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Dominant Grasses, Not Subordinate Forbs, Sustain Ecosystem Multifunctionality Across Spatial Scales

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ABSTRACT

Anthropogenic nitrogen (N) deposition and shifts in plant functional composition can alter grassland biodiversity and ecosystem functioning, yet their interactive effects on multifunctionality across spatial scales remain poorly understood. Using an eight-year field experiment that combined plant functional group removal with N addition in a subalpine meadow, we tested how N addition and the removal of dominant grasses or subordinate forbs affected biodiversity and multifunctionality from empirical α-scale plots to derived β- and γ-scale estimates based on four-plot assemblages. We found that grass removal reduced α- and γ-multifunctionality, whereas forb removal did not affect multifunctionality across scales under both ambient and N addition conditions. The decline in γ-multifunctionality after grass removal was associated mainly with reduced α-component multifunctionality. Grass removal increased plant α-diversity and β-diversity under ambient conditions, whereas forb removal reduced both α- and β-diversity, especially under N addition. However, neither plant nor soil α-diversity predicted α-multifunctionality and changes in plant β-diversity were not accompanied by comparable changes in β-multifunctionality, indicating that, within the range of diversity changes observed here, multifunctionality was more strongly tied to grass-associated functions than to plant diversity. N addition modified plant diversity responses to functional group removal but did not change multifunctionality responses. Our multiscale framework clarifies how dominant functional groups and biodiversity components contribute differently to ecosystem multifunctionality under nutrient enrichment, underscoring the need for integrated conservation approaches that safeguard both dominant and subordinate species.

1 | Introduction

Anthropogenic nitrogen (N) deposition, a pervasive and accelerating driver of global environmental change, has increased approximately threefold since the industrial revolution, with current rates exceeding critical thresholds for ecosystem integrity across vast regions of the globe (Bobbink et al. 2010; Liu et al. 2013; Ackerman et al. 2019). This massive alteration of the global N cycle is recognized as a primary threat to biodiversity and a key component of global change, contributing significantly to widespread biotic homogenization and the erosion of ecosystem resilience (Rockström et al. 2009; Stevens et al. 2015; Hautier et al. 2020; DeSiervo et al. 2023). The cascading effects of this nutrient enrichment fundamentally reshape plant community composition, disrupt biogeochemical cycles, and threaten ecosystem functioning across spatial scales (Olden 2006; Allan et al. 2015; van der Plas et al. 2016; Mori et al. 2018). For example, numerous local-scale N addition

experiments have demonstrated that increased N availability shifts plant functional composition by favoring fast-growing, resource-acquisitive species, while suppressing slow-growing, stress-tolerant species (Suding et al. 2005; Bobbink et al. 2010; Isbell et al. 2013; Zhang et al. 2014). Such functional turnover can profoundly alter ecosystem multifunctionality, the capacity of ecosystems to sustain multiple functions simultaneously, raising critical questions about the long-term provisioning of ecosystem services under continuing N enrichment (Maestre et al. 2012; Allan et al. 2015; Hautier et al. 2018). Although local-scale effects of N addition on ecosystem multifunctionality are increasingly well documented (Hu et al. 2023; Wang et al. 2023; Pichon et al. 2024; Yang et al. 2024), a critical gap remains in understanding how these local perturbations aggregate from individual plots to multi-plot assemblages, limiting our ability to predict the consequences of this global change driver for multifunctionality beyond the plot scale.

Recent theoretical advances, particularly the framework proposed by Chao et al. (2024), enable γ-multifunctionality of a set of local communities to be partitioned into α- and β-components (Figure 1b). In this framework, the α-component represents within-assemblage multifunctionality, whereas β-multifunctionality represents functional dissimilarity among plots within the assemblage. This decomposition is useful because biodiversity and ecosystem functioning may respond differently at local and among-plot scales. For example, local-scale studies have often reported positive associations between α-diversity and α-multifunctionality (Hector and Bagchi 2007; Isbell et al. 2011), whereas β-diversity, the dissimilarity in species composition among local communities, may contribute to spatial heterogeneity in ecosystem functioning (van der Plas et al. 2016; Chao et al. 2024; Li et al. 2025). Thus, the Hill–Chao framework provides a way to separate α-, β- and γ-scale responses of biodiversity and multifunctionality under environmental change. However, its application to N deposition and plant functional group manipulations remains limited.

Dominant and subordinate species may contribute to ecosystem multifunctionality in different ways. According to the mass ratio hypothesis, dominant species can exert strong effects on ecosystem processes because they contribute disproportionately to community biomass, resource capture and soil nutrient cycling (Grime 1998; Smith and Knapp 2003; Pan et al. 2016; Smith et al. 2020). In contrast, alternative perspectives emphasize the potential for rare species to sustain specific functions, such as invasion resistance and biomass at higher trophic levels (Zavaleta and Hulvey 2004; Bracken and Low 2012; Soliveres et al. 2016). N enrichment may change the relative roles of dominant and subordinate species by altering resource availability and competition for light and space, often favoring dominant species while suppressing subordinate species (Clark and Tilman 2008; LeBauer and Treseder 2008; Stevens et al. 2010; Dawson et al. 2012; Payne et al. 2013). Thus, a central question is whether multifunctionality across spatial scales is more sensitive to the removal of dominant grasses or subordinate forbs, and whether these effects are modified by N enrichment. Plant functional group removal provides a direct test of this question: dominant grass removal should have stronger effects than subordinate forb removal if dominant grasses sustain many

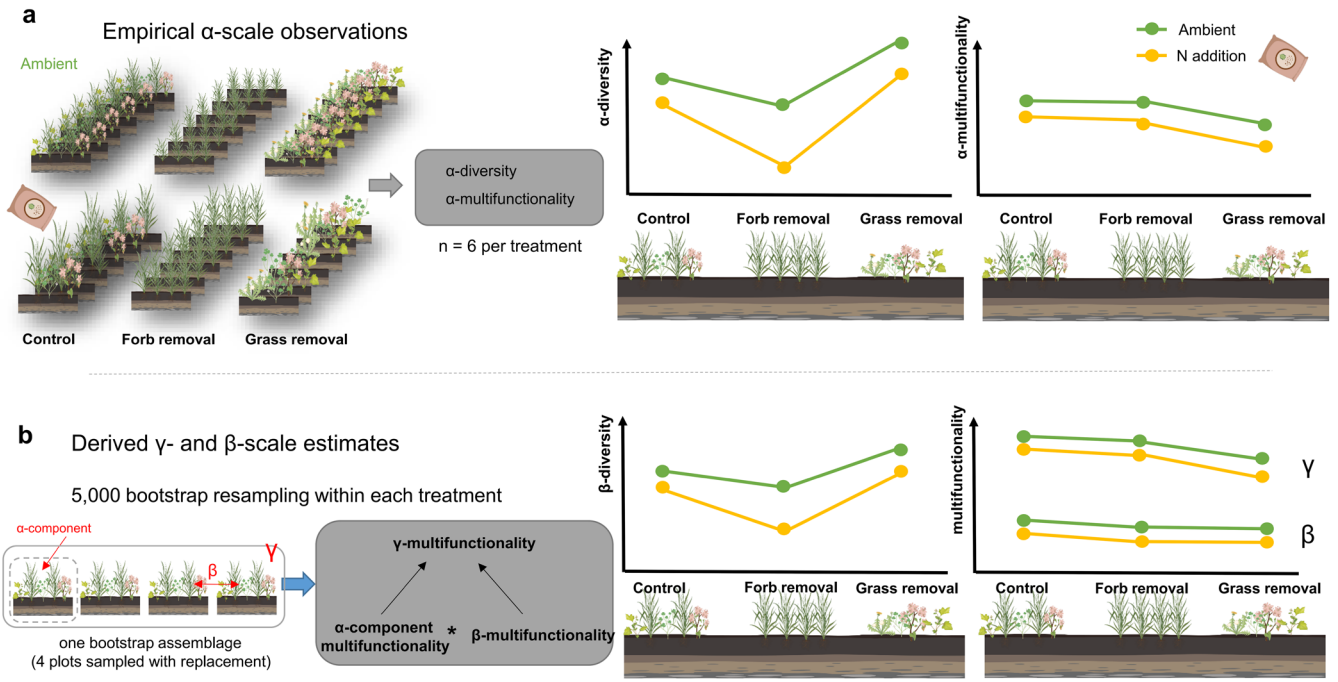

FIGURE 1 | Conceptual framework illustrating the partitioning of biodiversity and ecosystem multifunctionality across spatial scales and their responses to plant functional group removal and N addition. (a) α-diversity and α-multifunctionality were calculated from the original plot-level observations. These α-scale metrics were treated as empirical replicates, with six independent plots per treatment combination. (b) γ- and β-scale metrics of diversity and multifunctionality were treated as derived scale-dependent estimates rather than independent empirical replicates. Their uncertainty was propagated using plot-level bootstrap resampling within each treatment. β-multifunctionality was calculated as γ-multifunctionality divided by the α-component of multifunctionality. The right-hand panels illustrate the expected treatment-response patterns across plant functional group removal under ambient and N addition conditions.

productivity- or nutrient-related functions, whereas the strength of these effects may change under N addition.

We conducted an eight-year field experiment in a subalpine meadow on the eastern Tibetan Plateau, crossing ambient and N-addition conditions with control, dominant grass removal, and subordinate forb removal. In this ecosystem, grasses (2–3 species) account for approximately 65% of the aboveground biomass and thus represent the dominant functional group, whereas forbs contribute about 20% of biomass but account for most of the plant species richness (9–19 species). We quantified biodiversity and ecosystem multifunctionality at empirical α-scale plots and derived β- and γ-scale estimates based on four-plot assemblages, following the scaling framework proposed by Chao et al. (2024). Here, we formulated three hypotheses:

i. Grass removal will reduce α- and γ-multifunctionality because dominant grasses contribute strongly to biomass production, plant-derived inputs, and nutrient cycling. Grass removal may also increase plant diversity by releasing subordinate species from competition.
ii. Forb removal will reduce plant diversity but have weaker effects on measured multifunctionality because the remaining dominant grasses are expected to maintain much of the functional capacity of the community.
iii. N addition may modify the effects of plant functional group removal on biodiversity and multifunctionality, with the direction and strength of these responses differing between grass and forb removal.

By testing these hypotheses, our study clarifies how the loss of dominant grasses and subordinate forbs interacts with nitrogen enrichment to shape biodiversity and measured multifunctionality across experimentally defined spatial scales.

2 | Methods

2.1 | Study Site

Our subalpine meadow removal experiment was established in 2012 at Gannan Grassland Ecosystem National Observation and Research Station (34°44′ N, 102°53′ E, 2900 m) on the Qinghai-Tibet Plateau, in Hezuo, China. The mean annual air temperature is 2.4°C, ranging from −8.3°C during December–February to 11.9°C during June–August. The growing season is short, with about 50 frost-free days annually. The mean annual precipitation is 560 mm, with 85%–90% occurring during the growing season from June to September. The study site has been fenced since 2007 and has been grazed by Tibetan sheep for 20 days each year during January–February. This subalpine meadow is dominated by three grass species, *Roegneria nutans*, *Elymus nutans*, and *Leymus secalinus*.

2.2 | Experimental Design

A two-factor experiment of plant functional group removal and N addition was set up in early June 2012 (Figure S1). The removal treatments consisted of grass removal, forb removal, and no-removal control. Removing grasses (the dominant functional group in this system) substantially reduced community biomass, whereas removing forbs had a smaller effect on biomass but markedly decreased community species richness. A detailed list of removed species is provided in Table S1. The N addition treatment included two levels: control and N addition. Each treatment combination was replicated six times. In total, thirty-six 60 × 60 cm plots were randomly assigned within our study area. The plots were distributed across a 0.5 ha meadow with relatively homogenous vegetation cover and composition, and were separated by buffer zones 70–100 cm wide.

In all removal plots, aboveground biomass of target plant functional groups was manually removed twice per growing season, in early June and late July, from 2012 until sampling in 2020. The N-addition treatment was applied over the same experimental period, so plant removal and N addition were conducted concurrently throughout the experiment. The background N deposition is about 1.0–1.5 g N m^{-2} $year^{-1}$ at our site (Liu et al. 2015). To simulate future increases in N deposition on the Qinghai-Tibet Plateau, we added granular urea containing 42% N in N addition treatment plots at a rate of 5.0 g N m^{-2} $year^{-1}$ each June from 2012 to 2015. Subsequently, urea was added at a total rate of 10 g N m^{-2} $year^{-1}$ in early June (at a rate of 5.0 g N m^{-2}) and July (at a rate of 5.0 g N m^{-2}), respectively, from 2016 to 2020 (Li et al. 2022).

2.3 | Field Sampling

In August 2020, we estimated the percent cover of each plant species in each plot using the grid method described by Yang et al. (2012). To minimize edge effects, only the central 50 × 50 cm area of each plot was sampled. Aboveground plant materials were harvested and dried at 65°C until reaching a constant mass, after which they were weighed to determine aboveground living biomass and litter mass.

Five 0–10 cm-deep soil cores were excavated in each plot using a 3.8 cm diameter soil core sampler. Plant roots from two 0–10 cm soil cores were sieved through a 2-mm mesh, and the soil remaining on the roots was rinsed with water. The roots were oven-dried at 70°C to constant weight to determine root biomass. Other soil cores were mixed and sieved through a 2-mm mesh. Each soil sample was divided into three parts. The first part was air-dried and used to analyze soil total phosphorus, soil organic carbon, and soil total nitrogen. The second part was stored at 4°C for the analysis of dissolved organic carbon, soil moisture, microbial biomass carbon, carbon mineralization rate, net nitrogen mineralization rate, soil NH_4^+ and substrate-induced respiration (Section S1). The other part was stored at −20°C for soil microbial community analysis.

2.4 | Microbial Analyses

Soil bacteria, arbuscular mycorrhizal fungi (AMF), and protist diversity were determined using high-throughput sequencing. Soil DNA from 36 soil samples was extracted from 0.25 g soil using the E.Z.N.A. Soil DNA Kit (Omega Bio-tek, Norcross, GA, USA). The DNA extract was checked on 1% agarose gel, and the DNA

concentration and purity were determined by NanoDrop 2000 UV–vis spectrophotometer (Thermo Scientific, Wilmington, DE). The bacterial V3-V4 region of 16S rRNA genes was assessed using primer pairs 338F and 806R, and AMF communities were amplified using the forward primer AMV4-5NF and the reverse primer AMDGR. To characterize the diversity of protists, the hypervariable V4 region of the 18S rRNA gene was amplified using the general eukaryotic primers TAReuk454FWD1F and TAReukREV3R. All PCR products were sequenced using paired-end sequencing at Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China) on the Illumina NovaSeq PE250 platform. Valid sequences were clustered into different operational taxonomic units (OTUs) based on a 97% similarity cutoff. Finally, we obtained 8261 bacterial OTUs, 341 AMF OTUs, and 3678 protist OTUs.

2.5 | Ecosystem Functions

In each plot, 13 ecosystem functions were measured. These included aboveground living biomass, litter mass, root biomass, dissolved organic carbon, soil total phosphorus, soil organic carbon, soil C:N, substrate-induced respiration, microbial biomass carbon, carbon mineralization rate, net nitrogen mineralization rate, soil NH_4^+ and soil moisture (see Figure S2, Table S2 and Section S1). All measured functions are closely associated with plant productivity and plant-derived inputs, soil carbon and nutrient pools, microbial biomass and activity, biogeochemical process rates and soil water availability; detailed information is provided in Section S1.

2.6 | Assessing Biodiversity and Ecosystem Multifunctionality at Multiple Scales

We assessed biodiversity and ecosystem multifunctionality using empirical α-scale observations and derived β- and γ-scale estimates. Each plot was treated as an empirical α-scale community (Figure 1a). To estimate β- and γ-scale responses, we generated 5000 bootstrap assemblages for each treatment, with each assemblage consisting of four plots resampled with replacement from the six plots in that treatment. Following van der Plas et al. (2016), each four-plot assemblage was treated as a derived γ-scale assemblage for calculating γ-scale biodiversity and multifunctionality, with the corresponding β-scale metrics representing among-plot dissimilarity within that assemblage (Figure 1b). This bootstrap approach allowed us to estimate uncertainty in derived β- and γ-scale metrics while keeping assemblage size constant across treatments.

We quantified biodiversity using Hill-Chao numbers ($q \geq 0$), which determine the sensitivity of diversity estimates to species relative abundances. Because Simpson-based diversity ($q = 2$) integrates richness and evenness and can strongly predict ecosystem functioning across scales (Wang et al. 2021), we primarily used diversity of order $q = 2$. For plant diversity and each soil biotic group (soil bacteria, AMF and protists), empirical α-diversity, α-component diversity, γ-diversity and β-diversity were calculated as:

$$\mathrm{Div}_{\alpha,k} = 1 \Big/ \sum_{i=1}^{S_k} p_{ik}^2$$

$$\mathrm{Div}_{\alpha-\mathrm{component}} = \left[\frac{1}{4} \sum_{k=1}^{4} \frac{1}{Div_{\alpha,k}} \right]^{-1}$$

$$\mathrm{Div}_{\gamma} = 1 \Big/ \sum_{i=1}^{S_\gamma} p_i^2$$

$$\mathrm{Div}_{\beta} = \mathrm{Div}_{\gamma} \,/\, \mathrm{Div}_{\alpha-\mathrm{component}}$$

where p_{ik} is the relative abundance of species or taxon i in plot k, S_k is the number of species or taxa in plot k, p_i is the pooled relative abundance of species or taxon i across the four-plot assemblage, and S_γ is the total number of species or taxa in that assemblage. $\mathrm{Div}_{\alpha\text{-component}}$ is the within-assemblage diversity component used to derive Div_β, which represents spatial turnover among plots within the assemblage, rather than an additional empirical plot-level response. To represent soil biodiversity at each scale, we used a composite index described by Zhang et al. (2023), calculated as the average of min-max standardized soil bacterial, AMF and protist diversity metrics (Figure S3).

We used two complementary multifunctionality calculations. First, for empirical α-scale analyses, plot-level α-multifunctionality ($MF\alpha$) was calculated for each plot from its ecosystem-function vector using the "MF1_single()" function in the R package "MF.beta4" and used as the empirical α-scale response. Second, for derived β- and γ-scale analyses, we applied the decomposition framework of Chao et al. (2024) to each four-plot bootstrap assemblage using the "MF2_multiple()" function in "MF.beta4". In this framework, γ-multifunctionality ($MF\gamma$) represents multifunctionality at the assemblage scale, α-component multifunctionality ($MF\alpha\text{-component}$) is the within-assemblage component used to decompose $MF\gamma$ and derive β-multifunctionality (MF_β), and MF_β represents spatial dissimilarity or heterogeneity in multifunctionality among plots. These components satisfy:

$$\mathrm{MF}_{\gamma} = \mathrm{MF}_{\alpha-\mathrm{component}} \times \mathrm{MF}_{\beta}$$

$$\log\left(\mathrm{MF}_{\gamma}\right) = \log\left(\mathrm{MF}_{\alpha-\mathrm{component}}\right) + \log\left(\mathrm{MF}_{\beta}\right)$$

Because each bootstrap assemblage contained four plots, MF_β ranges from 1 to 4, with larger values indicating greater among-plot dissimilarity in multifunctionality within the assemblage. Full mathematical details of Hill-Chao multifunctionality are provided in Section S2.

2.7 | Statistical Analysis

We analyzed empirical α-scale responses and derived β−/γ-scale responses separately to match the structure of the data. For α-scale responses, α-multifunctionality, plant α-diversity and soil α-diversity were natural-log transformed to improve normality and analyzed using the 36 empirical plot-level observations. We used two-way ANOVA to test the effects of plant functional group removal, N addition and their interaction on each α-scale response variable. Tukey's post hoc tests were performed with the 'emmeans' package (Lenth 2021). When there was a significant interaction between removal

treatment and N addition, we compared removal treatments within each N treatment and, where needed, N treatments within each removal treatment; when only the removal main effect was significant, removal treatments were compared overall.

We quantified uncertainty in β-diversity, β-multifunctionality and γ-multifunctionality using 5000 bootstrap resamples. Treatment effects were evaluated using planned raw-difference contrasts. Grass removal and forb removal main contrasts compared each removal treatment with the control averaged over the two N treatments, and the N-addition main contrast compared N addition with ambient conditions averaged over removal treatments. Interaction contrasts tested whether the removal effect differed between ambient and N addition conditions. For multifunctionality, we also calculated log-scale contrasts to decompose the γ-multifunctionality response into α-component and β-multifunctionality. Bias-corrected (BC) 95% bootstrap confidence intervals (CIs) were used for β-scale contrasts to reduce bias in ratio-derived bootstrap distributions. Because β-scale metrics may also have skewed bootstrap distributions, we additionally recalculated all β-scale contrasts using bias-corrected and accelerated (BCa) 95% CIs, with the acceleration factor estimated by jackknife resampling. The BCa intervals gave identical support-status conclusions to the bias-corrected intervals; therefore, we present the bias-corrected intervals in the figures and tables. Percentile 95% bootstrap CIs were used for γ-multifunctionality and α-component multifunctionality contrasts. Effects were considered supported when the corresponding intervals excluded zero. We then performed multigroup structural equation modeling (SEM) using the 'piecewiseSEM' package (Lefcheck 2016) to examine α-scale pathways linking plant functional group removal, biodiversity and multifunctionality, and to test whether these pathways differed between ambient and N-addition conditions. The SEM used empirical plot-level observations, whereas β- and γ-scale biodiversity and multifunctionality, which were derived from resampled four-plot assemblages, were analyzed using bootstrap contrasts rather than pathway modeling. The a priori SEM included direct pathways from grass and forb removal to α-multifunctionality and indirect pathways through plant and soil α-diversity (Figure S4). Shipley's *d*-separation test was then used to confirm that no significant paths were missing, and model fit was considered acceptable when Fisher's C test was non-significant ($p > 0.05$).

To test the robustness of our results, we repeated the diversity analyses for $q = 0$ and 1 and the multifunctionality analyses for $q = 1$. Most of the results were consistent across different Hill-Chao numbers; therefore, we primarily present findings based on $q = 2$ in the main text. Because grass removal can directly reduce biomass production and plant-derived inputs, we conducted an additional sensitivity analysis to test whether multifunctionality responses depended on biomass-related functions. Specifically, we repeated the $q = 2$ multifunctionality analyses after excluding aboveground living biomass, litter mass and root biomass from the multifunctionality index. All analyses were performed in R version 4.4.3 (R Core Team 2025).

3 | Results

3.1 | Impact of Plant Functional Group Removal and N Addition on Multifunctionality Across Spatial Scales

Using the empirical α-scale observations, two-way ANOVA showed that plant functional group (PFG) removal significantly affected α-multifunctionality ($F_{2,30} = 6.273$, $p = 0.005$; Figure 2a), whereas N addition had no significant main effect ($F_{1,30} = 2.909$, $p = 0.098$) and did not interact with PFG removal ($F_{2,30} = 0.004$, $p = 0.996$). Tukey comparisons showed that grass removal reduced α-multifunctionality by 14.3% relative to the control ($t_{30} = -2.53$, $p = 0.043$) and by 18.8% relative to forb removal ($t_{30} = -3.41$, $p = 0.005$); forb removal did not differ from the control ($t_{30} = 0.88$, $p = 0.655$).

For β-multifunctionality, planned bootstrap contrasts showed no clear response to PFG removal, N addition or their interaction, as all 95% CIs included zero (Figure 2b). At the γ-scale, grass removal reduced multifunctionality relative to the control (95% CI [−1.429, −0.075]), whereas forb removal and N addition had no significant effects. No interaction between N addition and PFG removal was clearly supported for γ-multifunctionality (Figure 2c). Log-scale decomposition showed that this grass-removal response was associated mainly with the α-component multifunctionality, whereas the β-multifunctionality component was close to zero (Figure 2d). The multifunctionality results were similar when calculated at $q = 1$ (Figure S5). After excluding aboveground living biomass, litter mass and root biomass, PFG removal, N addition and their interaction had no significant effects on α-, β- or γ-multifunctionality (Figure S6).

3.2 | Responses of Plant and Soil Biodiversity Across Spatial Scales

PFG removal significantly affected plant α-diversity ($F_{2,30} = 42.50$, $p < 0.001$), whereas N addition did not ($F_{1,30} = 1.18$, $p = 0.285$). There was a significant interaction between N addition and PFG removal ($F_{2,30} = 4.78$, $p = 0.016$). Specifically, under ambient conditions, grass removal tended to increase plant α-diversity by 35.4% relative to the control ($t_{30} = 2.24$, $p = 0.080$), whereas forb removal decreased it by 37.3% ($t_{30} = -3.46$, $p = 0.005$). Under N addition, grass removal did not significantly affect plant α-diversity ($t_{30} = -1.39$, $p = 0.361$), whereas forb removal decreased it by 63.1% ($t_{30} = -7.38$, $p < 0.001$; Table S3). Within removal treatments, N addition increased plant α-diversity in control plots by 53.0% ($t_{30} = 3.15$, $p = 0.004$), but not under grass removal ($t_{30} = -0.48$, $p = 0.633$) or forb removal ($t_{30} = -0.78$, $p = 0.442$). Soil α-diversity showed no significant main effects of PFG removal ($F_{2,30} = 0.75$, $p = 0.479$) or N addition ($F_{1,30} = 0.13$, $p = 0.720$), and no interaction between them ($F_{2,30} = 0.004$, $p = 0.996$; Figure 3c; Table S3).

For β-diversity, planned bootstrap contrasts showed clear responses for plants but not soils (Figure 3b,d). Main-effect contrasts showed that grass removal increased plant β-diversity relative to

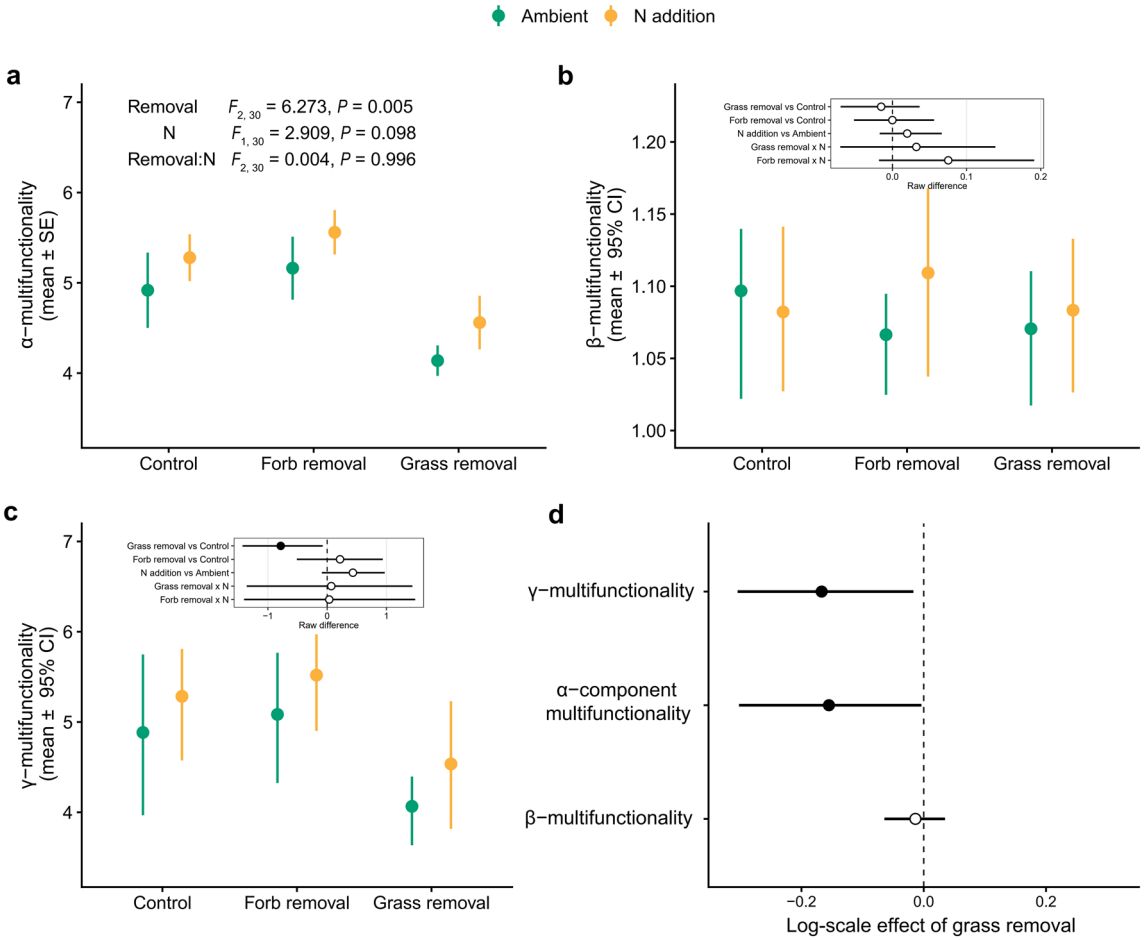

FIGURE 2 | Effects of plant functional group removal and nitrogen addition on ecosystem multifunctionality across spatial scales. Shown are treatment responses of (a) α-, (b) β-, and (c) γ-multifunctionality, and (d) the log-scale effect of grass removal relative to the control. Points and vertical lines represent treatment means ± SE for α-multifunctionality and treatment means with percentile 95% confidence intervals for derived β- and γ-scale estimates at the four-plot γ-scale. Insets show planned raw-difference contrasts; bias-corrected 95% confidence intervals are used for β-multifunctionality and percentile 95% confidence intervals for γ-multifunctionality. In the insets and panel (d), filled points indicate intervals excluding zero. Post hoc comparisons for α-multifunctionality are provided in Table S3.

the control (95% CI [0.050, 0.504]), whereas forb removal decreased it (95% CI [−0.298, −0.156]). The overall N-addition main contrast showed no clear effect. Because the interaction between N addition and forb removal was supported (95% CI [−0.373, −0.027]), we examined simple contrasts and found that forb removal decreased plant β-diversity under both ambient conditions (95% CI [−0.212, −0.045]) and under N addition (95% CI [−0.416, −0.202]), with a larger reduction under N addition. Conversely, N addition increased plant β-diversity in control plots (95% CI [0.026, 0.361]), whereas this effect was not supported in the removal treatments. Soil β-diversity showed no clear main effects or interactions, as all 95% CIs included zero (Figure 3d; Table S4). Biodiversity responses were generally similar when diversity was calculated at $q = 0$ and 1 (Figure S7; Tables S3 and S4).

3.3 | α-Scale Pathways Linking PFG Removal, Biodiversity and Multifunctionality

We used multigroup SEM to examine whether α-scale pathways differed between ambient and N-addition conditions. The model showed acceptable fit to the data (Fisher's C = 5.27, df = 2, $p = 0.07$; AIC = 33.27; Figure 4). Under ambient conditions, grass removal had a direct negative effect on α-multifunctionality (standardized path coefficient = −0.43, $p < 0.05$) and a positive effect on plant α-diversity (0.39, $p < 0.05$). Forb removal decreased plant α-diversity (−0.60, $p < 0.01$). Under N addition, the direct negative effect of grass removal on α-multifunctionality remained significant (−0.52, $p < 0.05$), but the positive grass-removal effect on plant α-diversity was no longer detected. In contrast, the negative effect of forb removal on plant α-diversity was stronger under N addition (−0.96, $p < 0.001$). Plant α-diversity was not significantly associated with α-multifunctionality under either ambient or N-addition conditions, and soil α-diversity pathways were not supported.

Overall, grass removal reduced α-multifunctionality mainly through a direct pathway, whereas the observed α-diversity responses to PFG removal did not translate into detectable α-multifunctionality pathways in the SEM.

4 | Discussion

Our findings show that dominant grasses, rather than subordinate forbs, played a key role in maintaining measured multifunctionality across experimentally defined spatial scales.

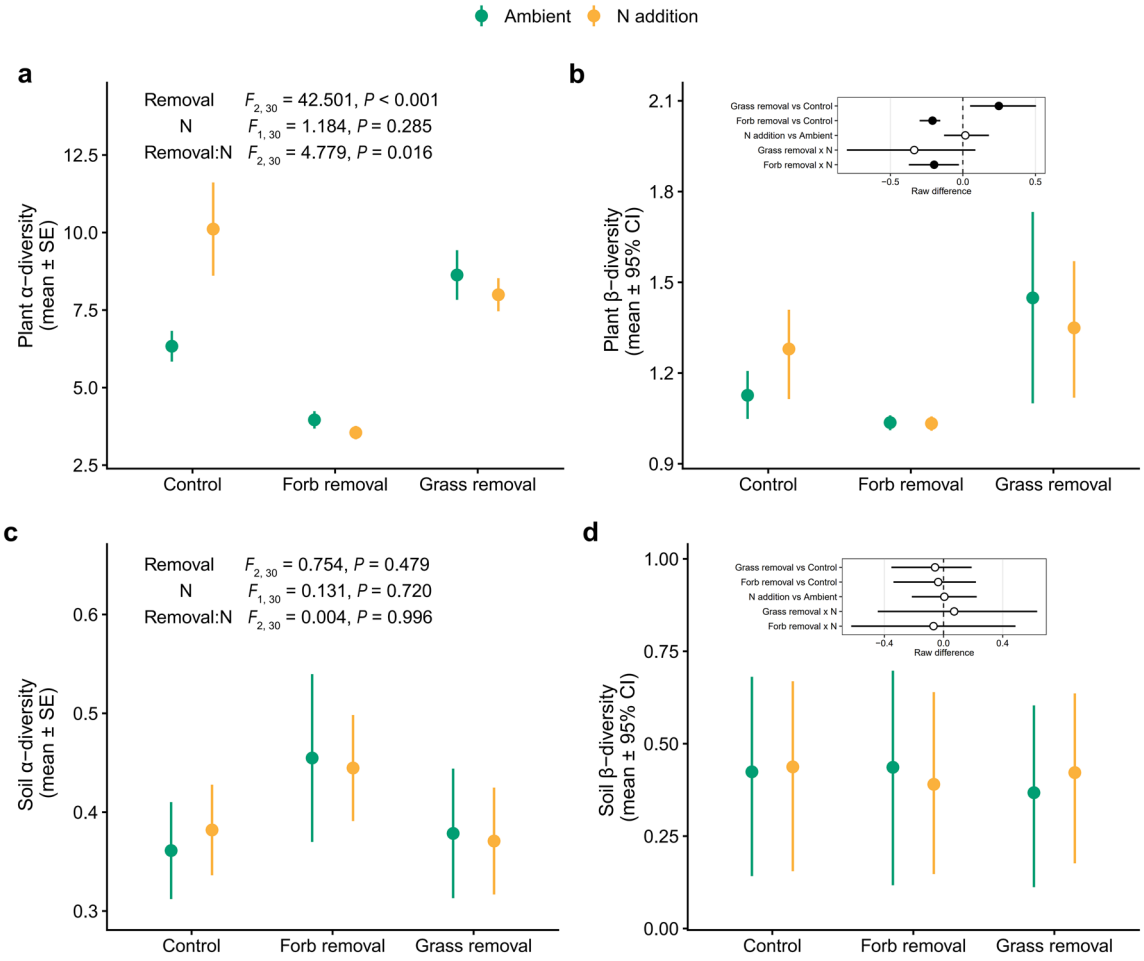

FIGURE 3 | Effects of plant functional group removal and nitrogen addition on plant and soil diversity. Shown are (a) plant α-diversity, (b) plant β-diversity, (c) soil α-diversity and (d) soil β-diversity. Points and vertical lines represent treatment means ± SE for α-diversity and treatment means with percentile 95% confidence intervals for derived β-scale estimates at the four-plot γ-scale. Insets show planned raw-difference contrasts using bias-corrected 95% confidence intervals; filled points indicate intervals excluding zero. Post hoc comparisons for α-diversity and β-diversity are provided in Tables S3 and S4, respectively.

Consistent with Hypotheses i and ii, the loss of dominant grasses reduced both empirical α-multifunctionality and derived γ-multifunctionality. Forb removal, by contrast, caused pronounced losses of plant diversity but weaker changes in multifunctionality across scales. In relation to Hypothesis iii, N addition modified the effects of functional group removal on plant diversity, but these changes did not lead to stronger α- or γ-multifunctionality responses. Together, these findings suggest that dominant grasses sustained measured α- and γ-multifunctionality mainly through biomass-linked functions, with no detectable diversity-mediated pathway in the α-scale SEM.

4.1 | Dominant Grasses Sustained Measured Multifunctionality Across Experimentally Defined Spatial Scales

Our results showed that grass removal reduced both α- and γ-multifunctionality (Figure 2a,c). This pattern is consistent with the mass ratio hypothesis, which predicts that ecosystem processes are strongly influenced by the most abundant species and their associated biomass (Grime 1998; Smith et al. 2020). In this meadow, dominant grasses likely affected α- and γ-multifunctionality through two linked pathways. First, a dominance-related effect may have arisen because grasses were the dominant functional group and contributed to resource capture and soil nutrient cycling (Smith and Knapp 2003; Zhang et al. 2017; Eisenhauer et al. 2018). Grass removal also reduced soil NH_4^+ availability, suggesting that dominant grasses contributed to soil N cycling and that this grass-associated function was not rapidly compensated by the remaining community in this alpine ecosystem (Marshall et al. 2011; Figure S2; Table S2). Second, a biomass-related effect likely contributed because grasses accounted for most aboveground biomass, and their removal reduced biomass production and plant-derived inputs, as reflected by lower aboveground living biomass (Figure S2; Table S2). Consistent with this interpretation, after excluding aboveground living biomass, litter mass and root biomass, plant functional group removal, N addition and their interaction showed no clearly supported effects on α-, β- or γ-multifunctionality calculated from the reduced function set (Figure S6), indicating that biomass-related functions accounted for much of the grass-removal response. These functions are ecologically meaningful because biomass production, litter inputs and root biomass represent plant-derived inputs linking

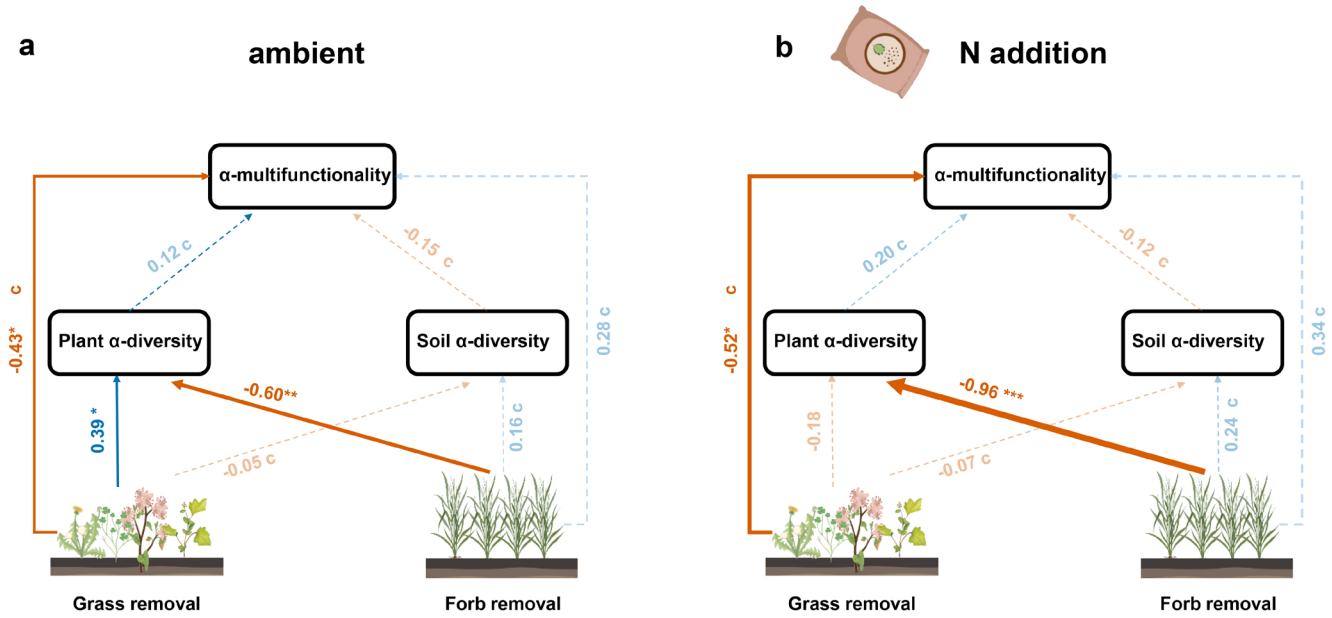

FIGURE 4 | Multigroup structural equation model links plant functional group removal, plant and soil α-diversity and α-multifunctionality using the empirical plot-level observations under (a) ambient and (b) N-addition conditions. Model fit: Fisher's $C = 5.27$, df = 2, $p = 0.07$; AIC = 33.27. Solid arrows indicate significant paths ($p < 0.05$), whereas dashed arrows indicate non-significant paths ($p > 0.05$). Blue and orange arrows indicate positive and negative standardized path coefficients, respectively. Arrow thickness is proportional to the standardized path coefficient, with the numbers on the arrows representing the coefficients and asterisks indicating significance: ***$p < 0.001$; **$p < 0.01$ and *$p < 0.05$. Paths without (c) markers represent unconstrained paths that differed between the ambient and N addition treatments as determined using type III ANOVA.

aboveground productivity with belowground carbon and nutrient cycling (Wardle et al. 2004; Zavaleta et al. 2010).

In addition, the scale decomposition further clarified how the grass-removal effect was expressed across spatial scales. Under the Hill–Chao framework, log γ-multifunctionality is additively decomposed into log α-component multifunctionality and log β-multifunctionality (Chao et al. 2024). In our study, grass removal reduced α- and γ-multifunctionality but had little effect on β-multifunctionality, indicating that the decline in γ-multifunctionality was transmitted mainly through reduced α-component multifunctionality (Figure 2d). This suggests that grass removal caused relatively consistent losses of local functioning across plots, so these local losses accumulated to reduce γ-multifunctionality. By contrast, β-multifunctionality contributed little to the grass-removal response, possibly because grass removal did not substantially alter among-plot functional heterogeneity or because the remaining communities were functionally similar for the measured ecosystem functions (Rosenfeld 2002).

4.2 | Plant Diversity Changes Did Not Consistently Translate Into Measured Multifunctionality Across Scales

Grass removal altered plant diversity in a way consistent with competitive release of subordinate species. Under ambient conditions, grass removal increased plant β-diversity and tended to increase plant α-diversity (Figure 3), which may reflect reduced competition for light, space, or belowground resources after dominant grasses were removed (Grime 1998; Hautier et al. 2009; Borer et al. 2014). However, such release may depend on dark diversity, that is, whether suitable subordinate species are present in the surrounding species pool but absent from the realized plot community (Pärtel et al. 2013). In contrast, forb removal reduced both plant α- and β-diversity, consistent with the strong contribution of forbs to plant species richness in this meadow (Table S1).

At the α-scale, plant and soil α-diversity were not detectably associated with α-multifunctionality in the SEM (Figure 4). This does not imply that α-diversity is generally unimportant for α-multifunctionality, as many studies have shown positive biodiversity–multifunctionality relationships, especially across broad diversity gradients or communities that differ strongly in composition and functional traits (Hector and Bagchi 2007; Zavaleta et al. 2010; Isbell et al. 2011; Li et al. 2025). In our experiment, however, changes in plant diversity mainly reflected shifts in subordinate species, whereas changes in measured multifunctionality were more closely linked to dominant grass loss, likely because the selected functions were strongly associated with biomass-related processes. This interpretation is consistent with studies showing that ecosystem functioning under non-random species loss can depend strongly on dominant species, community functional structure, phylogenetic diversity and environmental context, rather than on species-based diversity indices alone (Smith and Knapp 2003; Mouillot et al. 2011; Wang et al. 2022; Pichon et al. 2024).

At the β-scale, grass removal increased plant β-diversity under ambient conditions, whereas β-multifunctionality showed little response to plant functional group removal (Figures 2b and 3b). Thus, increased plant compositional turnover did not translate into a detectable increase in multifunctional turnover among plots for the measured ecosystem functions. This pattern

differs from multiscale studies in which β-diversity promoted γ-multifunctionality when compositional turnover was linked to functional differentiation among local communities (van der Plas et al. 2016; Chao et al. 2024; Li et al. 2024, 2025). In our experiment, this decoupling may have occurred because grass removal increased turnover mainly among subordinate plant species, whereas the measured ecosystem functions were more strongly influenced by the local loss of dominant grasses. In addition, replacing species may have been functionally similar for the measured functions, so greater compositional turnover did not generate comparable multifunctional turnover among plots (Rosenfeld 2002; Omidipour et al. 2021; van der Plas et al. 2023).

It is important to note that the diversity gradient created by our removal treatments was modest relative to studies that manipulate species richness across a broad range. Thus, our findings do not challenge the well-established positive relationship between biodiversity and ecosystem functioning, but rather highlight that in systems where dominant grasses contribute disproportionately to biomass, their loss can override the contributions of subordinate diversity to the measured functions (Grime 1998).

4.3 | N Addition Altered Plant Diversity Responses but Did Not Amplify Measured Multifunctionality Responses

N addition did not simply reduce plant diversity; instead, its effects depended on community context. In control plots, N addition increased both plant α- and β-diversity, suggesting that moderate nutrient enrichment may have relaxed nutrient limitation and allowed more species to occur locally and across plots in this subalpine meadow (Li et al. 2022). Similar positive diversity responses to nutrient addition have been reported in nutrient-limited high-latitude or high-elevation systems, particularly where environmental constraints or grazing can delay competitive exclusion (Madan et al. 2007; Boutin et al. 2017). This positive response was not maintained after functional group removal. Under N addition, the grass-removal effect on plant α-diversity disappeared, whereas forb removal caused stronger losses of both α- and β-diversity (Figure 3). These patterns suggest that N enrichment changed the outcome of species release and species loss after functional group removal. One plausible mechanism is intensified competition for light and space under higher productivity, which may change the composition of subordinate species after dominant grasses are removed and strengthen diversity losses after forb removal (Clark and Tilman 2008; Hautier et al. 2009; Eskelinen et al. 2022).

These N-induced diversity changes did not translate into amplified responses of measured multifunctionality. N addition showed no supported interaction with functional group removal for α-, β- or γ-multifunctionality, and the SEM indicated that, under N addition, grass removal still had a direct negative effect on α-multifunctionality whereas plant α-diversity was not detectably associated with α-multifunctionality (Figure 4). Thus, measured multifunctionality remained more closely linked to dominant grass-associated and biomass-linked functions than to N-induced changes in plant diversity. This context dependency is consistent with studies showing that biodiversity–multifunctionality relationships vary with species identity, functional composition, nutrient availability and spatial scale (Allan et al. 2015; Hautier et al. 2018; van der Plas et al. 2023).

4.4 | Caveats

Two caveats should be considered when interpreting these findings. First, our γ-scale communities were constructed by resampling four α-scale plots with replacement within each treatment. This design allowed us to apply a spatial partitioning framework for multifunctionality (van der Plas et al. 2016; Chao et al. 2024), but the resulting γ-scale assemblages were derived from non-independent plot combinations and should be interpreted as experimentally defined aggregates rather than as naturally replicated metacommunities. Future larger-scale experiments should include broader spatial extents, more independent γ-scale communities, and greater sampling intensity to test whether similar scale-dependent patterns emerge under natural landscape heterogeneity (Gonzalez et al. 2020; Liang et al. 2021). Second, our study was conducted at a single subalpine meadow site over 8 years, and the measured multifunctionality index included several biomass-related functions. Comparative studies across sites, longer time scales, contrasting ecosystem types, and additional ecosystem functions are therefore needed to determine whether broader biodiversity effects on multifunctionality emerge under other environmental contexts or over longer periods.

5 | Conclusion

Our results suggest that maintaining the dominant grass functional group may be important for sustaining the productivity- and nutrient-related functions measured in this study. However, we caution against interpreting this as a call to prioritize dominant species over biodiversity conservation. Subordinate species, although contributing less to the measured functions in the short term, may help maintain functions and trophic interactions not captured by our measurements, particularly under future environmental change. A comprehensive management strategy should therefore aim to protect both dominant functional group and overall biodiversity, recognizing that their relative importance may vary across functions, spatial scales, and environmental contexts.

Author Contributions

Xi Zhou: conceptualization, methodology, investigation, visualization, writing – original draft, writing – review and editing, formal analysis, data curation. **Wang Yu:** writing – original draft, formal analysis. **Yann Hautier:** methodology, writing – review and editing, formal analysis. **Akira S. Mori:** formal analysis, writing – review and editing, methodology. **Paul Kardol:** writing – review and editing. **Xinyu Xu:** writing – review and editing. **Yi Xu:** investigation. **Yuehua Deng:** investigation. **Pengfei Zhang:** writing – review and editing. **Jiahao Liang:** formal analysis. **Wenjin Li:** funding acquisition, writing – original draft, writing – review and editing, methodology, conceptualization, supervision, data curation.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available in the Figshare at https://doi.org/10.6084/m9.figshare.33060863.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Section S1**. Ecosystem functions and their ecological relevance. **Section S2**. Calculation details for biodiversity and ecosystem multifunctionality. **Figure S1:** Pictures of each treatment in August 2020 at the end of the experiment. **Figure S2:** The effects of plant functional group removal on (a) aboveground living biomass (g/m^2), litter mass (g/m^2), root biomass (kg/m^2), soil total phosphorus (mg/g); dissolved organic carbon (mg/kg), soil organic carbon (g/kg), soil C: N, substrate induced respiration (mL/kg/h), microbial biomass carbon (mg/kg), carbon mineralization rate (mg/g/day), net nitrogen mineralization rate (mg/kg/day), soil NH4+ (mg/kg), soil moisture (%). Values are means ± SE. Points represent treatment means and error bars represent SE. Information about two-way ANOVA is provided in Table S2. (b) Pairwise Pearson correlations among normalized ecosystem functions used for multifunctionality calculations. Only the lower triangle is shown. Positive correlations are shown in red and negative correlations in blue. **Figure S3:** Microbial α- and β-diversity across diversity orders. The left three columns show α-diversity based on the 36 empirical plot-level observations, and the right three columns show β-diversity based on 5000 bootstrap resamples. Points represent treatment means; error bars represent SE for α-diversity and percentile 95% confidence intervals for β-diversity. **Figure S4:** Our a priori structural equation model (SEM) depicting potential direct and indirect path effects of grass removal and forb removal on α-multifunctionality. **Figure S5:** Treatment effects on α-, β-, and γ-multifunctionality at $q = 1$. Panel a shows α-multifunctionality calculated from the 36 empirical plot-level observations and analysed using two-way ANOVA.

Panels b and c show derived β- and γ-scale estimates at the four-plot γ-grain; uncertainty was propagated using 5000 bootstrap resamples within each treatment. Points represent treatment means, with SE for α multifunctionality and percentile 95% confidence intervals for β- and γ multifunctionality. Insets show planned raw-difference contrasts, using bias-corrected 95% confidence intervals for β-multifunctionality and percentile 95% confidence intervals for γ-multifunctionality. Filled points indicate intervals excluding zero. **Figure S6:** No-biomass sensitivity analysis of multifunctionality at $q = 2$. Treatment responses of α-, β-, and γ-multifunctionality after excluding biomass-related ecosystem functions. The keys are the same as Figure S5. **Figure S7:** Treatment responses of plant and soil α- and β-diversity at $q = 0$ and $q = 1$. The keys are the same as Figure S5. Information about two-way ANOVA is provided in Table S3 and planned bootstrap contrasts for β-diversity are provided in Table S4. **Table S1:** The removal species lists. **Table S2:** The results of two-way ANOVA for the effects of removal (Removal), N addition (N), and their interaction (Removal × *N*) on the individual ecosystem function. **Table S3:** Two-way ANOVA and Tukey post hoc comparisons for α multifunctionality and α-diversity. Analyses were based on empirical plot-level observations. Significant *p* values are shown in bold. **Table S4:** Planned bootstrap contrasts for plant and soil β-diversity. β-diversity represents derived β-scale estimates at the four-plot γ-grain, with uncertainty propagated from 5000 bootstrap resamples within each treatment. Panel A shows planned raw-difference contrasts for plant and soil β-diversity at $q = 0$ and 1. Panel B and C show simple contrasts within each N or removal background for plant β-diversity at $q = 2$, because the Forb removal × *N* interaction was supported.