# Nutrient supply shifts successional paths but not speed of grassland recovery from disturbance

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## Abstract

Disturbance and environmental change may cause communities to converge to a steady state, diverge towards multiple alternative states, or remain in long-term transience. Yet, empirical tests of these successional trajectories are rare, especially in systems experiencing multiple concurrent anthropogenic drivers of change. We compared competing models of succession in grassland communities subjected to disturbance and nitrogen fertilization using data from a long-term (22-year) experiment. Regardless of disturbance, after a decade communities settled on equilibrium states largely determined by resource availability, with species turnover declining as communities approached dynamic equilibria. Species favored by the disturbance were those that eventually came to dominate the highly fertilized plots. Furthermore, disturbance made successional pathways more direct, revealing an important interaction effect between nutrients and disturbance as drivers of community change. Our results underscore the dynamical nature of grassland succession, demonstrating how community properties such as beta-diversity change through transient and equilibrium states.

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# Abstract

Disturbance and environmental change may cause communities to converge to a steady state, diverge towards multiple alternative states, or remain in long-term transience. Yet, empirical tests of these successional trajectories are rare, especially in systems experiencing multiple concurrent anthropogenic drivers of change. We compared competing models of succession in grassland communities subjected to disturbance and nitrogen fertilization using data from a long-term (22-year) experiment. Regardless of disturbance, after a decade communities settled on equilibrium states largely determined by resource availability, with species turnover declining as communities approached dynamic equilibria. Species favored by the disturbance were those that eventually came to dominate the highly fertilized plots. Furthermore, disturbance made successional pathways more direct, revealing an important interaction effect between nutrients and disturbance as drivers of community change. Our results underscore the dynamical nature of grassland succession, demonstrating how community properties such as beta-diversity change through transient and equilibrium states.

# Introduction

Temporal change in ecological communities has long fascinated ecologists, prompting a rich study of succession that underpins many theories in community ecology (Clements, 1916; Gleason, 1926; Pickett *et al.*, 2009; Chang *et al.*, 2019). In the modern era of rapid environmental change, background disturbance and temporal dynamics are now interacting with multiple global change drivers known to influence community composition and structure (Chang *et al.*, 2019). Consequently, there is a renewed interest in updating and revising our understanding of succession using contemporary approaches that capture the complex determinants of community composition change through data-theory integration (Avolio *et al.*, 2021). For example, a recent metaanalysis of global change experiments in herbaceous plant communities determined that the effects of multiple global change drivers on communities were often synergistic, but lagged, and often were only detectable over long ($\geq$ 10 years) timescales (Komatsu *et al.*, 2019). Building on foundational theory of ecological succession and community assembly is essential for understanding community responses to anthropogenic drivers of change, and to improve conservation and restoration outcomes in a rapidly changing world (Chang *et al.*, 2019; Komatsu *et al.*, 2019).

Early models in successional theory predicted that communities change directionally over time towards a climax system (Clements, 1916; Lepš & Rejmánek, 1991; Pickett *et al.*, 2009). Inherent in this historical paradigm of succession is convergence: a process where spatially segregated communities equilibrate to the same species composition and structure regardless of any underlying spatiotemporal variability in starting conditions. The final stable state community, which persists in perpetuity until a disturbance restarts the successional process, is comprised of a suite of coexisting species with stable population dynamics (Law, 1999). Resource-based competition models of community structure (e.g. $R^*$ theory) predict that nutrient supply rate is the primary determinant of equilibrium plant community assemblages

(Tilman & Wedin, 1991; Wilson & Tilman, 1991, 1993; Tilman, 1985) and that spatially segregated communities with the same nutrient supply rate will converge on the same species composition, regardless of disturbance or variable starting conditions assuming all species can reach all locations (Inouye & Tilman, 1988). A change in nutrient supply, however, is predicted to shift the equilibrium plant community towards a new, possibly irreversible stable state (Suding *et al.*, 2004). For example in the grassland system we focus on here, Isbell *et al.* (2013a) found that plant communities persisted in a low-diversity state more than two decades after the cessation of nutrients, suggesting that fertilization caused a regime shift.

Directly contradicting a highly predictable, resource-based competition paradigm of succession, more recent theory has emphasized how stochastic and heterogeneous factors including small scale abiotic variation, probabilistic dispersal, colonization, and local extinction can lead to divergent communities that exhibit alternative stable states (Fukami & Nakajima, 2011; Fukami, 2015; Shoemaker *et al.*, 2020; Miller *et al.*, 2021). Priority effects including niche preemption and niche modification from early arriving species can result in multiple, alternative steady state pathways across locations that had different species arrival order, even under the same environmental conditions (Fukami, 2015). For example, Chase's (2003) classic study demonstrated that succesional trajectories in aquatic mesocosms with intermediate productivity exhibited alternative stable states depending on the initial density of a single organism (snails). Theoretical modelling further suggests that divergence between spatially segregated communities is more likely to occur with a large species pool, low connectivity, high productivity, and infrequent disturbance (Chase, 2003). In parallel, divergence in community composition can also depend on small-scale local heterogeneity (spatial contingencies) (Young *et al.*, 2017; Kardol *et al.*, 2007) or the environmental drivers experienced at a single point in time, especially early in succession (Werner *et al.*, 2020). One empirical study examining patterns of plant succession after the eruption of Mt. St. Helens, found evidence for multiple successional pathways within and among sites, which the authors attribute to the interaction of disturbance, spatial contingencies, and other chance factors (Chang *et al.*, 2019).

Though many community theories tend to focus on final equilibrium dynamics, it has long been recognized that many real-world communities persist for long periods without reaching an equilibrium (Cowles, 1899; Noy-Meir, 1975; Hastings, 2004; Fukami & Nakajima, 2011; DeAngelis & Waterhouse, 1987; Huston, 1979). In many cases of long-term transience, disturbance occurs frequently enough that species are consistently reshuffled, and the community never reaches a point where composition is stable (Fukami, 2015). Even simple models of community assembly can produce prolonged transience when there is sufficient temporal variability. For example, a Lotka-Volterra competition model of a grassland system with periodic disturbance and seasonal changes, led to sustained dynamic transience, where modelled species compositions continued to shift towards equilibria without enough time to reach the predicted state before shifting yet again (Geijzendorffer *et al.*, 2011). Though several long-term studies demonstrate dynamics that are consistent with long-term transience, few studies provide quantitative metrics, such as the rate of community turnover, to accurately identify transience. One exception is a recent study examining a long-term restoration project in the drylands of China, which demonstrated a prolonged period of transience of shrub dominance (around 37 years), followed by a rapid shift to the desired, restored, state characterized by low shrub cover and high grass cover (Chen *et al.*, 2019).

Identifying successional trajectories in practice necessitates high spatial and temporal replication that can capture the long-term behavior of the system (Hastings, 2004, 2010; Inouye & Tilman, 1988). Thus, despite a rich body of theoretical work on succession and community assembly, there are few experimental studies with time series sufficiently long to capture asymptotic trends in community structure and composition and to distinguish between competing theories of successional dynamics (Hastings, 2004, 2010). For this study, we analyzed a long-term (22 year) experiment from the Cedar Creek Ecosystem Reserve in

Minnesota, USA to examine competing models of community succession in a prairie system experiencing two simultaneous anthropogenic drivers of change: disturbance and nutrient addition. This an ideal system to test competing theories of succession due to a long history of study at Cedar Creek (Tilman, 1987; Wilson & Tilman, 1991, 1993; Isbell *et al.*, 2013a), including a rich natural history of the common species in this system (Sullivan *et al.*, 2018; Catford *et al.*, 2019). We focus on the joint effects of both drivers, as both community response to nutrient supply (Tilman, 1987; Wilson & Tilman, 1991, 1993; Isbell *et al.*, 2013a) and recovery from agricultural disturbance (Holt *et al.*, 1995; Debussche *et al.*, 1996; Li *et al.*, 2016; Pickett *et al.*, 2009) have been well-studied individually, but their joint effects are less understood (Seabloom *et al.*, 2020). If the community tended towards alternative stable states or prolonged periods of transience, disturbance would alter the predictions from adding nutrients alone. An earlier short-term analysis of successional change from this experiment found that species composition changed rapidly in response to nutrient addition, but that a 4 year timeseries was not sufficient to determine community convergence to resource-controlled equilibria versus divergence caused by alternative stable states or long-term transience (Inouye & Tilman, 1988). A more recent analysis from this experiment suggests an interactive effect between nutrients and disturbance on species diversity and total biomass that emerges after approximately a decade of recovery (Seabloom *et al.*, 2020); however this study did not evaluate the joint effects of nutrients and disturbance on community composition nor successional trajectories.

Here, we revisited Inouye and Tilman's (1988) initial investigation of succession, extending from their focus on convergence versus divergence to additionally ask: 1) Do communities tend towards equilibrium states, remain in long-term transience, or reach alternative stable states? 2) Do resources determine long-term composition? and 3) Are successional trajectories altered by disturbance? To address whether communities settled into a steady state, or persisted in long-term transience, we examined the rate of community compositional change between years, and the directionality of those changes. If communities progressed along a successional pathway towards a stable equilibrium (single or multiple equilibria), then we expected community turnover to decline, and community trajectories to display low sinuosity in multivariate space as they headed towards equilibria. To address whether fertilization led to the development of resource-controlled stable equilibria, or if disturbance or variable starting conditions yielded alternative stable states, we looked for evidence of convergence or divergence in composition between spatially segregated communities both within and across experimental treatments.

# Materials and methods

## Study site and data collection

Data were collected in mixed-grass prairie habitats at the Cedar Creek Ecosystem Science Reserve in Minnesota, USA (CDR, Lat: 45.4 Long: 93.2 W) from 1982 to 2004. CDR has well-drained sandy soils that are low in nitrogen (N), resulting in low productivity relative to other grasslands worldwide (Fay *et al.*, 2015). The mean annual temperature from 1982 to 2004 was 6.7°C (± 0.02 SE) and the mean annual precipitation was 818 mm (± 35 SE). There were two notable drought years in the time series (1987 and 1988) where annual precipitation was below 600 mm.

The experimental design is described briefly here, with additional details in Tilman (1987) and Seabloom *et al.* (2020). In 1982, identical disturbance by nutrient addition experiments were established in three abandoned agricultural fields that were last tilled and farmed in 1968 (Field A), 1957 (Field B), and 1934 (Field C). Within each field, two experimental grids (35 X 55 m) were established for nutrient application, one in an area that was thoroughly disked in the spring of 1982 (E002), and another in an adjacent area that remained intact (E001). Each grid consisted of 54, 4 X 4 m vegetation plots, receiving one of eight nutrient treatments (applied annually in mid-May or late-June) plus an unmanipulated control in a randomized block design, with 6 replicate plots per field. Importantly, the disturbance occurred once at the beginning of the experiment, while the nutrient additions were applied annually throughout the experiment.

For this study, we compared community trajectories with increasing N across the undisturbed (E001) and disturbed (E002) grids. To capture a gradient of increasing resource supply, we focused our analyses on the following treatments: No nutrients, 0 g N + $\mu \cdot$ m$^2$ $\cdot$ year$^{-1}$ , 1 g N+ $\mu \cdot$ m$^2$ $\cdot$ year$^{-1}$ , 3.4 g N+ $\mu \cdot$ m$^2$ $\cdot$ year$^{-1}$ , and 9.5 g N+ $\mu \cdot$ m$^2$ $\cdot$ year$^{-1}$. Matching previous studies in the system (Clark & Tilman, 2008; Seabloom *et al.*, 2020), we chose 9.5 g N+ $\mu \cdot$ m$^2$ $\cdot$ year$^{-1}$ as our maximum "high" N treatment because previous work in this study system and elsewhere indicates this treatment overcomes N limitation without inducing toxicity (Elser *et al.*, 2007; Isbell *et al.*, 2013a; Fay *et al.*, 2015). Throughout the experiment nitrogen was added as $NH_4NO_3$ and the micronutrients ($\mu$) consisted of of P, K, Ca, Mg, S and citrate-chelated trace metals ($P_2O_5$ at 20 g$\cdot$ m$^2$ $\cdot$ year$^{-1}$, $K_2O$ at 20 g$\cdot$ m$^2$ $\cdot$ year$^{-1}$, $CaCO_3$ at 40 g$\cdot$ m$^2$ $\cdot$ year$^{-1}$, $MgSO_4$ at 30 g$\cdot$ m$^2$ $\cdot$ year$^{-1}$, $CuSO_4$ at 18 µg$\cdot$ m$^2$ $\cdot$ year$^{-1}$, $ZnSO_4$ at 37.7 µg$\cdot$ m$^2$ $\cdot$ year$^{-1}$, $CoCO_2$ at 15.3 µg$\cdot$ m$^2$ $\cdot$ year$^{-1}$, $MnCl_2$ at 322.0 µg$\cdot$ m$^2$ $\cdot$ year$^{-1}$, and $NaMoO_4$ at 15.1 µg$\cdot$ m$^2$ $\cdot$ year$^{-1}$).

Beginning in 1982, vegetation was sampled by clipping a 10 X 300 cm strip each year at the ground level. After clipping, biomass was sorted into previous year's growth (litter), and current year's growth (live biomass). Live biomass was sorted by species, dried, and weighed to the nearest 0.01 g. All plots in all fields were sampled annually from 1982 to 2004, with the exception of years 1995 (only E001 sampled), 2001 (only E001 sampled), and 2003 (only E001 and field C in E002 sampled). Due to a change in the fire regime of E001 after 2004, we restrict analyses to years before 2004. Prior to all multivariate analyses, we applied a $ln(1 + x)$ data transformation where $x$ = biomass (in g) of individual plant species within a plot in a given year.

# Statistical Analyses

## Explained variability and overall trends in community composition

To assess how the disturbance event in 1982, yearly fertilization regime, initial conditions, and historical contingencies of each field explained community composition throughout the experiment, we used a Permutational ANOVA (PERMANOVA) using the *adonis* function from package *vegan* (Oksanen *et al.*, 2008) in R version 4.1.2 (R Core Team, 2020) with the Bray-Curtis dissimilarity matrix generated from log-transformed species' biomass data between all plots in all fields in both experiments (E001 and E002) for each year from 1982 to 2004. The Bray-Curtis matrix of a given year was the dependent variable, with three additive categorical independent variables: disturbance, nutrient treatment, and field identity. We included field as a fixed effect, as there are only three replicates, and we were additionally interested in how differences among fields may explain differences in composition through time. We examined yearly trends in the explained variation (partial $R^2$) for each independent variable over the 22 years of the experiment in the PERMANOVA model, evaluating which variables explained the most variation in community composition, and the longevity of those effects.

To provide species-level and community-level context for our multivariate results, we report trends in biomass, species richness (alpha diversity), and the relative proportion of plant functional groups across nutrient and disturbance regimes. To determine which plant species were driving variation in species composition we performed an indicator species analysis using the *multipatt* function from package *indicspecies* (De Caceres & Legendre, 2009) in R. Indicator species analyses assess the association between species patterns and combinations of groups of sites, and perform permutation tests for statistical significance of the best matching associations (Cáceres & Legendre, 2009). For the indicator species analysis we grouped sites according to disturbance treatment (E001 and E002), nutrient treatment (focusing on control and high nutrient plots only) and timing relative to the start of the experiment (early: 1982 - 1985, and late: 2000 - 2004). We constrained the indicator species analysis to include species associated with one to three of the aforementioned groups.

## Assessing convergence vs. divergence

To test if plots converged or diverged in community composition over time, we calculated the average Bray-Curtis distance of replicate communities within treatments to their group centroids using the *betadisper* function from the R package *vegan* (Oksanen *et al.*, 2008) in R. If the distance between plots to their group centroids declined over time, this indicated convergence. We evaluated the trends of the average distance to the centroid over the 22-year time series for each nutrient by fertilization treatment. One way to evaluate whether systems have reached an equilibrium is to look for asymptotic behavior, or stasis in the eventual long-term dynamics of the system (Hastings, 2004; Isbell *et al.*, 2013b). Thus, we performed AIC model selection to determine whether a linear or saturating function best described convergence within a treatment over the 22-year time series. For our saturating function, we utilized the model $y = Asym + (R_0 - Asym) * exp(-exp(lrc) * year + \epsilon)$ due to the ease of interpretation of estimated parameters, where $Asym$ represents the horizontal asymptote of the response variable, $R_0$ represents the response at year 0 and $lrc$ represents the natural log of the rate constant.

To test if plots receiving different nutrient treatments diverged in composition, we calculated the distances between treatment group centroids each year using the *betadisper* function from package *vegan* (Oksanen *et al.*, 2008), assessing dispersion trends over the time series. If the distance between group centroids increased over time, this indicated divergence among plots receiving different nutrient treatments. As above, we performed AIC model selection to determine whether a linear or saturating function best described the trend over time. For both convergence within a give a nutrient treatment, and divergence between nutrient treatments, we compared model parameters across disturbance treatments (E001 and E002) to see if disturbance altered the rate or asymptotic behavior of the system.

## Direction and speed of succession

To examine how communities changed through time with disturbance and nutrient addition, we conducted a suite of community trajectory analyses (CTA) (De Cáceres *et al.*, 2019) by projecting data from spatially segregated communities (plots) into a multivariate space, and analyzing various geometric properties of their trajectories over time. We defined a multivariate space to study temporal compositional dynamics, using the abundance-based Bray-Curtis dissimilarities in community composition among all plots across the entire time series. We conducted a principal coordinates analysis (PCoA) using the *pcoa* function from package *ape* (Paradis & Schliep, 2019) to summarize the Bray-Curtis dissimilarity matrix of plots through time in Euclidean space.

To visualize the directionality of succession, we plotted yearly and decadal trends across disturbance and fertilization regimes. Then, to quantitatively assess directionality of successional pathways, we calculated a sinuosity index (ie: how meandering is a given community's path in multivariate space), using the index defined by Benhamou (2004):

$$S = 2[p(\frac{1+c}{1-c}) + b^2]^{-0.5}$$

where $p$ is step length (here, annual step length), $b$ is the the coefficient of variation of step lengths, and $c$ is the mean cosine of turning angles. Sinuosity indices express the amount of angular change over a given path length with smaller values representing straighter, or more directional paths, and larger values representing more meandering paths.

Lastly, we assessed the speed of succession across disturbance and fertilization regimes, to address whether communities tended towards a stable state with lower temporal turnover, or persisted in a state of long-term transience with high turnover. We did so by calculating trajectory lengths between subsequent annual surveys in the same plots using the *trajectorylengths* function from package *ecotraj* (De Cáceres *et al.*, 2019) and regressing inter-annual trajectory distances (also in Bray-Curtis distance) over time. Inter-annual trajectory distances practically represent the amount of species turnover between years, with larger values indicating more turnover and smaller values indicating similar species composition between years. As above, we performed AIC model selection to determine whether a linear or saturating function best described changes in the speed of succession, and if that varied by nutrient and disturbance treatments.

# Results

### Explained variability and overall trends in community composition

Prior to the disturbance event and beginning of fertilization, variation in community composition was dominated by among-field spatial variation, likely reflective of differences in age since abandonment. Variation in community composition explained by the disturbance event in 1982 was high (15%) in the first year, but quickly declined to almost 0% explained over a period of about five years (Figure 1a). Meanwhile, the variation explained by annual fertilization increased over the first decade, and then plateaued at about 40% (range 35-45% from years 9 to 22) (Figure 1b). During the first 5 years, the three fields initially varied in composition, but then began to converge due to treatment effects, although variation among fields still persisted after 20 years of fertilization (range 15-25% from years 9 to 22 , Figure 1c). Total aboveground biomass fluctuated throughout the experiment (Figure S1). Immediately after the disturbance in 1982, biomass was greater in the disturbed grids, and especially in the fertilized and disturbed plots; however this increase in biomass from disturbance dissipated around year 5 of the experiment (Figure S1).

Early successional communities that established after the disturbance in 1982 included the C3 grasses *Panicum oligosanthes* and *Agrostis scabra*, the C4 grass *Setaria lutescens*, and a variety of forbs including *Polygonum convolvulus* and *Rumex acetosella* (Table 1). After two decades, high nutrient plots in both intact and disturbed grids were characterized by the C3 grasses *Agropyron repens* and *Poa pratensis*. Meanwhile, control (unfertilized) plots in both intact and disturbed grids were characterized by the C4 grasses *Schizachyrium scoparium* and *Sorghastrum nutans*. Legumes disappeared from high nutrient plots, but persisted in control plots, regardless of disturbance (Table 1). For both the disturbed and undisturbed grids, community composition changed annually, with fertilization moving successional trajectories towards higher values of PCoA 1 and lower values of PCoA 2 (Figure 2 a-j). Moreover, changes in communities during the first decade of the experiment were more directional in PCoA ordination space, especially in high nutrient plots, whereas changes in the second decade were more meandering in ordination space (Figure 2 a-j).

Consistent with the notion that fertilization increasingly explained community composition over the first decade of the experiment, annual fertilization yielded predictable changes in the dominance of plant functional types throughout the experiment (Figure S2). For both disturbed and undisturbed grids, unfertilized plots had a more even distribution of plant types over the time-series, while the highly fertilized plots quickly became dominated by C3 grasses (Figure S2). Plot-level species richness ($11.65 \pm 0.24$ species in year 1) declined throughout the experiment at a similar rate in both disturbed and undisturbed grids (Figure S3). The rate and overall amount of decline varied across fertilization treatments, with species richness plateauing to $8.55 \pm 0.30$ species for the control plots and $5.67 \pm 0.21$ species for the highly fertilized plots (Figure S3)

## Assessing convergence vs. divergence

For both intact and disturbed grids, communities that received the same annual nutrient supply converged in composition (Figure 3 a, b). The average Bray-Curtis distance between each community (plot) to its group centroid (18 plots with the same nutrient X disturbance regime) was best fit with a decreasing saturation function through time (Table S1) where community change was rapid during the first decade and plateaued during second decade to an average distance between 0.23 and 0.37 (Table S2). The asymptote, representing within-treatment spatial beta diversity, was determined by fertilization regime, with a lower distance (spatial beta diversity) in medium and high nutrient treatments compared to low nutrient and control plots (Figure 3 a, b Table S2). Interestingly, this relationship was most pronounced in disturbed grids, indicating an interaction effect between fertilization and disturbance on the overall level of convergence in grassland plant communities that emerges after about 10 years of recovery, affecting the asymptotic level of beta diversity of plots within the same treatment (Figure 3 a, b).

While communities within a given nutrient treatment converged in composition, communities receiving different nutrient treatments diverged through time (Figure 3 c, d). Bray-Curtis distances between group centroids (across nutrients treatments) were best fit with an increasing saturation function (Table S3) where the overall level of divergence accumulated rapidly in the first decade of the experiment, and began to slow towards an asymptote in the second decade (Figure 3 c, d). The distance between centroids in the intact grid plateaued at $0.174 \pm 0.007$ while the distance between centroids in the disturbed grid plateaued at $0.170 \pm 0.010$ ($Asym$ parameter in Table S4).

## Direction and speed of succession

The sinuosity of community change, indicating how meandering a community's successional pathway is in multivariate space, showed strong differences with both nutrient addition and disturbance (Figure 4 a and b). For both intact and disturbed grids, increasing fertilization moved pathways towards higher values of PCoA 1 (Figure 4 c and d). In the intact grids, plots that did not receive fertilizer had higher sinuosity, and increasing nutrient supply rate decreased sinuosity, indicating more directional change in community composition (Figure 4 a). Meanwhile in the disturbed grids, sinuosity was lower overall, and did not vary significantly among fertilization treatments, with overlapping confidence intervals amongst all treatments. (Figure 4 b). Sinuosity also varied by decade, with lower sinuosity in the first decade of the experiment, as communities were in a transient successional state, and higher sinuosity in the second decade, as communities reached an equilibrium state (Figure S4). Higher sinuosity in the equilibrium state suggests communities settled on a dynamic equilibrium, rather than a point equilibrium at stasis.

Interannual community trajectory distance, a measure of temporal turnover, declined by over 50% throughout the duration of the experiment for both disturbed and intact grids (Figure 5). This relationship was best described with a linear function with a negative slope rather than a saturating function, ( Table S5). All estimated slope parameters for intact and disturbed grids were $< 0$ including 95% confidence intervals. Though highly fertilized plots in both intact and disturbed grids initially had higher levels of community turnover (as indicated by intercept parameters in Table S6), community turnover declined at a similar level for both fertilized and unferilized plots (as indicated by overlapping slope parameters between nutrient treatments in Table S6). This suggests that neither disturbance or fertilization had a strong effect on the rate of community turnover (Figure 5).

# Discussion

Collectively, our results demonstrated that both disturbed and undisturbed grassland communities tended towards resource-mediated equilibrium states (Figure 4 c, d). Species turnover declined linearly over time (Figure 5), while most other community metrics displayed asymptotic long-term behavior, suggesting the emergence of equilbria (Figure 3). Successional trajectories emerged from two processes: convergence within communities with the same nutrient treatments (Figure 3 a, b) and divergence between communities with different nutrient treatments (Figure 3 c, d). Though patterns of convergence and divergence were similar across disturbed and undisturbed grids, disturbed plots that were unfertilized or received low levels of nitrogen settled on a higher distance between group centroids compared to their undisturbed counterparts, indicating greater levels of spatial beta diversity at equilibria (Figure 3 b). Though the equilibrium state was mostly determined by nutrient conditions, disturbance made the successional pathway towards arriving at the equilibrium state more direct (lowered sinuosity in Figure 4 b), revealing an important interaction effect between drivers. In particular, species that were favored by the pulse disturbance event in 1982, were those that came to dominate the highly fertilized plots, (Table 1).

Plant communities at Cedar Creek persisted in a distinct phase of transience for approximately 10 years before they settled on resource-mediated equilibrium states (Figure 1 b, Figure 3). Inter-annual rates of change in community composition decreased as the communities reached these states (Figure 5); however communities still experienced relatively high levels of community turnover in the later stages of succession. These results align with a study examining post-agricultural secondary succession in New Jersey where temporal turnover of dominant species decreased over time, but remained relatively high at later succesional stages (Li *et al.*, 2016). Both our study and Li *et al.* (2016) suggest that communities undergoing succession can ultimately settle on a "dynamical equilibrium" or steady state distribution, rather than a point equilibrium with highly consistent communities. At a steady state equilibrium, community turnover can still be quite high as communities sample compositions within the steady state distribution (Naselli-Flores *et al.*, 2003; Shoemaker *et al.*, 2020)(Compositional changes between 1992 and 2004 in Figure 2).

Multiple factors, including demographic stochasticity, environmental fluctuations, and small-scale spatial heterogeneity (Furey *et al.*, 2022) can yield temporal turnover and variability in community composition at this dynamical equilibrium. Cross-system comparisons could shed light on dynamical equilibria, and how the amount of turnover and variability at an ecosystem's steady state distribution may depend on species traits (e.g. fast-growing versus slow-growing systems, seedbanks), the size of the species pool, and the inherent amount of environmental variability under which the system has evolved (Chase, 2003; Fernandez-Going *et al.*, 2012).

The long-term nature of this experiment reveals that the effects of disturbance and nutrient additions operate on different time scales, but both have sustained, long-term impacts. Consistent with theory, the disturbance (pulse perturbation) initially had a strong effect on species abundances, but had minimal impacts on long-term composition (Figure 1) (Bender *et al.*, 1984). Early in succession, we found a higher number of indicator species, and distinct communities in the disturbed grids, characterized by forbs including *Erigeron canadensis*, *Polygonum convolvulus*, and the C4 grass *Setaria lutescens* (Table 1). These species tend to have high fecundity and dispersal ability (Sullivan *et al.*, 2018), and germinate quickly from the seedbank after disturbance. Meanwhile annual fertilization (a press perburtation) created a sustained change in species composition, and the emergence of new, nutrient-mediated equilibrium states (Bender *et al.*, 1984), with unfertilized plots dominated by the C4 grass *Schizachyrium scoparium* which is a strong competitor for nitrogen (Wilson & Tilman, 1991), and highly fertilizated plots dominated by the rhizomatous C3 grass *Agropyron repens* (Table 1). In our study, initial starting conditions varied in two ways: the fields (which varied in time since agricultural abandonment) and the disturbance treatment in 1982. Though among field differences at Cedar Creek were initially strong, these effects declined as the effect of fertilization explained more variation in community composition in the first decade of the experiment (Figure 1 b, c). Collectively, these findings support Inouye and Tilman's prediction based on a few years of data collection (1988) that communities at Cedar Creek would eventually move towards resource-mediated equilibrium states regardless of variation in starting conditions .

While grassland community shifts in this system were mostly attributed to nutrient addition, the single pulse disturbance event in 1982 resulted in some long-term unexpected systematic community differences. Disturbed plots that were unfertilized or received low levels of nitrogen settled on a higher distance between group centroids compared to their undisturbed counterparts, indicating greater levels of spatial beta diversity at equilibria (Figure 3 c ,d). Other investigators have found long-lasting effects of pulse perturbations on ecological communities. For example, in a microcosm experiment with protozoa, there was a long-term effect of "ghost of disturbance past," where communities that received intense pulse disturbances settled on a community with lower species richness than communities than received less or no disturbance (Jacquet & Altermatt, 2020). Future cross-system comparisons across environmental gradients and communities with different levels of species richness will be helpful to inform when disturbance is likely to lead to predictable successional dynamics, systemic community changes, or alternative stable states (Török *et al.*, 2021)

Despite both intact and disturbed plots settling on long-term equilibria, the initial disturbance treatment made successional pathways more direct, as evidenced by a reduction in the sinuosity of community trajectories (Figure 4). This is likely because the disturbance favored species that also eventually dominated the fertilized plots (Table 1). Soil disturbances, such as tilling, typically create environments with high levels of light and soil resources and low levels of competition. For example, tilling aerates the soil which can cause a short-term release of nutrients such as nitrogen (Hassink, 1992; Kristensen *et al.*, 2003). A variety of models suggest that recently disturbed patches will be colonized by fast-growing species that are poor resource competitors (Bolker & Pacala, 1999; Pacala & Rees, 1998) consistent with the competition-colonization tradeoff, a stabilizing force for coexistence (Hastings, 1980; Levins & Culver, 1971). This relationship is apparent at our study site, where early successional species (e.g., C3 grasses) also have low nitrogen-use efficiency (Tilman, 1994), and come to dominate in fertilized plots (Tilman & Wedin, 1991; Isbell *et al.*, 2013a). The coupling of species that prefer disturbance and high-resource requirements may not occur in other systems or for other types of disturbance. For example, some types of disturbances, such as fires, reduce limiting nutrient availability. Similarly, severe disturbances that initiate primary succession (e.g., glaciation or volcanoes) can create very harsh low nutrient environments. In these cases, the early colonizers must be nutrient efficient (e.g., nitrogen-fixers), which are ultimately at a disadvantage in higher nutrient environments (Tognetti *et al.*, 2021).

# Conclusion

Our study simultaneously tested competing theories of community assembly (HilleRisLambers *et al.*, 2012; Kraft *et al.*, 2015), stability (Connell & Slatyer, 1977; Hallett *et al.*, 2018), and coexistence (Tilman, 1985) while informing potential future avenues of theoretical development using community metrics that capture the speed, sinuosity, and direction of community change over time (De Cáceres *et al.*, 2019). Taken together, our results imply that grassland communities at Cedar Creek tended towards resource-mediated equilibrium states regardless of initial conditions, but that disturbance caused changes to successional pathways that persisted for over two decades. In particular, disturbance moved successional trajectories towards changes eventually induced by fertilization. Importantly, our results also demonstrated that the rate of community turnover can remain high as communities approach equilibrium states (Figure 5). As such, we advocate that future work on succession and community assembly theory should incorporate fluctuation dependent coexistence and stochastic theory, as communities at their dynamical equilibrium can be highly variable

(Hallett *et al.*, 2019; Shoemaker *et al.*, 2020; Aoyama *et al.*, 2022).

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Table 1: Indicator species analysis depicting plants associated with groups based on disturbance (E001 and E002), nutrients (control (9) and high nutrient plots (6)), and timing relative to the start of the experiment (early: 1982 - 1985, and late: 2000 - 2004). We allowed for species to be associated with 1 to 3 groups. Species associations were generated using permutation tests for statistical significance. We report only the species with the strongest group associations (test statistic > 0.4), and their associated functional group. Plant functional groups: F = Forb, L = Legume C3 = C3 grass, C4 = C4 grass, S = Sedge.

| Intact | | | | Disturbed in 1982 | | | | Timing |
|---|---|---|---|---|---|---|---|---|
| Control | | High nutrients | | Control | | High nutrients | | |
| *Agrostis scabra* | C3 | *Panicum oligosanthes* | C3 | *Agrostis scabra* | C3 | *Agropyron repens* | C3 | Early (1982 - 1985) |
| | | | | *Panicum oligosanthes* | C3 | *Agrostis scabra* | C3 | |
| | | | | | | *Panicum oligosanthes* | C3 | |
| *Aristida basiramea* | C4 | *Aristida basiramea* | C4 | *Aristida basiramea* | C4 | *Setaria lutescens* | C4 | |
| | | *Schizachyrium scoparium* | C4 | *Setaria lutescens* | C4 | | | |
| | | | | *Sorghastrum nutans* | C4 | | | |
| *Cyperus sp.* | S | | | *Cyperus sp.* | S | *Cyperus sp.* | S | |
| *Achillea millefolium* | F | *Achillea millefolium* | F | *Ambrosia artemisiifolia* | F | *Ambrosia artemisiifolia* | F | |
| *Anemone cylindrica* | F | *Ambrosia artemisiifolia* | F | *Artemisia ludoviciana* | F | *Artemisia ludoviciana* | F | |
| *Artemisia ludoviciana* | F | *Anemone cylindrica* | F | *Berteroa incana* | F | *Chenopodium album* | F | |
| *Berteroa incana* | F | *Berteroa incana* | F | *Crepis tectorum* | F | *Erigeron canadensis* | F | |
| *Crepis tectorum* | F | *Crepis tectorum* | F | *Erigeron canadensis* | F | *Lepidium densiflorum* | F | |
| *Erigeron canadensis* | F | *Hedeoma hispida* | F | *Erigeron strigosus* | F | *Mollugo verticillata* | F | |
| *Erigeron strigosus* | F | *Lepidium densiflorum* | F | *Hedeoma hispida* | F | *Polygonum convolvulus* | F | |
| *Hedeoma hispida* | F | *Polygonum tenue* | F | *Lepidium densiflorum* | F | | | |
| *Polygonum tenue* | F | *Potentilla recta* | F | *Mollugo verticillata* | F | | | |
| *Potentilla recta* | F | *Rumex acetosella* | F | *Polygonum convolvulus* | F | | | |
| *Rumex acetosella* | F | *Solidago nemoralis* | F | *Rumex acetosella* | F | | | |
| *Solidago nemoralis* | F | | | | | | | |

| Intact |  |  |  | Disturbed in 1982 |  |  |  | Timing |
|---|---|---|---|---|---|---|---|---|
| Control |  | High nutrients |  | Control |  | High nutrients |  |  |
| *Agropyron repens* | C3 | *Poa pratensis* | C3 | *Agropyron repens* | C3 |  |  | Late (2000- 2004) |
|  |  | *Poa pratensis* | C3 |  |  | *Poa pratensis* | C3 |  |
| *Schizachyrium scoparium* | C4 |  |  | *Schizachyrium scoparium* | C4 |  |  |  |
| *Sorghastrum nutans* | C4 |  |  | *Sorghastrum nutans* | C4 |  |  |  |
| *Achillea millefolium* | F | *Asclepias syriaca* | F | *Euphorbia corollata* | F | *Asclepias syriaca* | F |  |
| *Asclepias syriaca* | F | *Chenopodium album* | F | *Viola pedatifida* | F | *Euphorbia corollata* | F |  |
| *Euphorbia corollata* | F |  |  |  |  |  |  |  |
| *Viola pedatifida* | F |  |  |  |  |  |  |  |
| *Lathyrus venosus* | L |  |  | *Lathyrus venosus* | L |  |  |  |

**Table 1 – continued from previous page**

# Figures

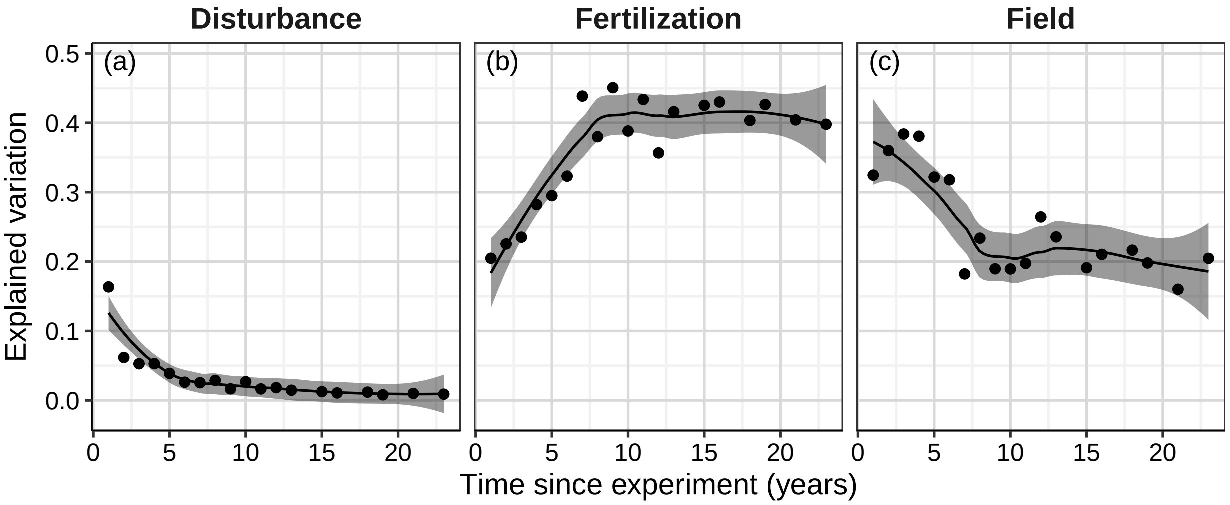

Figure 1: Variation in aboveground plant community composition explained by (a) disturbance (single plowing event in 1982), (b) fertilization (annual nutrient addition) and (c) field identity (A, B, C) from 1982 to 2004. Plotted values are the $R^2$ of each independent variable from a PERMANOVA model. Lines represent loess fits with 95% confidence intervals.

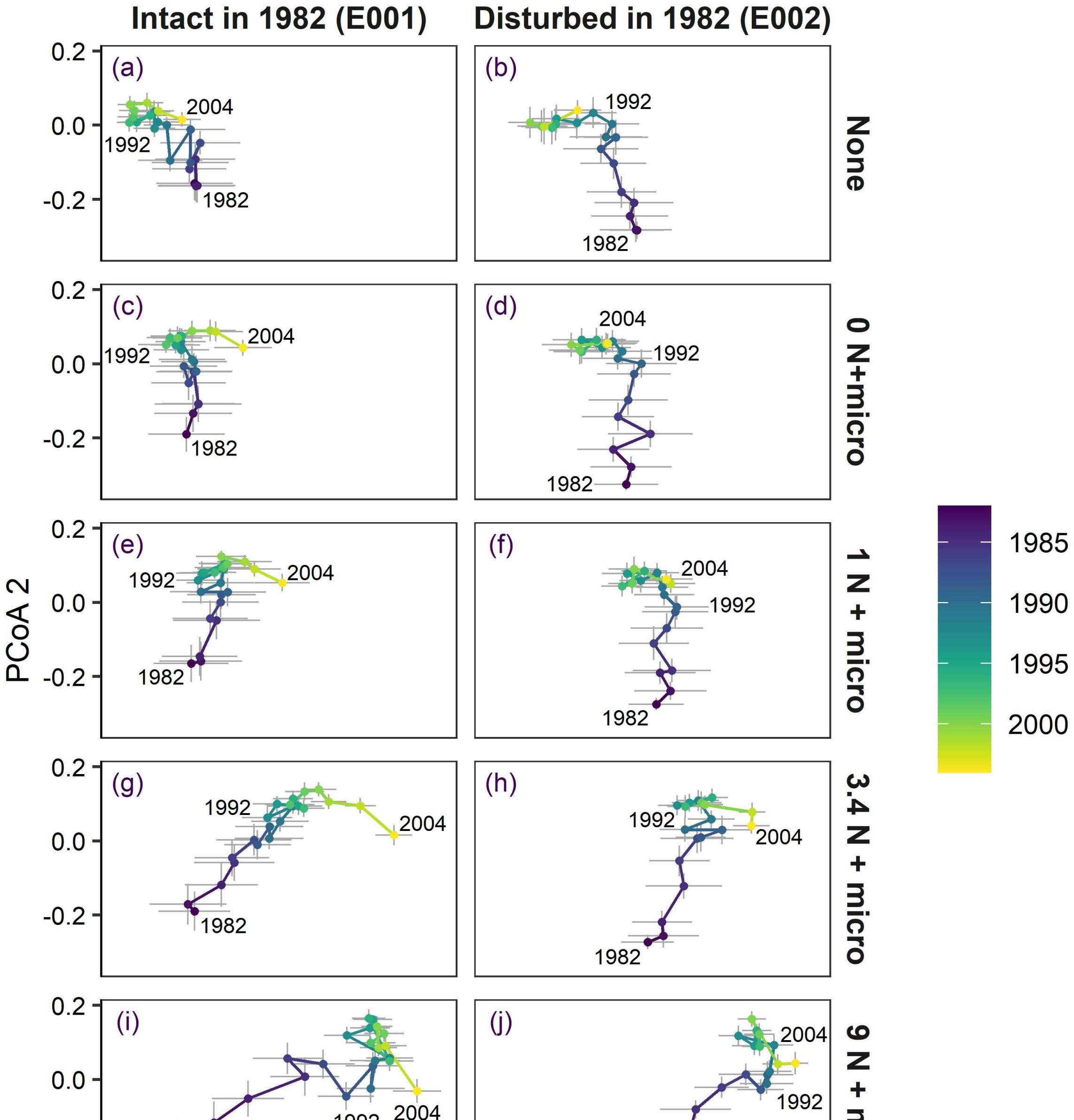

Figure 2: Yearly community trajectories in PCoA ordination space. Points represent mean PCoA scores ± 1 SE (in gray bars) from aboveground plant community composition data in intact (left column) and disturbed (right column) communities across annual fertilization regimes (rows; n=18 plots per treatment). Points are connected through time to show yearly trajectories throughout the experiment from 1982 (purple) to 2004 (yellow).

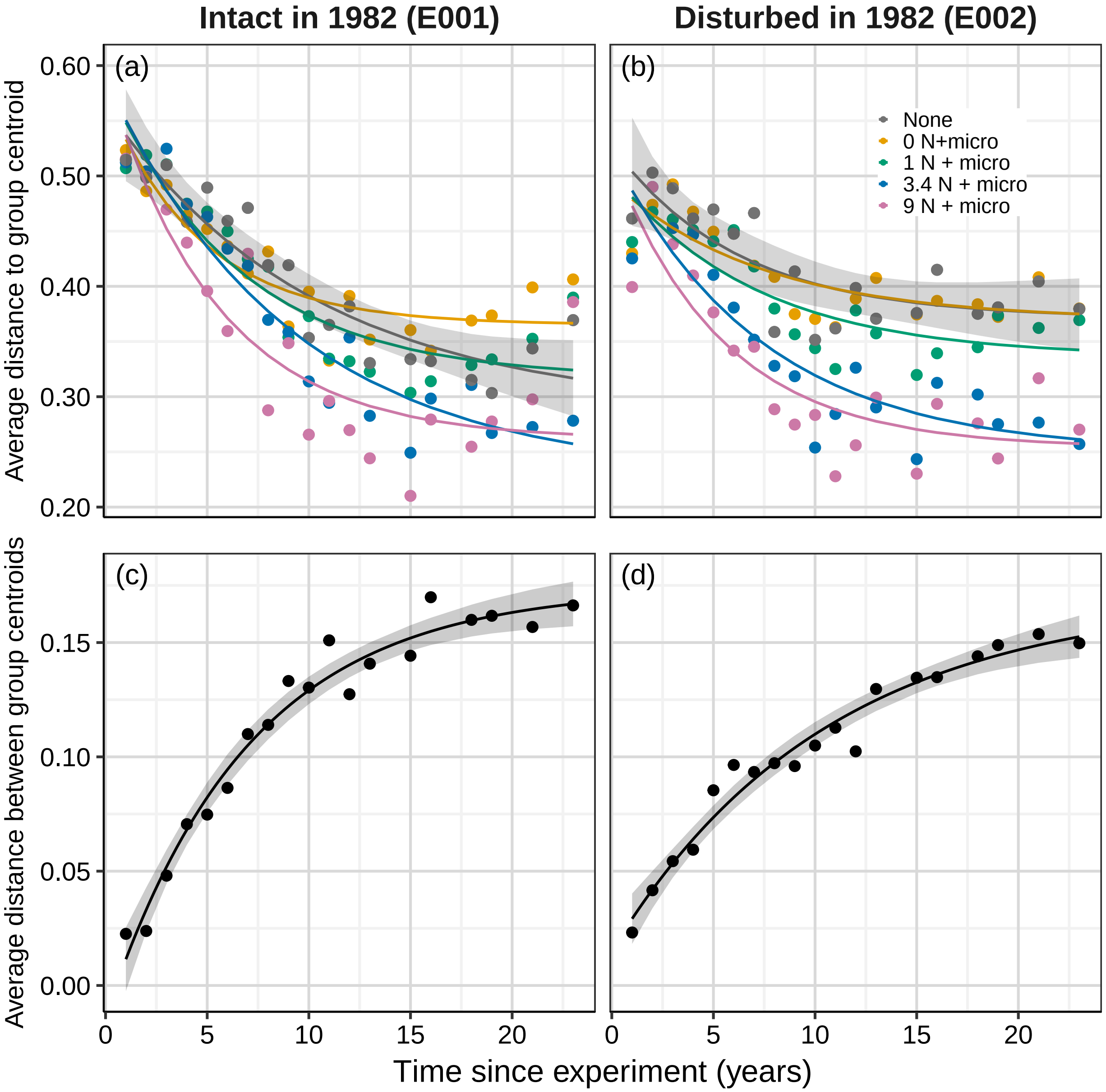

Figure 3: (a & b) Average Bray-Curtis distance from each plot to its treatment group centroid through time in intact (a) and disturbed plots (b). Lines represent asymptotic regressions, with 95% confidence intervals shown for the control. AIC values of competing models and and parameter estimates for regressions are in Table S1 and S2.(c & d) Average Bray-Curtis distance between treatment group centroids through time in intact (c) and disturbed plots (d). Lines represent asymptotic regressions with 95% confidence intervals. AIC values of competing models and parameter estimates for regressions are in Table S3 and S4.

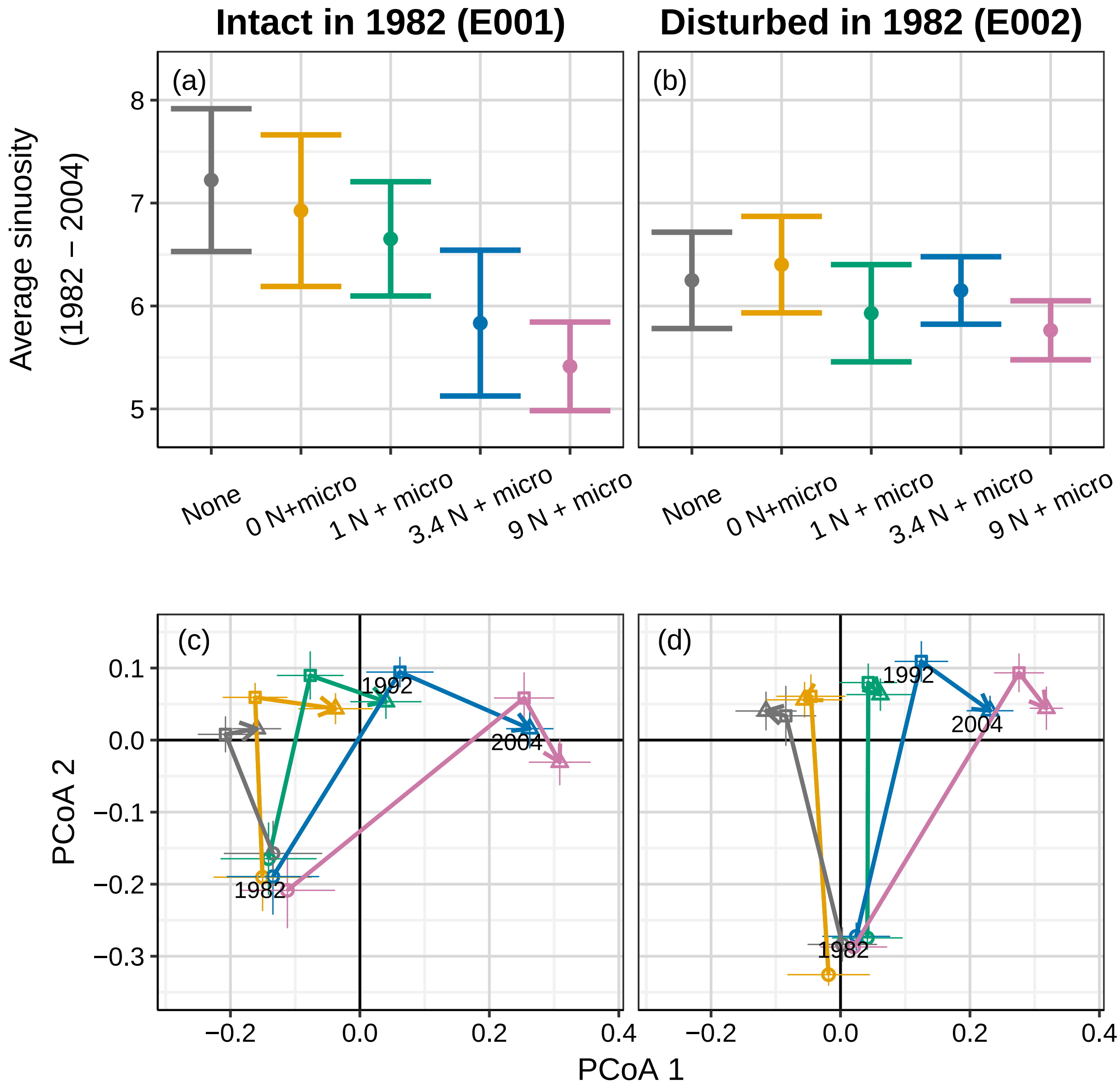

Figure 4: Community trajectories including average sinuosity (a & b) and decadal trajectories (c & d) in PCoA ordination space in intact (a & c) and disturbed (b & d) communities across different levels of N fertilization. Trajectories in c & d are shown for each decade (1982 to 1992 and 1992 to 2004). Each point represents the average sinuosity across the entire time series (a & b) of 18 communities with the same disturbance X nutrient treatment and the average PCoA score in a given year (b & c) or . Error bars represent 95% confidence intervals (a & b) and ± 1 SE (c & d).

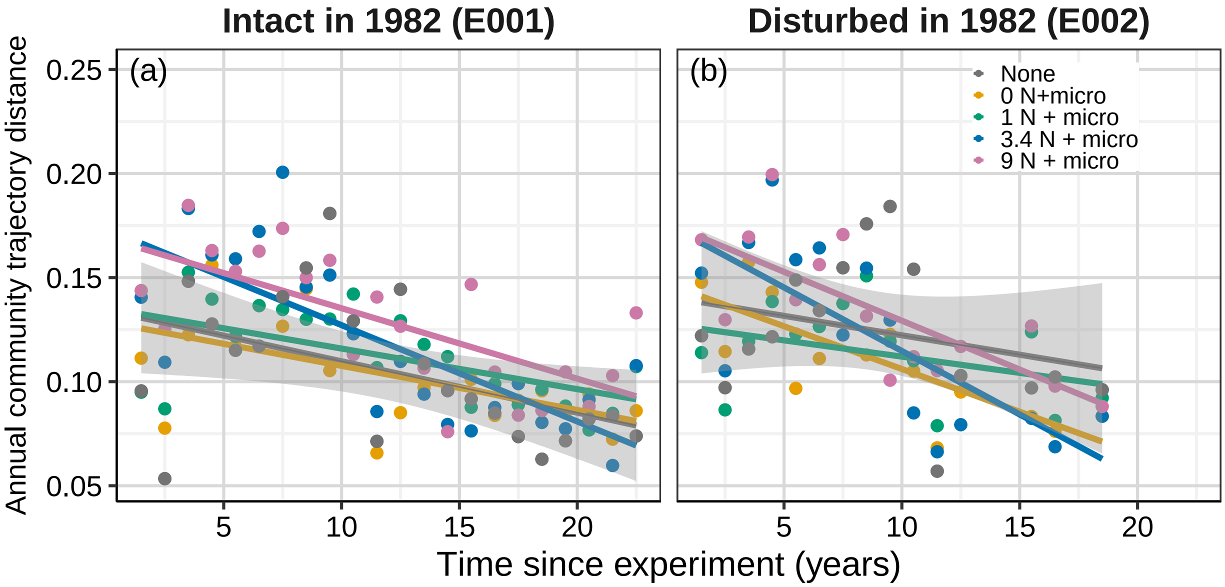

Figure 5: Annual community trajectory distance (temporal species turnover) in aboveground plant community composition in intact (a) and disturbed (b) communities across fertilization treatments. Points represent the average trajectory distance of 18 replicates per treatment. Lines represent linear regression fits, with 95% confidence intervals shown for the control for clarity.