Title: Nutrient addition in grasslands worldwide reveals proportional plant diversity decline across spatial scales but little change in beta diversity

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Abstract:

Plant diversity decline under nutrient addition in local grassland communities is typically ascribed to the loss of rare species, species with particular traits ill-suited for high nutrient levels, and displacement of many localized species with a few widespread species. Whether these changes result in stronger diversity decline and vegetation homogenization at larger spatial scales (aggregated local communities) remains largely unknown. Using a standardized replicated fertilization experiment in 70 grasslands on six continents, we found proportional species loss at local and larger spatial scales but no vegetation homogenization under nutrient addition. Moreover, nutrient addition drove proportional species loss across spatial scales irrespective of species abundance, provenance, life form, or distribution. These results demonstrate that nutrient addition poses a potential threat to all plant functional groups including widespread dominant species that may be critical for ecosystem functions and services.

One-Sentence Summary:

Nutrient addition did not lead to biotic homogenization in experimental grasslands across the world.

Main Text:

Excessive agricultural fertilization and burning of fossil fuels have led to increased availability and redistribution of soil nutrients globally (*1*). Nutrient addition often reduces grassland plant diversity at local scales (i.e. α diversity) (*2*, *3*), typically ascribed to disproportionate loss of rare species and/or species with particular traits ill-suited for high nutrient levels (*4*). Rare species often are lost or displaced more than dominant species regardless of their traits (*4–6*) because small populations are more susceptible to extinction. Here, rare and dominant species are relative to their abundance within sites (e.g. rare species have lower percent cover)(*7*). Localized species are often rarer than widespread species and are therefore more likely to be lost (*8*). Widespread species refer to species that occur in all local communities, while localized species refer to species that occur only in a few local communities within sites. Importantly, localized species are not alway rare, in fact, localized species can be dominant species too (*9*). For instance, across multiple communities sampled, species A is only found at one community with 100% cover, species A is a localized species but also a dominant species. Whereas species B occurs at all communities with a cover of 10%, species B is a widespread species but also a rare species. Additionally, the niche breath of localized species may be narrow, thus, they may be less flexible to adapt to change in nutrient levels than widespread species, leading to their increased loss (*10*). Furthermore, legume species may be lost more than non-legume species due to decreased competitive advantages under high soil nutrients (*4*, *11*). Similarly, native species may be displaced more than non-native species given that non-native species can adapt better to nutrient-rich conditions (*4*, *12*, *13*). Whether these changes result in stronger diversity decline at larger spatial scales (i.e. γ diversity; across aggregated local communities) remains largely unknown. Investigating plant diversity change across scales is crucial to bridge the knowledge between changes in plant diversity seen at local scales and that unfolding at larger scales to support and guide biodiversity conservation/management.

The disproportionate loss of plant diversity at the local community scale suggests that nutrient addition may cause vegetation homogenization at larger spatial scales (i.e. decreasing β diversity among local scales) (*14*). This is because, according to the resource competition model, nutrient addition may homogenize soil nutrients and consistently select for a few highly competitive (for light) widespread species across local communities (Fig. 1A and Fig. 1B) (*15*, *16*). However, nutrient addition may also cause vegetation differentiation (i.e. increasing β diversity) because higher nutrient levels may promote both stochastic and density-dependent processes including priority effects and heterogeneity of diversity-limiting processes such as herbivory (*17–19*). Alternatively, adding nutrients may have weak effects on β diversity if nutrients impact plant diversity through scale-independent processes such as host-pathogen interactions (*20*). In this context, three independent meta-analyses show that grassland plant diversity decline are proportional at local and larger spatial scales under nutrient addition (*20–22*). Besides these three key studies, many single-site experiments and a few multi-site experiments have investigated scale-dependent plant diversity change but often found mixed results, with vegetation homogenization (*23–25*), differentiation (*21*, *26–31*), or no effects all reported (*32*, *33*). However, no study has so far integrated abundance- and trait-based plant diversity change (or lack thereof) seen at local scales to understand plant diversity change at larger spatial scales under nutrient addition.

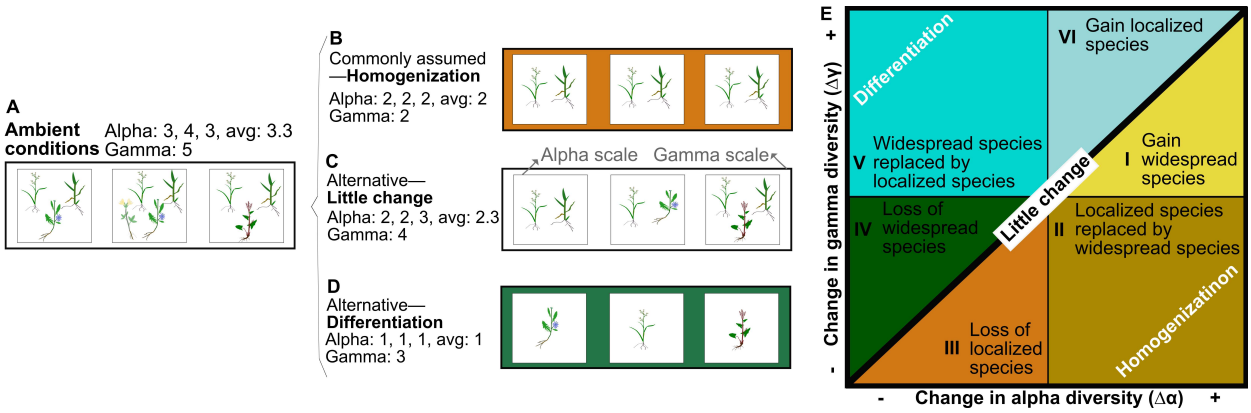

Fig 1. Effects of nutrient addition on beta diversity and an integrated framework to understand plant diversity change across spatial scales. Nutrient addition may cause vegetation homogenization (decrease β diversity), little change, or vegetation differentiation among local communities due to different responses of plant diversity at local and larger spatial scales (i.e. alpha and gamma diversity). This, in turn, is a result of different responses of localized and widespread species to nutrient addition. Species occurring in all local communities within sites are referred to as widespread species, otherwise, localized species.

A recently developed conceptual framework enables us to investigate this important question by explicitly considering change in alpha (Δα), gamma (Δγ), and beta (Δβ) diversity simultaneously through changing localized and/or widespread species (Fig. 1E) (*34*). This framework extends Whittaker's multiplicative β diversity and quantifies Δβ as Δγ/Δα. Accordingly, homogenization may be attributed to I: gain widespread species ($\Delta\gamma < \Delta\alpha$, and $\Delta\gamma > 0$, $\Delta\alpha > 0$); II: Localized species replaced by widespread species ($\Delta\gamma < \Delta\alpha$, $\Delta\gamma < 0$ and $\Delta\alpha > 0$); III: Loss of localized species ($\Delta\gamma < \Delta\alpha$, and $\Delta\gamma < 0$, $\Delta\alpha < 0$). Differentiation may be attributed to IV: Loss of widespread species ($\Delta\gamma > \Delta\alpha$, and $\Delta\gamma < 0$, $\Delta\alpha < 0$); V: Widespread species replaced by localized species ($\Delta\gamma > \Delta\alpha$, $\Delta\gamma > 0$ and $\Delta\alpha < 0$); VI: gain localized species ($\Delta\gamma > \Delta\alpha$, and $\Delta\gamma > 0$, $\Delta\alpha > 0$). We synthesize scale-dependent plant diversity change in response to nutrient addition using a long-term globally distributed standardized nutrient addition experiment in 70 grasslands on 6 continents (i.e. NutNet; Fig. S1 and Table S1; ref (*35*)). Overall, we found adding nutrients did not lead to vegetation homogenization within sites. Instead, nutrient addition result in proportional plant species loss at local and larger spatial scales. Moreover, such propotional species loss occurred irrespective of species abundance, traits, provenance, life form, or distribution under nutrient addition.

Results and discussion

First, we examined the effects of nutrient addition on alpha and gamma diversity over time. It is well established that plant diversity is a multidimensional concept that has multiple facets (*7*, *31*, *37*). We focus on species richness or Hill number (*36*) with q of 0 for this analysis, because species richness is the most commonly examined plant diversity metric (*37*). Extending our understanding of scale-dependent plant diversity change (*20–22*, *32*), we found that, overall, adding nutrients decreased α and γ diversity through time (Fig. 2; Table S2; See Fig. S2 for model fit; see Fig. S3 for plant diversity change at individual sites).

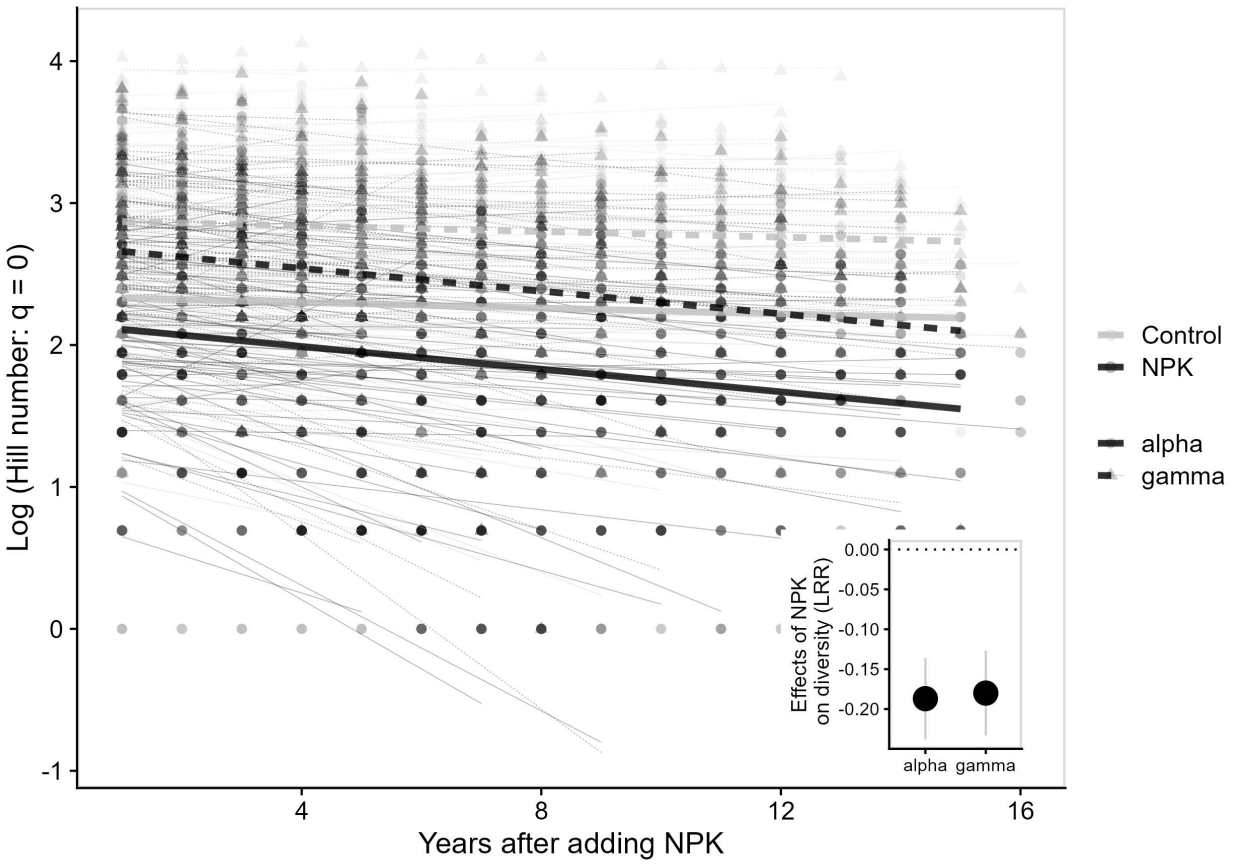

Fig. 2. Predicted alpha and gamma diversity over time under ambient and nutrient addition treatments at 70 NutNet sites. Alpha diversity is the average number of species over 3 blocks, gamma diversity is the total number of species across 3 blocks for each treatment at each site. Species are recorded within 1 × 1 m sampling units. Lines are fitted using linear mixed-effect models with random slope for each site. Thick lines are predicted overall effects across sites, thin lines are predicted site-level trends. Dots are raw data. The inset plot shows the estimated overall difference of plant diversity 4 years after adding nutrients relative to the ambient conditions. Error bars are 95% confidence intervals. See Table S2 for details about model fitting and 95% confidence intervals for estimated intercepts and slopes. See Fig. S3 for plant diversity change at individual sites.

Second, we used estimated changes in alpha and gamma diversity (i.e. $\Delta\alpha$ and $\Delta\gamma$) 4 years after adding nutrients relative to the control to quantify change in beta diversity ($\Delta\beta$). Estimates of $\Delta\alpha$ and $\Delta\gamma$ at individual sites were mapped as x- and y-coordinates, and $\Delta\beta$ was the difference between $\Delta\gamma$ and $\Delta\alpha$ (Fig. 2; Fig. 3A). Overall, adding nutrients had no significant effects on beta diversity (Table S3). A previous study across 34 NutNet sites also finds little change in beta diversity as well community composition dissimilarity under nutrient addition (*32*). At the site level, nutrient addition did not strongly impact beta diversity at 69 sites, while it caused homogenization (lowered β) at 1 site (Fig. 3A). Moreover, at 38 sites, plant diversity change across spatial scales was due to localized and widespread species loss at the same speed, at 23 sites due to loss of localized species only, at 7 sites due to loss of widespread species only, and at 2 sites due to gain of localized and widespread species at the same speed. Without considering 95% confidence intervals, at 36 sites, plant diversity change across spatial scales was due to that loss of widespread species only, at 28 sites due to loss of localized species only, at 3 sites due to

localized and widespread species loss at the same speed, at 2 sites due to gain localized species, at 1 site due to widespread species replaced by localized species. Our results suggest that nutrient addition may mainly impact plant diversity through local processes (e.g. intensifying interspecific competition) rather than through larger-scale processes (e.g. favoring highly competitive species).

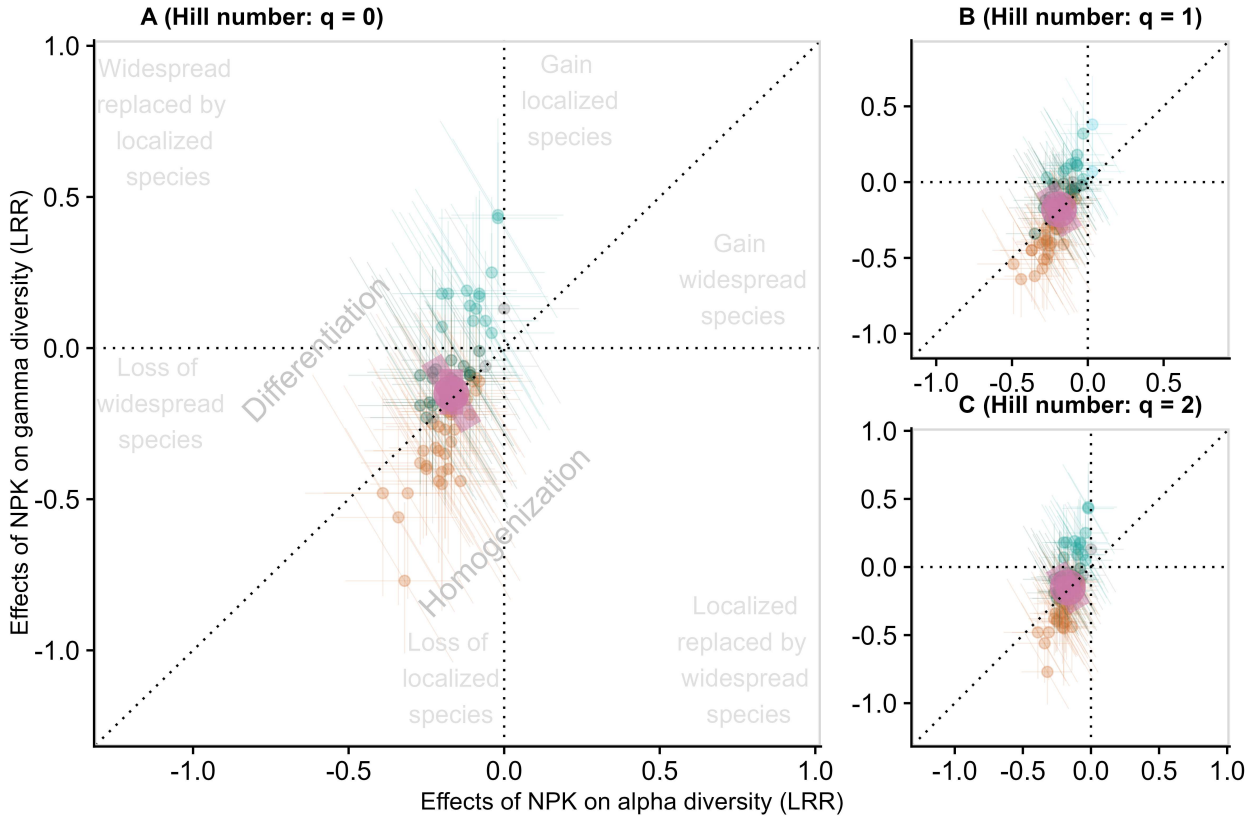

Fig. 3. Predicted effects of nutrient addition on alpha, gamma, and beta diversity (Δα, Δγ, and Δβ) at individual sites and the overall effects. Diversity is calculated using Hill numbers with q of 0, 1, and 2, corresponding to species richness, Shannon diversity, and Simpson diversity. In A, $\Delta\alpha$ and $\Delta\gamma$ are the diversity changes 4 years after nutrient addition from Fig. 2. Δβ is calculated as the difference between Δγ and Δα on the log scale. We extracted 1000 draws from the posterior distribution of the estimated Δγ and Δα and calculated the difference between them to estimate uncertainty of Δβ at individual sites. Colors for small dots correspond to 6 distinct scenarios of Δα and Δγ induced by changes in widespread and localized species in Fig. 1E. The reddish purple large dot is the overall effects of nutrient addition on alpha, gamma, and beta diversity. See Table S3 for estimated overall means and 95% confidence intervals of Δα, Δγ, and Δβ, see Table S4 for site-level estimates.

Third, we investigated effects of nutrient addition on plant diversity accounting for species abundance by using Hill numbers q of 1 and 2 (*36*). An increase in q indicates greater weights of more abundant species. The overall effects were basically the same to that of species richness (Fig. 3B and Fig. 3C; Table S3). Additionally, we did these analyses using slopes (i.e. rate of change in alpha, gamma, and beta diversity), the results were similar to those of using intercepts

(Fig. S4). Our results are in contrast to a previous nitrogen-addition study across 34 grasslands in the US, which shows that rare species were six times more likely to be lost than dominant species in local communities (*4*). This may be due to many reasons including 1) different forms and elements of nutrients added (i.e. NPK vs N), 2) different ways of defining rare species, and 3) different spatial extent used. However, that study also found dominant species can be lost after nitrogen addition(*4*). Similarly, a study based on 49 NutNet sites spanning six continents shows that both rare and abundant species can be lost after adding nutrients in local communities (*38*). Extending these studies, we show that, overall, dominant, common, and rare species were lost under nutrient addition, but the loss was proportional across spatial scales.

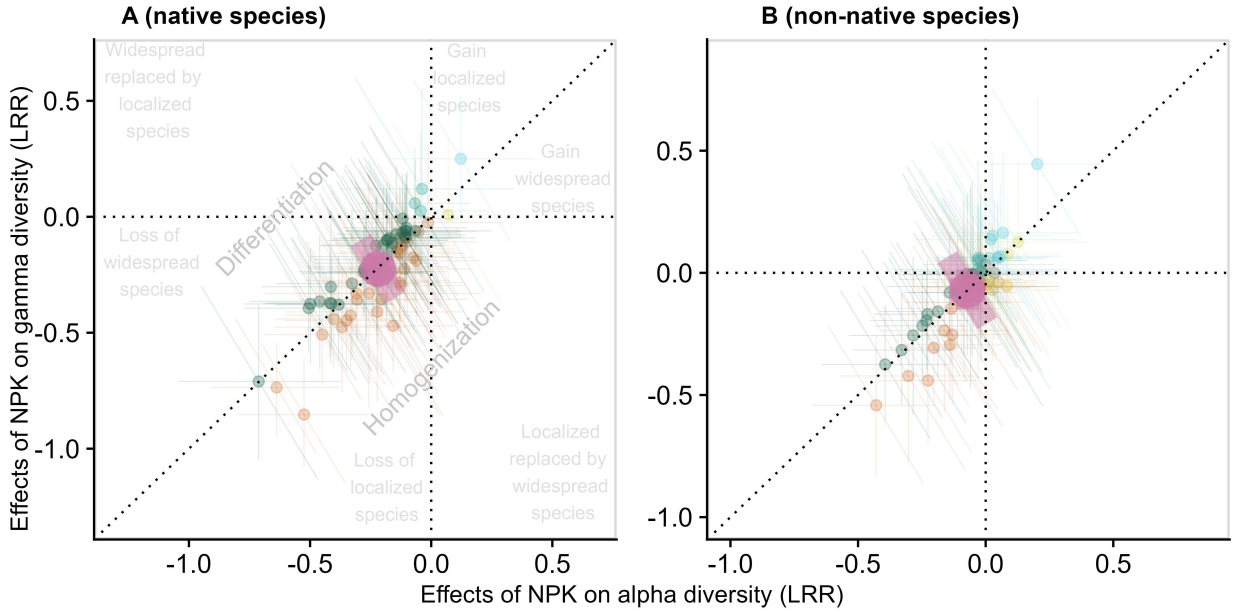

Fig. 4. Predicted effects of nutrient addition on alpha, gamma, and beta diversity (Δα, Δγ, and Δβ) at individual sites and the overall effects for native and non-native species. Diversity refers to the Hill number with q of 0. Colors and dots are the same as Fig. 3. See Table S5 for estimated overall means and 95% confidence intervals for $\Delta\alpha$, $\Delta\gamma$, *and* $\Delta\beta$ for native and non-native species.

Fourth, we investigated whether plant traits influence diversity change across spatial scales under nutrient addition. In line with previous studies (*4*, *20*), we found that nutrient addition decreased native plant diversity much stronger than non-native species diversity (Fig. 4A and Fig. 4B; Table S5). Nutrient addition decreased native species 3 times higher than that of non-native species, but it decreased alpha and gamma diversity at the same magnitude for both species groups (effects on non-native species were not statistically significant; Table S5). Therefore, nutrient addition did not affect beta diversity within native or non-native species group (Table S5). For species with different life forms, nutrient addition decreased forb species diversity the most, followed by woody, graminoid, and legume species diversity (Fig. 5; Table S6). This may be related to species pool size for these life forms (*20*) (site maximum gamma diversity for forbs, graminoids, woody and legumes: 37, 16, 11, 10 species, respectively). Most NutNet sites had forbs and graminoids, but not woody and legume species. Nutrient addition decreased alpha and gamma diversity at a similar magnitude for forbs and graminoids (Table S6). It decreased gamma diversity slightly more than alpha diversity for woody and legume species (Table S6). However, overall, nutrient addition did not significantly lead to vegetation homogenization or

differentiation for either of these functional groups.

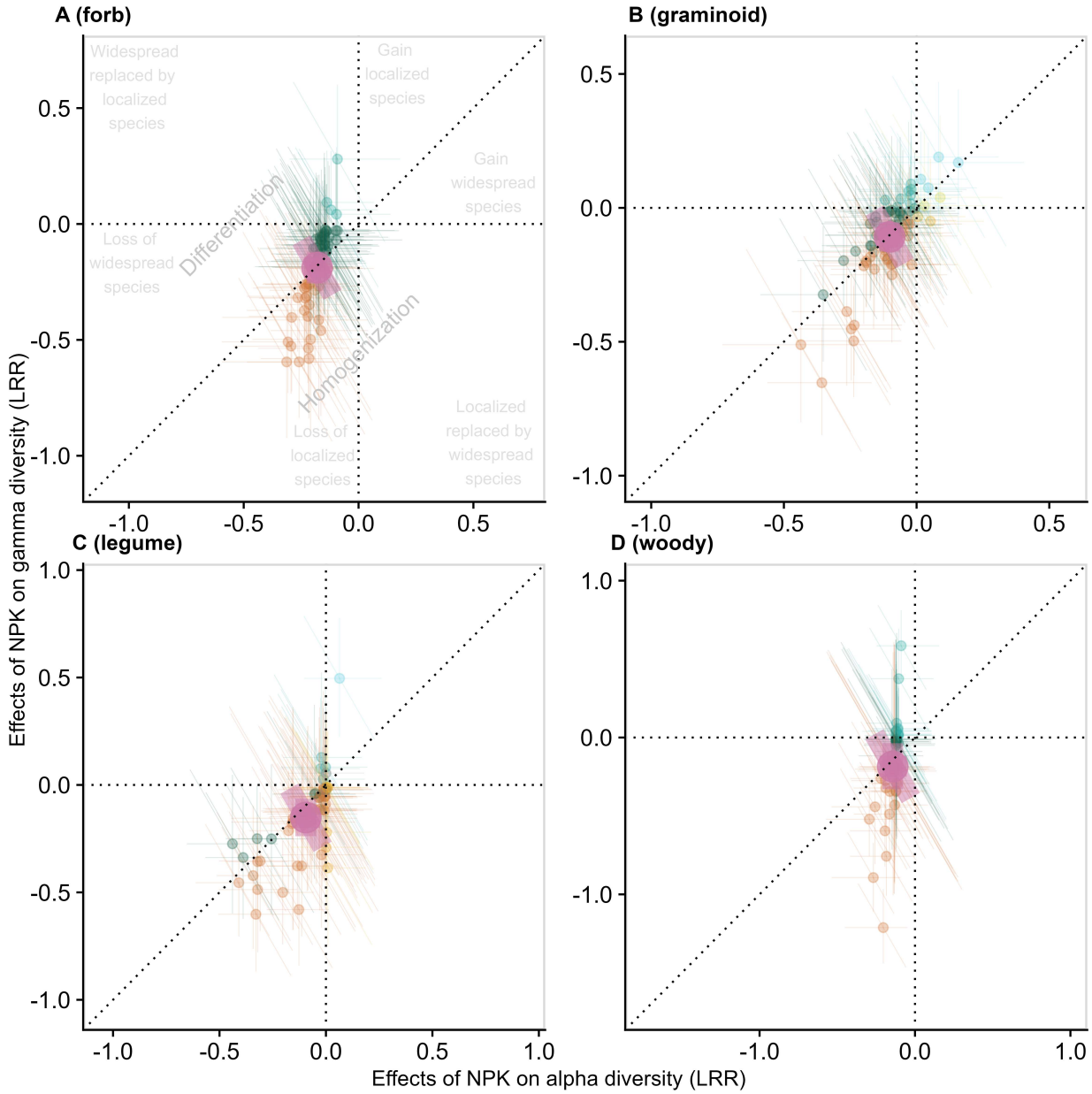

Fig. 5. Predicted effects of nutrient addition on alpha, gamma, and beta diversity (Δ*α*, Δ*γ*, *and* Δ*β*) at individual sites and the overall effects for forb, graminoid, legume, and woody species. Diversity refers to the Hill number with q of 1. Colors and dots are the same as Fig. 3. See Table S6 for estimated overall means and 95% confidence intervals for Δα, Δγ, and Δβ for forb, graminoid, legume, and woody species.

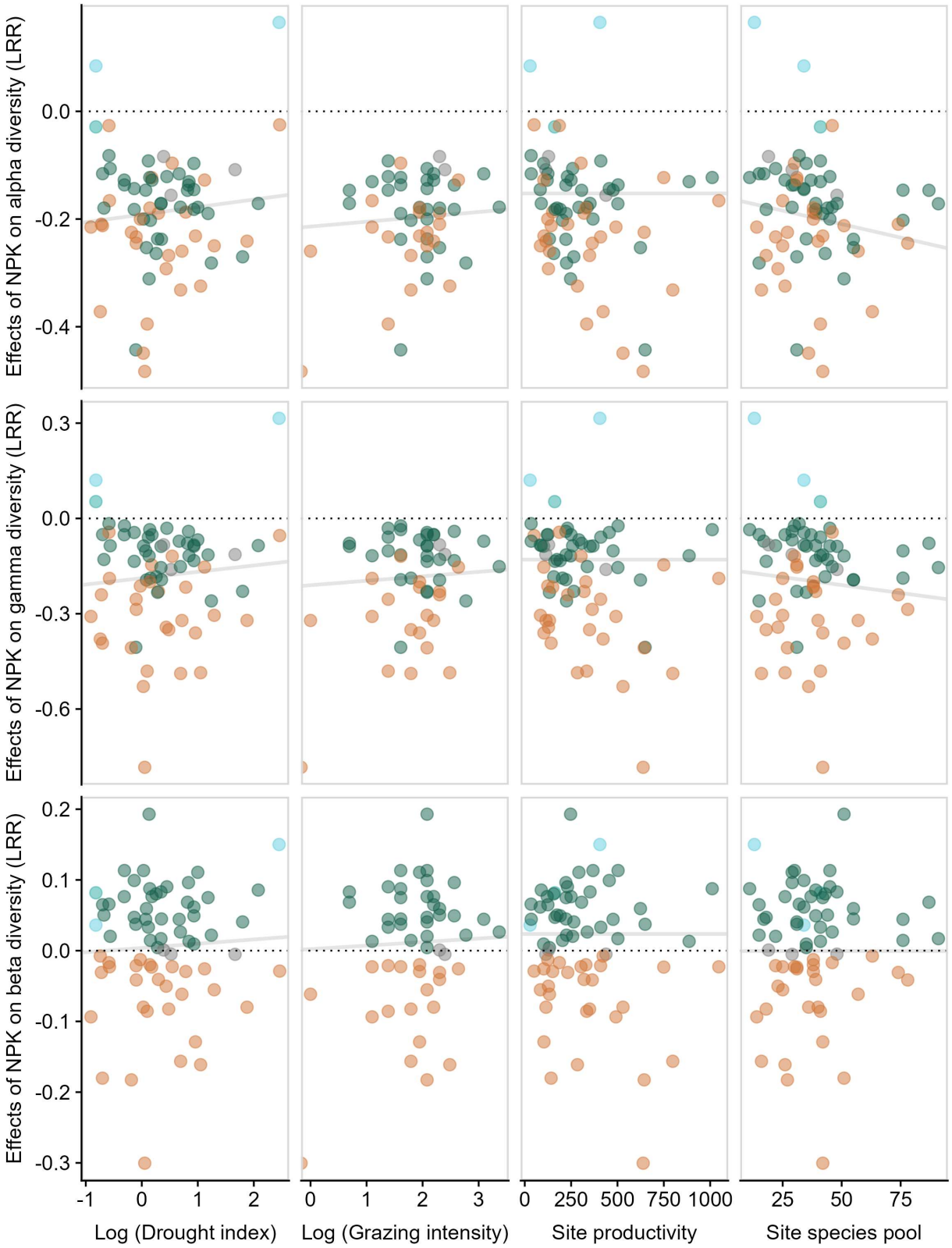

Fig. 6. Bivariate relationship between effects of nutrient addition on alpha, gamma, and beta diversity (Δα, Δγ, and Δβ) and site covariates. Δα, Δγ, and Δβ correspond to Fig. 3A. Colors for small dots correspond to 6 distinct scenarios of Δα and Δγ induced by changes in

widespread and localized species in Fig. 1E. Solid grey lines represent non-significant relationships (95% confidence intervals overlap with 0). See Table S7 for the number of sites used, estimated 95% confidence intervals for intercepts and slopes.

Finally, we investigated whether the effects of nutrient addition on alpha, gamma, and beta ($\Delta\alpha$, $\Delta\gamma$, and $\Delta\beta$) through changing widespread and localized species was mediated by site characteristics. Although the overall patterns of $\Delta\alpha$, $\Delta\gamma$, and $\Delta\beta$ were persistent using different measures of plant diversity, large variations existed among sites (Fig. 3A; Fig. 6). A natural question is then whether sites distributed in six distinct scenarios categorized by localized and widespread species share similarities (Fig. 3A; sites were categorized without considering 95% confidence intervals). We included four site characteristics that have been shown in previous literature to influence $\Delta\alpha$, $\Delta\gamma$, and $\Delta\beta$ in grasslands: drought index, grazing intensity, site productivity, and site species pool (*17*, *20*, *21*, *27*). However, we failed to detect any strong correlation between these site covariates with change in alpha, gamma, or beta diversity under nutrient addition (Fig. 6; Table S7).

To address the robustness of our results, we performed sensitivity tests including using longer-term time series and using subsets of sites with higher number of blocks. A few single-site experiments investigated scale-dependent plant diversity change over time (*24*, *25*, *27*, *39*), and two studies from one single system show that nutrient addition may only cause vegetation homogenization in the longer term (> 3 years) but not short term (*24*, *25*). Therefore, we test whether effects of nutrient addition on plant diversity across spatial scales would be consistent with the main results using 29 and 16 sites that have added nutrients for 10 and 14 years, respectively (Fig. S5 ~ Fig. S8 for 10-year post treatments; Fig. S9 ~ Fig. S12 for 14-year post treatments). The overall effects were similar, but nutrient addition weakly caused vegetation differentiation especially for sites adding nutrients for 14 years (Fig. S5 and Fig. S9). Moreover, we used 16 and 11 sites that had four or five spatial blocks in order to generate larger estimates of $\Delta\beta$, nutrient addition weakly caused vegetation differentiation (Fig. S13 and Fig. S14). Overall, these results were similar to that present in the main text.

Whether the results found here can be extrapolated to a management relevant scale remains to be tested. Our analyses focus on effects of nutrient addition on plant diversity changes across spatial scales within sites, thus, large-scale ecological processes such as seed dispersal may not be well captured. Species dispersal is an important factor that can influence vegetation differentiation or homogenization at landscape scales (*40*). We know that proportional species loss across spatial scales can go down to 0.01 m^2 (*20*), but we still do not know whether such proportional species loss can extend to landscape scales and beyond under nutrient addition.

Conclusions

Our analysis provides a comprehensive synthesis of the impact of nutrient addition on scale-dependent plant diversity change in grasslands worldwide. We found no vegetation homogenization within sites, but proportional plant diversity declines at local and larger spatial scales under nutrient addition, irrespective of species abundance, traits, provenance, life form, or distribution. These results demonstrate that eutrophication poses a potential threat to all functional groups including widespread dominant species that are critical for ecosystem functions and services.

Methods

Experimental setup

The experimental sites used in this study are part of the Nutrient Network (NutNet, Fig. S1 and Table S1). For the analyses here, we used plots under one of two treatments: Control and Fertilized by $NPK_{+\mu}$. $NPK_{+\mu}$ treatment plots were fertilized with nitrogen (N), phosphorus (P), and potassium (K) with a combination of micronutrients and macronutrients (Fe, S, Mg, Mn, Cu, Zn, B, and Mo) as a one-time addition to the potassium treatment ($K_{+\mu}$). The micronutrient mix was applied once at the start of the experiment at a rate of $100 gm^{-2}$. N was supplied as time-release urea ($(NH_2)_2CO$), P was supplied as triple superphosphate ($Ca(H_2PO_4)_2$), and K as potassium sulfate (K_2SO_4). N, P, and K were added annually at rates of 10 g m^{-2} y^{-1}. Nutrients were added to plots of 5 m × 5 m in a randomized block design. More details in experimental design can be found in ref (*35*).

Data were retrieved in November 2023. We selected 70 sites where 1) Control and $NPK_{+\mu}$ were applied for ≥ 4 years; 2) each site had at least 3 blocks. These sites are distributed in 6 continents and include a wide range of grassland types. See Fig. S1 and Table S1 for details of site codes, grassland types, geolocation, and experimental years used.

Sampling protocol

All NutNet sites followed standard sampling protocols. Specifically, a 1 × 1 m subplot within each plot was permanently marked for annual measurement of plant properties. Species cover (%) was estimated visually for individual species in the subplots; thus the total cover of living plants may exceed 100% for multilayer canopies. At most sites, cover was recorded once per year at peak biomass before fertilization. At some sites with strong seasonality, cover was recorded twice per year to include a complete list of species. For those sites, the maximum cover for each species and total biomass were used in the analyses. The taxonomy was adjusted within sites to ensure consistent naming over time. That is, when individuals could not be identified as species, they were aggregated at the genus level but referred to as "species" for simplicity. Aboveground biomass was measured within the treatment plot, but adjacent to the permanent subplot. All aboveground biomass within two 1 × 0.1 m strips (in total 0.2 m^2) were clipped, strips were moved from year to year to avoid resampling the same location. For subshrubs and shrubs occurring within strips, we collected all leaves and current year's woody growth. Biomass (g m^{-2}) was dried at 60 °C (to constant mass) before weighing to the nearest 0.01 g.

Quantifying change in α, γ, and β diversity

First, we calculated α and γ diversity using Hill numbers with q ranging from 0 to 2 (an increase in q indicating greater weights of abundant species) (*36*). Here, q is a continuous variable, we use q of 0, 1, 2, corresponding to species richness, Shannon diversity, and Simpson diversity. α diversity was the average over 3 blocks for each treatment, γ diversity was calculated based on total species (and total cover for q of 1 and 2) occurring over 3 blocks for each treatment at each site. Second, we investigated how α and γ diversity changed over time. We fitted multilevel (also referred as mixed effects or hierarchical) models for α and γ diversity (as the response variable; on the log scale) separately. In the model for either α and γ diversity, we included the interaction of treatment and experimental years as the fixed variable, treatment (NPK and Control) was a categorical fixed variable, and the experimental year was a continuous fixed variable. For α diversity, we included random intercepts and slopes among sites and blocks (nested within sites), model was coded as HillDiv.log ~ trt * year_trt + (trt * year_trt | site_code/block). For γ diversity, we included random intercepts and slopes among sites, model was coded as

HillDiv.log ~ trt * year_trt + (trt * year_trt | site_code). In both models, we subtracted 4 from each observed year so as the treatment intercept term was an estimate of the change in plant diversity 4 years after nutrient addition compared with the control (i.e. the intercepts). Models described above were fitted using the Hamiltonian Monte Carlo (HMC) sampler Stan and coded using the R package 'brms' in R (*41–43*). Models were fitted with 6 chains and 3000 iterations (1000 interaction for warm up). Visual inspection of the HMC chains and Rhat summaries showed excellent convergence (all Rhats < 1.02; Table S2). We visually checked posterior predictive plots to determine how well models can reproduce the data (Fig. S2). Third, we used intercepts in α and γ diversity (i.e. Δα and Δγ) at individual sites extracted from the above models to quantify change in β diversity (Δβ). Similarly, we used slopes in α and γ diversity (i.e. Δα and Δγ) at individual sites extracted from the above models to quantify rate of change. We calculated Δβ as the difference between Δγ and Δα. We extracted 1000 draws from the posterior distribution of the estimate Δα and the corresponding estimate Δγ and calculated the difference between Δγ and Δα from these 1000 points to estimate uncertainty of Δβ at individual sites.

We tested trait-based plant diversity change across spatial scales. First, we ran similar analyses for native and non-native species because previous studies suggest that non-native species may lead to biotic homogenization (*44*). Native species were classified by site PIs. Second, we investigated effects of nutrient addition on plant diversity for different life forms including forb, graminoid, legume, and woody species because previous studies have shown that different functional groups may show distinct responses to nutrient addition (*4*, *11*, *45*).

We tested whether effects of nutrient addition on plant diversity across spatial scales depend on short and long term. We reran analysis using 29 NutNet sites each with more than 10-year post treatments and 16 NutNet sites each with more than 14-year post treatments. We estimated plant diversity change under nutrient addition after 10 and 14 years of treatments. Second, we tested the effects using 14 and 11 NutNet sites that have 4 or 5 blocks (respectively) separately, because three blocks were somewhat limited in spatial extent.

Site covariates

We investigated whether the effects of nutrient addition on alpha, gamma, and beta diversity through changing widespread and localized species was mediated by site characteristics. We included 4 site characteristics that have been shown in previous literature to influence Δα, Δγ, and Δβ in grasslands: site species pool, site productivity, drought index, and grazing intensity (*17*, *20*, *27*, *46*). We quantified the drought index as sum annual evapotranspiration/precipitation, and averaged it during the first four experimental years at each site. We quantified the site species pool as the number of species occurring from year_trt 0 to 3 at each site and site productivity as the average biomass from year_trt 0 to 3 in the control treatment. We quantified an integrated grazing intensity from vertebrate herbivores at each site Specifically, herbivore species (> 2 kg) that consume grassland biomass were documented at each site by site PIs, and each species was assigned an importance value from 1 (present, but low impact and frequency) to 5 (high impact and frequency). An index value was calculated for each site as the sum of herbivore importance values for all herbivores following ref (*47*, *48*). We fitted linear regression models for Δα, Δγ, and Δβ as the response variable separately, each of the site characteristics was used as a predictor variable.

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Author contributions

Conceptualization: QC, JC, SAB, EL, WSH; **Methodology**: QC, JC, SAB; **Investigation and data collection**: all author except QC, JC, SAB, EL, EHG; **Visualization**: QC; **Writing—original draft**: QC; **Writing—review & editing**: all authors. See table S9 for more details for

the contribution of each author.

Competing interests: Authors declare that they have no competing interests.

Data and materials availability: The NutNet data are publicly available on the Environmental Data Initiative (EDI) (https://doi.org/10.6073/pasta/583874460a0af70f93d 3eee2f22f9a13). The raw data used and processed data generated in this study and the R codes used to produce results in this study will be made publicly available once the manuscript is accepted.

Supplementary Materials

Figs. S1 to S14

Tables S1 to S9