.02
	-	-	-	*Quercus alba*	0.55	0.02
	-	-	-	*Lonicera japonica*	0.52	<0.01
p75-80	*Hexastylis arifolia*	0.49	0.04	-	-	-
p81-86	*Oxydendrum arboreum**	0.57	0.05	*Vaccinium pallidum*	0.73	<0.01
	-	-	-	*Oxydendrum arboreum*	0.57	0.05
	-	-	-	*Smilax rotundifolia*	0.56	0.04
	-	-	-	*Desmodium nudiflorum*	0.55	0.03

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Appendix S3. Correlations among several temporal beta diversity

We computed three temporal beta diversity: (a) Sorensen temporal beta diversity, based on community incidence data, and can be decomposed into turnover and nestedness, (b) Temporal beta indices (TBI) calculated using species cover data, and can be broken down into species loss (B index), species gains (C metric), and overall temporal beta diversity (D index), and (c) community stability measure based on the species cover data. We also calculated the difference between the B and C indices to determine if there was net increase in species richness in each site. These temporal beta diversity metrics measure different aspects of community dynamics.

Sorensen temporal beta diversity was significantly positively and highly correlated with turnover and D metric and moderately correlated with the C metric, while it was negatively correlated with nestedness (Table S2). A significantly high positive relationship of Sorensen temporal beta diversity with turnover indicates that species replacement contributes the most to temporal beta diversity than nestedness. Additionally, the D metric overall temporal beta diversity was significantly correlated was positively correlated with species turnover and species gains, indicating that community shifts was mainly characterized by new floristic entrants in the NEON sites. The B-C metric difference was significantly positive related to species gains (C metric) and negatively correlated to species gains (B metric). Community stability was not correlated with other temporal beta diversity measures (Table S2).

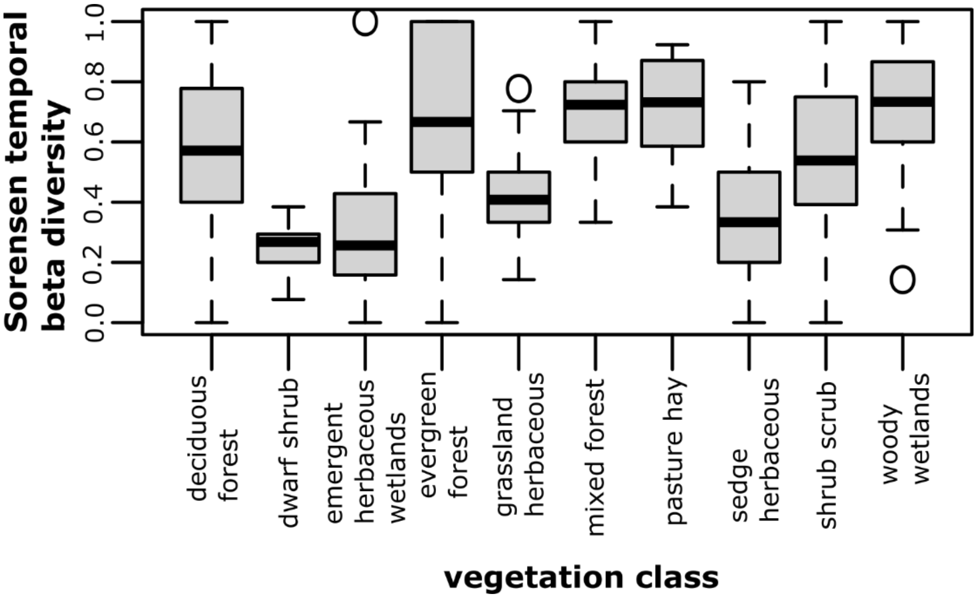
Table S3. Relationship among the calculated temporal beta diversity metrics across the 1105 1 m^2 plots spread across the 20 NEON sites. ***Stability metric had results for only 1070 1 m^2 plots***

Metric	Beta.sim	Beta.sne	Beta.sor	B metric	C metric	D metric	B-C diff	Stability
Beta.sne	-0.55***	-						
Beta.sor	0.90***	-0.13***	-					
B metric	0.31***	-0.16***	0.26***	-				
C metric	0.39***	-0.03	0.46***	-0.5***	-			
D metric	0.69***	-0.17***	0.73***	0.41***	0.58***	-		
B-C diff	0.07*	-0.07*	0.14***	-0.85***	0.88***	0.13***	-	
Stability	-0.05	0.01	-0.05	-0.02	-0.09**	-0.05	-0.11***	-

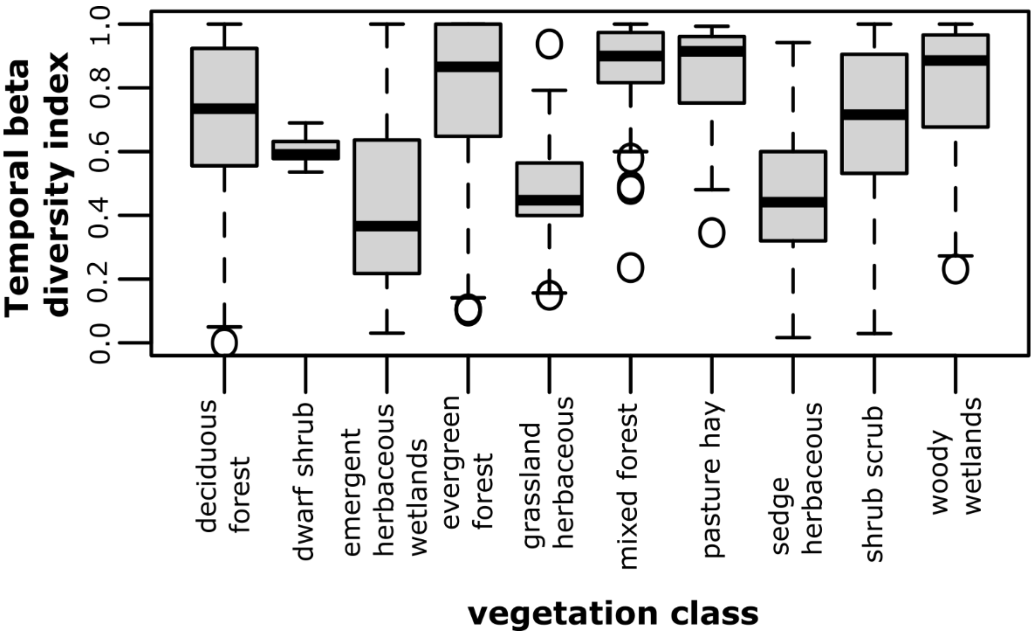
*P<0.05, **P<0.01, ***P<0.001

Appendix S4. Pairwise difference among vegetation classes representing all NEON sites

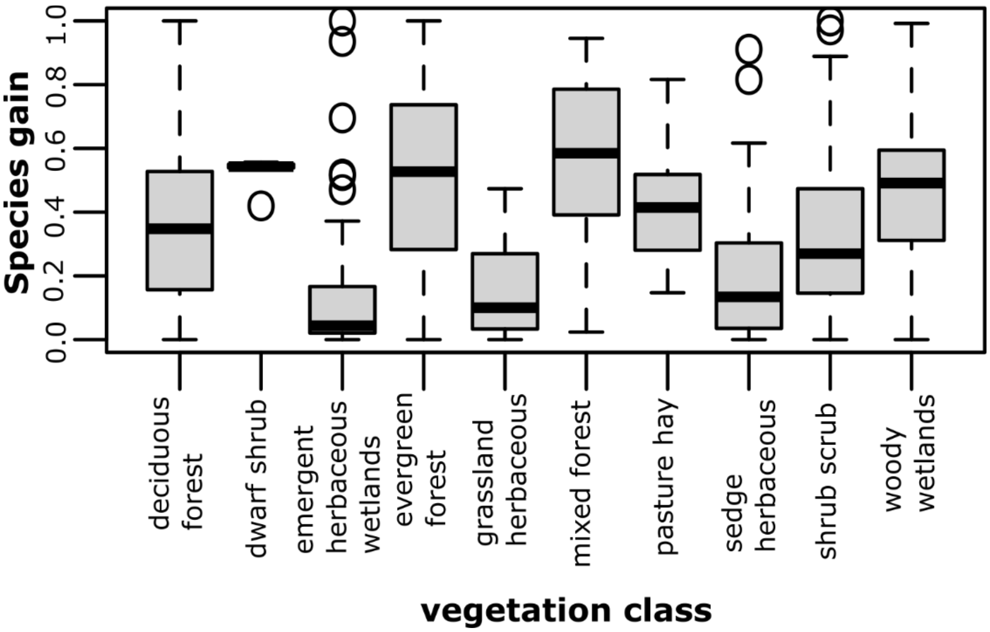
Boxplots showed that deciduous forest, evergreen forest, mixed forests, pasture hay, shrub scrub, and woody wetlands ecosystems experienced relatively high Sorensen temporal beta diversity (Figure S2a), TBI (Figure S2b), while dwarf scrub, emergent herbaceous wetlands, grassland herbaceous, and sedge herbaceous sites have lower Sorensen beta diversity values (Figure S2a). Additionally, emergent herbaceous wetlands, grassland herbaceous, and sedge herbaceous sites have lower TBI values (Figure S2b). Species gains were higher in sites in the evergreen forest and mixed forest (Figure S2c), while loss was the highest in pasture hay (Figure S2d).



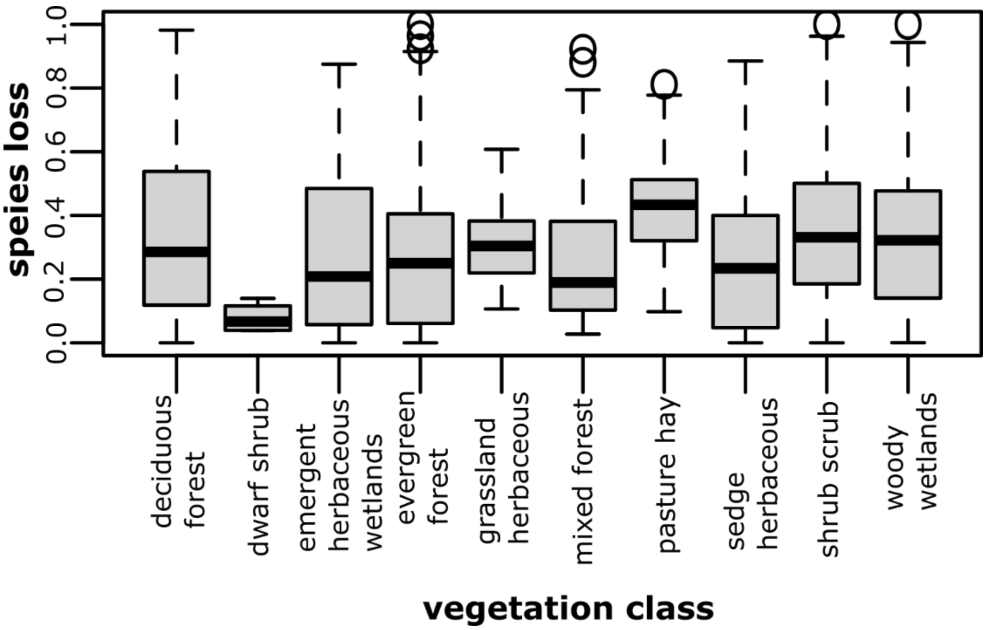
a) Sorensen temporal beta diversity



b) TBI



c) C metric



d) B metric

Figure Sup. Methods 2. Boxplots showing the distribution of (a) Sorensen temporal beta diversity (b) TBI, (c) C metric, and (d) B metric across vegetation classes.

Table S4. Relationship between temporal beta diversity measures of NEON plots and climatic and edaphic variables using Pearson's moment correlation tests

Predictor variables	**Sorensen**		**TBI**		**Stability**	
	r	***p***	***r***	***p***	***r***	***p***
Aridity index	-0.02	0.69	0.03	0.30	0.01	0.79
Soil pH	0.16	<0.01	0.13	<0.01	-0.07	0.03
Soil nitrogen	-0.05	0.10	-0.05	0.13	0.08	0.01
Soil organic carbon density	-0.25	<0.01	-0.22	<0.01	0.03	0.42
Soil bulk density	0.41	<0.01	0.39	<0.01	-0.03	0.36
Soil coarse fragments	0.27	<0.01	0.24	<0.01	-0.07	0.02
Soil sand content	0.12	<0.01	0.07	0.02	0.09	<0.01
Soil silt content	-0.19	<0.01	-0.08	<0.01	-0.05	0.09
Soil fine organic carbon	-0.38	<0.01	-0.35	<0.01	0.01	0.73
Mean of monthly Tmax	0.50	<0.01	0.46	<0.01	0.02	0.47
Standard deviation of monthly Tmax	-0.41	<0.01	-0.37	<0.01	-0.05	0.13
Coefficient of variation of monthly Tmax	-0.46	<0.01	-0.46	<0.01	-0.03	0.40
Mean of monthly Tmin	0.48	<0.01	0.44	<0.01	0.03	0.38
Standard deviation of monthly Tmin	-0.40	<0.01	-0.38	<0.01	-0.04	0.21
Coefficient of variation of monthly Tmin	-0.04	0.18	0.01	0.79	-0.04	0.18
Mean of monthly Tmean	0.49	<0.01	0.46	<0.01	0.03	0.39
Standard deviation of monthly Tmean	-0.40	<0.01	-0.37	<0.01	-0.04	0.15
Coefficient of variation of monthly Tmean	-0.37	<0.01	0.33	<0.01	-0.05	0.11
Mean of monthly precipitation	0.13	<0.01	0.17	<0.01	0.09	<0.01
Standard deviation of monthly precipitation	0.17	<0.01	0.20	<0.01	0.09	<0.01
Coefficient of variation of monthly precipitation	-0.19	<0.01	-0.24	<0.01	-0.04	0.19
Mean of actual evapotranspiration	0.44	<0.01	0.39	<0.01	0.01	0.84
Standard deviation of actual evapotranspiration	0.05	0.11	0.09	<0.01	0.03	0.40