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Key Points:

- Experimental warming and drought alter functional diversity and soil processes in Pacific Northwest grasslands
- Increased asymbiotic N fixation may compensate for lower symbiotic fixation in stressed ecosystems
- Plant-soil responses to climate stress vary with species composition across a latitudinal gradient

Supporting Information:

Supporting Information may be found in the online version of this article.

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Evidence for the Coupling of Plant Functional Diversity and Soil Biogeochemistry Under Climatic Stress in Pacific Northwest Grasslands

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Abstract Increasing warming and drought severity are projected for the Pacific Northwest (PNW) and are expected to negatively impact species composition and ecosystem function. In this study, we test the hypothesis that the impact of climatic stress (i.e., experimental warming and drought) on PNW grasslands are mediated by interactions between plant functional diversity and soil biogeochemical processes, including symbiotic nitrogen (N) fixation in legumes and free-living asymbiotic nitrogen fixation (ANF) by soil microorganisms. To test this hypothesis, we measured the response of plants and soils to three years of warming (+2.5°C) and drought (−40% precipitation) in field experiments replicated at three different sites across a ~520-km latitudinal gradient. We observed interactive effects of warming and drought on functional diversity and soil biogeochemical properties, including both positive and negative changes in ANF. Although direct measurements of symbiotic nitrogen fixation (SNF) rates were not conducted, the observed variations in ANF, in conjunction with changes in legume cover, suggest a compensatory mechanism that may offset reductions in SNF. Generally, high ANF rates coincided with low legume cover, suggesting a connection between shifts in species composition and N cycling. Our ANF estimates were performed using isotopically labeled dinitrogen ($^{15}N_2$) in tandem with soil carbon (C), phosphorus (P) and iron (Fe), pH, and moisture content. Along the latitudinal drought severity gradient, ANF rates were correlated with changes in species composition and soil N, P, moisture, and pH levels. These results highlight the importance of soil-plant-atmosphere interactions in understanding the impacts of climatic stress on ecosystem composition and function.

Plain Language Summary In this study, we used field experiments replicated across a 520-km latitudinal gradient, involving several years of warming and drought manipulations. Our results show the interplay between warming, drought, plant functional diversity, and soil biogeochemical cycles. We observed a shift in species composition, with a decline in legume cover and associated symbiotic N fixation, counterbalanced by an increase in soil diazotroph activity and asymbiotic N fixation. These dynamics are presented within a broader analysis of soil chemical and physical properties which show the importance of biogeochemical processes in ecosystem management and conservation under climate change scenarios.

1. Introduction

Species diversity and ecosystem function are changing in response to a changing climate; however, there is still limited experimental data on how plant-soil interactions mediate ecosystem responses to increasing temperature and decreasing precipitation (Farinacci et al., 2024; Malik & Bouskill, 2022). Plant functional diversity and soil biogeochemical processes are often linked, as plant-soil interactions provide compensatory mechanisms that can attenuate climate-induced stress from individuals to ecosystems (Silva & Lambers, 2021). Such mechanisms include fast but long lasting soil microbiome responses to changes in plant growth, litter deposition, and root exudation (Gargallo-Garriga et al., 2018; Spencer et al., 2023) and slow climate-induced shifts in ecosystem productivity and heterotrophic respiration (Maxwell & Silva, 2020; Tang et al., 2020), which can impact nutrient cycling and functional diversity (Busch et al., 2018). In this study, we distinguish between functional changes in symbiotic nitrogen fixation (SNF), occurring in plant root nodules, and asymbiotic nitrogen fixation (ANF), which occurs freely in the soil. A tradeoff between SNF and ANF is expected and could be relevant for climate

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impact prediction (Bomfim et al., 2019), but it is unclear how warming and drought influence free-living soil diazotrophs relative to plant functional groups that perform symbiotic N_2 fixation, such as rhizobia in legumes.

In many parts of the world, unmanaged ecosystems obtain most of their N via biological N_2 fixation, either through plant-associated or free-living diazotrophs (N_2 fixers) like bacteria or archaea inhabiting the soil. Unlike SNF, ANF is more evenly distributed in space (Sullivan et al., 2014) and it can account for nearly half of the N_2 fixed via SNF rates in temperate grasslands (Reed et al., 2011). Free-living N_2 fixers can also increase soil organic C and the availability of mineral elements under drought, decoupling the effects of moisture and nutrient limitation on plant growth (Pérez Castro et al., 2020). As a result, it is difficult to predict how the supply of N and related biogeochemical processes will affect ecosystems response to a changing climate.

To address this limitation, we devised experiments in PNW Mediterranean grasslands, measuring the impact of multiannual warming and drought on plant community composition and soil biogeochemical processes, focusing on N cycling and its major biotic and abiotic controls. Our goal was to test if climate-induced changes in grasslands species composition and microbial processes are modulated by a tradeoff between ANF and legume cover, the latter of which serves as a proxy for SNF. We asked three questions about how climate affects communities and biogeochemical processes: (a) how does warming and drought affect plant functional groups and soils properties in grassland communities? (b) Are climatic effects mediated by plant functional diversity and soil biogeochemical features? (c) How does plant and soil responses vary across sites and seasons? We hypothesized that warming and drought would lead to a decline in plant functional diversity and SNF, with a corresponding compensatory increase in soil ANF. We also hypothesized that warming increases soil ANF due to an increase in diazotroph activity in warmer environments (i.e., across a latitudinal gradient and in response to experimental warming at each site), with a secondary effect of seasonality on ANF expected at the onset of the relatively colder rainy season. To test these predictions, we quantified changes in ANF rates and N, P, Fe, pH, moisture, and temperature in response to warming and drought at three grassland sites along a 520-km latitudinal gradient established in 2016 (Reed et al., 2019).

2. Materials and Methods

2.1. Site Description and Experimental Design

We conducted this study in three experimental grassland sites: Northern (central-western Washington; characterized as cool and wet), Central (central-western Oregon; characterized as warm and wet), and Southern (southwestern Oregon; characterized as warm and dry) (Figure 1). At each site, we surveyed the vegetation and collected composite soil samples from five co-located plots under control (ambient), warming (+2.5°C by six 2000-W infrared heaters), and drought (−40% precipitation by rain-out shelters) conditions during the fall and spring seasons. The climate data (Table 1) show distinct baselines across experimental sites: the Northern site has average temperatures ranging from 4.3 to 12.8°C with substantial precipitation differences between seasons (6.45 mm in the fall vs. 0.82 mm in the spring). The Central site maintains milder temperatures (11.6–13.0°C) with fairly consistent precipitation (1.4 mm in the fall to 1.9 mm in the spring). The Southern site, warmer, averages from 10.3 to 10.6°C with a sharp increase in precipitation in the spring (0.73–6.2 mm). This natural variation of climatic conditions provides a range of possibilities to test how environmental stressors such as warming and drought impact grassland ecosystems in the PNW.

2.1.1. Vegetation Manipulation

In control and drought plots, we quantified the moderator effect of plant composition by comparing pasture (unmanipulated plant composition; $n = 10$) and restored prairie (manipulated composition; $n = 15$) grassland plots. The restored prairie plots were mowed, raked, sprayed with herbicide, and seeded with a mix of 29 native grasses and forbs. Initial manipulation happened between 2014 and 2015; afterward, we annually seeded manipulated plots with 14 native species of grasses and forbs between 2015 and 2017 (Reed, Bridgham, et al., 2021; Reed, Peterson. et al., 2021; Reed, Pfeifer-Meister, et al., 2021). The pasture plots were unmanipulated and were dominated by a few species of perennial pasture grasses (*Schedonorous arundinaceus, Agrostis capillaris, and Alopecurs pratensis*). Over the years, non-native species have self-seeded into these plots, but restored prairie plots maintained higher plant diversity than the low diversity pasture plots. For additional details on the experiment and manipulations see Reed et al. (2019) and Dawson et al. (2022). We used a point intercept

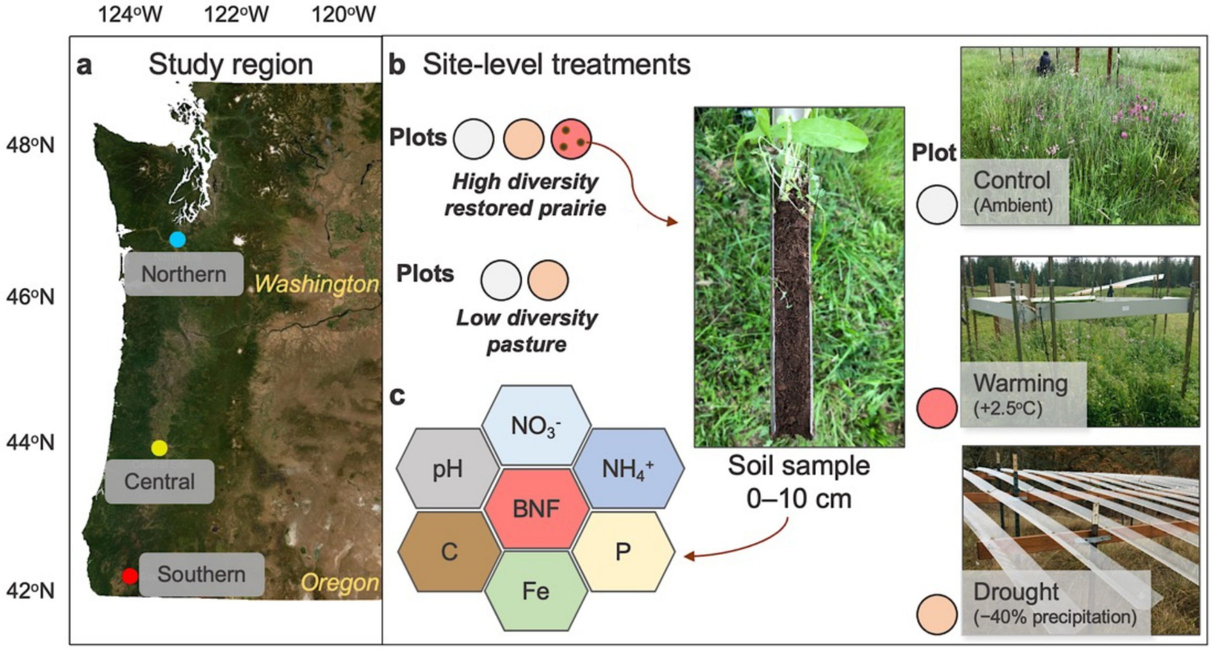

Figure 1. Study sites along the U.S. Pacific Northwest, (a) from southwestern Oregon (Southern) to central-western Oregon (Central) to central-western Washington (Northern). At each site (b), soil samples were collected for the inference of biological N fixation (BNF) during the fall and spring seasons from pasture and restored prairie plots representing three climate treatments: control, drought, and warming (with control and drought treatments represented across all plots and warming treatments applied to high diversity restored prairie plots only). The soils (c) were incubated for ANF measurements and analyzed for soil biogeochemical variables controlling ANF.

Table 1
Soil and Environmental Variables in Each Study Site Along the PNW Latitudinal Gradient

Variable	Site Descriptive statistics	Northern Fall 2018	Northern Spring 2019	Central Fall 2018	Central Spring 2019	Southern Fall 2018	Southern Spring 2019
Total daily precipitation (mm)	Mean[a]	6.45	0.82	1.4	1.9	0.73	6.2
	Minimum[b]	0	0	0	0	0	0
	Maximum[c]	36.6	4.6	14.0	14.5	8.4	66.3
Air temperature (°C)	Mean	4.3	12.8	11.6	13.0	10.3	10.6
	Minimum	−3.9	8.5	7.4	12.6	9.7	10.1
	Maximum	8.4	16.9	19.9	13.5	11.0	11.2
Total C (g/kg)	Mean	78.5	72.5	86.7	95.9	44.6	38.7
Total N (g/kg)	Mean	6.4	5.8	6.5	7.2	4.1	3.5
pH (H_2O)	Mean	5.8	5.8	5.7	5.8	6.4	6.3
Soil temperature (°C)	Mean	5.9	12.9	7.9	13.8	7.1	16.9
	Minimum	1.0	7.5	1.1	7.8	3.7	8.9
	Maximum	8.5	17.4	16.9	22.7	10.7	32.5
Soil volumetric water content	Mean	0.31	0.27	0.22	0.28	0.24	0.27
	Minimum	0.22	0.21	0.17	0.22	0.18	0.16
	Maximum	0.46	0.37	0.35	0.36	0.35	0.40

[a] Mean denotes the average among monthly mean soil temperatures measured in control plots at each studied site during the sampling month. [b] Minimum denotes the monthly minimum soil temperature, as described in "a". [c] Maximum denotes the monthly maximum soil temperature, as described in "a". The soil temperature values derive from sensors installed in each studied plot. The precipitation data derived from each site's meteorological station.

technique to survey plot-level plant community composition and calculate diversity and percent cover of legumes (members of Fabaceae).

2.1.2. Climate Manipulation

To heat the canopy by ~2.5°C, we installed six 2000-W infrared heaters (Kalglo Electronics, Bethlehem, Pennsylvania, USA) in each warming plot, which we turned on by 2016. Previously, we had heated the Southern and Central plots from 2010 to 2012 (Pfeifer-Meister et al., 2016; Reynolds et al., 2017; Wilson et al., 2016); the Northern plots were not heated until this experiment. Heaters were controlled by a dimmer switch using the control plot average to determine intensity. We turned off the heaters at all sites in August and September 2017 due to high fire risk. Control plots were ambient temperature plots with false wooden "heaters" installed to mitigate possible shading effects. To create the effect of drought, we installed rain-out shelters that covered 40% of the drought plots with clear acrylic shingles (MultiCraft Plastics, Eugene, OR) beginning February 2016 for the restored prairie plots and February 2017 for the pasture plots. The plastic sheeting used no more than 8% of absorbed light transmittance (Yahdjian & Sala, 2002). The geographical coordinates of the plots sampled across the three sites where the experiment was conducted is shown in Table S1 in Supporting Information S1 and the effects of climate treatment relative to natural variation in control plots, including mean soil volumetric water content and temperature are shown in Figure S1 in Supporting Information S1.

2.2. Field Sampling Design

2.2.1. Vegetation Survey and Plant Functional Data

We measured plant cover at peak standing biomass (mid-May, late-May, and mid-June at the southern, central, and northern sites, respectively) for three years (2017–2019). We used a point intercept technique to survey plot-level plant community composition. Using a 1 m^2 quadrat in a fixed location within each plot, we dropped 25 equally spaced pins through the plant canopy to the soil surface. We counted each plant hit on a pin to the species level and multiplied each hit by 4 to standardize to 100. The resulting cover is >100% absolute cover due to the multilayering nature of the canopy. We assigned a cover of 0.4% to species which were present in the quadrat but not hit by a pin. To determine biological N-fixing plant cover, we calculated relative cover as the proportion of absolute cover belonging to legumes, that is, members of the plant family Fabaceae as in Blesh (2018). Although not all species were present at all sites or all plots, all species included in this study have a cool-season C_3 metabolism, and all major functional groups (annual and perennial forbs, grasses, and legumes) are distributed across all experimental sites (Dawson et al., 2022).

2.2.2. Soil Sampling

Each site was sampled in the fall (November–December 2018) and spring (April–May 2019). The fall sampling included 9 high-diversity and 6 low-diversity plots for each site. We used a sterile soil corer to collect three soil samples (0–10 cm depth) in each plot and combined these into one composite sample. Each composite soil sample was placed into a sterile bag and stored at 4°C during sampling. An additional soil sample was taken from the same plots to determine bulk density (BD). After the sampling was completed in each site, samples were immediately transported to the Soil Plant Atmosphere Research Laboratory at the University of Oregon for the following measurements (summarized in Table 1).

2.3. Laboratory Measurements

2.3.1. Soil Physical Properties

We determined soil water holding capacity (WHC), gravimetric water content, and BD immediately after sampling. We calculated gravimetric water content (weight of water divided by soil dry weight) by oven-drying ~5 g of soil at 100°C for 48 hr (Table S2 in Supporting Information S1). We measured BD, (g cm^{-3}) as the total dry weight (oven-dried at 105°C for 48 hr) of the sample divided by the volume of the core sampler (68.71 cm^3) We determined WHC by placing oven-dried soils in a funnel lined with filter paper over a glass beaker. We added deionized water to the beaker until the soil surface glistened and left the soil to drain through the funnel. Once draining was finished, we placed the soil in an oven at 100°C for 48 hr to determine soil moisture content at 100% WHC. We stored all soils at −20°C while we measured these variables.

2.3.2. Soil Chemical Analyses

After oven-drying for 48 hr, we sieved soil samples to 2 mm, ground them using a ceramic mortar, and chemically characterized the soils to determine the most important factors known to influence ANF (Reed et al., 2011). We measured total soil C and N (PDZ Europa ANCA-GSL elemental analyzer), available P and available Fe by Mehlich-3 (M3) extraction (Mehlich, 1984) followed by colorimetric determination (Dominik & Kaupenjohann, 2000; Murphy & Riley, 1962); inorganic nitrogen (Ni, sum of NH_4^+, and NO_3^-) using 1M KCl extractions followed by colorimetric determination (Forster, 1995), soil pH in H_2O (2:1) and $CaCl_2$ (0.5 M) and bulk density.

2.3.3. Asymbiotic N Fixation Measurements

ANF rates were quantified following Bomfim et al. (2019). To provide optimal conditions for microbial activity without affecting diffusion of air into samples (Hicks et al., 2003) we brought all samples to 50% WHC in experimental vials before applying labeled gas. We incubated three replicates of each soil sample in 12-ml sealed gas vials (Exetainer, Labco, UK) containing 1 g of sample (dry weight) and 2 mL of ^{15}N-labeled dinitrogen gas (98 atom% $^{15}N_2$, Cambridge Isotope Laboratories) while we incubated three corresponding controls in 12 ml vials containing the same sample (∼1 g, dry weight) but not receiving any isotopically labeled gas. All vials were incubated at 25°C in the dark for 24 hr, a period we chose to give ample time for fixation without exhausting the oxygen in the vial. Afterward, we removed the samples from the vials and measured ANF rates according to Hsu and Buckley (2009). We oven-dried all samples at 40°C and ground them in separate ceramic mortars to avoid cross-contamination. N, ^{15}N, and C content was determined at the University of California Stable Isotope Facility using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK). The amount of N fixed (ng N g dry weight^{-1} hr^{-1}) was calculated in relation to background (control) levels (Table S3 in Supporting Information S1).

2.4. Statistical Analysis

We ran all analyses on R version 3.6.2 (R Core Team, 2019).

2.4.1. Plant Functional Diversity

We used Rao's Quadratic Entropy, or Rao's Q index, as a measure that captures plant diversity by integrating both the abundance of species and the functional trait differences between them. Unlike diversity indices that may only account for species counts or abundance, Rao's Q combines these aspects considering not only how many species are present but also how different they are from each other in terms of functional traits, aimed at understanding the interplay between species characteristics and their distribution (Rocchini et al., 2024). We calculated Rao's Q in R with the picante package (Kembel et al., 2010) using the community composition data and phylogenetic data derived from the megatree in V.PhyloMaker (Figure S1 in Supporting Information S1; Jin & Qian, 2019; Smith & Brown, 2018). Relative legume absolute percent cover calculated as legume cover divided by total cover within the quadrat. We then logit-transformed the data using R's logit function to normalize the data as a proportion.

2.4.2. Soil ANF and Biogeochemistry

To quantify the effect of each treatment on soil ANF, we calculated the effect size using 95% confidence intervals for each plant diversity treatment and site across both fall and spring seasons. Effect sizes are presented in the text as percentages for clarity. For statistical analyses, effect sizes were calculated as the logarithm with base 10 (Log_{10}) to represent the response ratio of soil ANF relative to controls at each site and for each treatment as follows:

$$\mathrm{RR} = \mathrm{Log}_{10} \frac{\mathrm{ANF}_{\mathrm{treatment}}}{\mathrm{mean\ ANF}_{\mathrm{control}}} \tag{1}$$

We determined the effect of site, plant diversity, climate treatment, and seasonal effects on soil ANF effect sizes and biogeochemical variables using a two-way analysis of variance (Table S4 in Supporting Information S1). Mean effect size values were compared by least squares means adjusted for Tukey's HSD test at a 95% significance level.

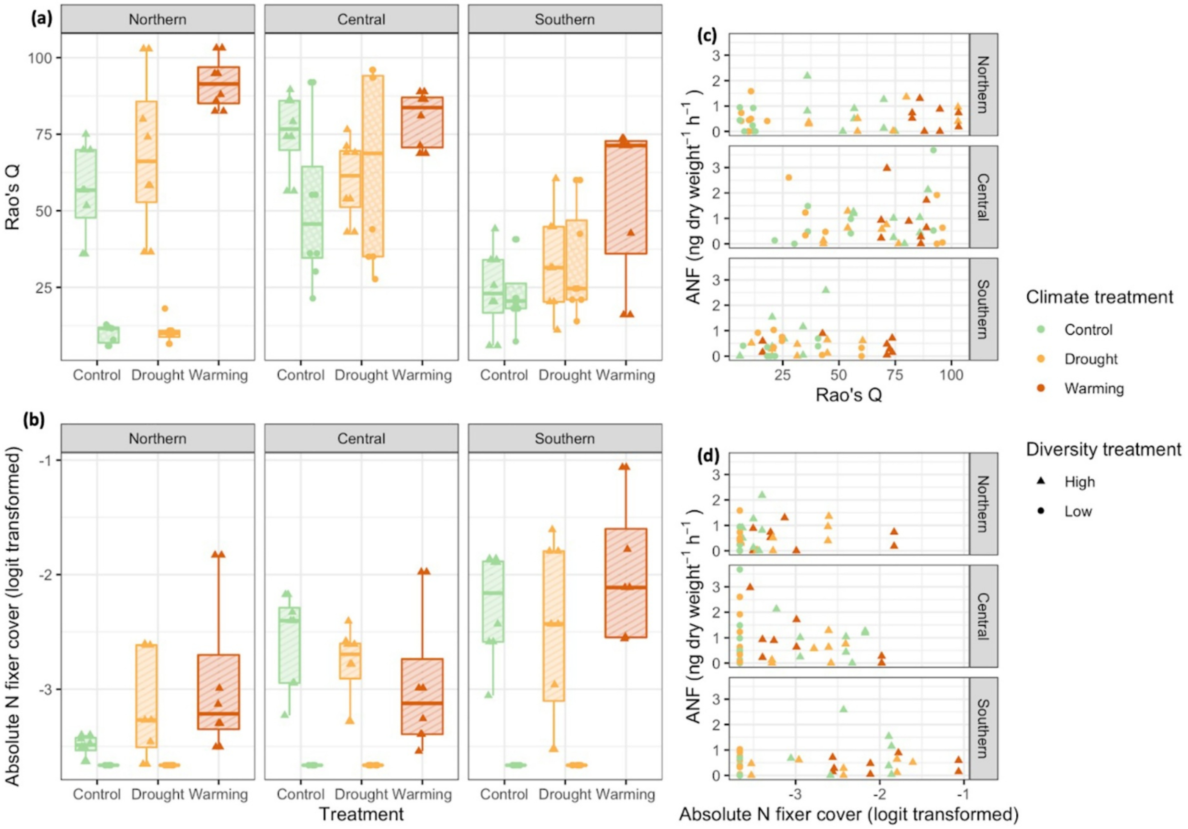

Figure 2. Treatment effect on Rao's Q (a), symbiotic N-fixing plant cover (b), and correlation with soil asymbiotic N fixation (c)–(d) (ANF; in ng N g dry weight^{-1} hr^{-1}), indicating a compensatory mechanism by which ANF rates become generally higher as N-fixing plants become less abundant and symbiotic N fixation declines. Table S4 in Supporting Information S1 shows significant effects of climate treatment, site, and diversity treatment, with climate treatment having a pronounced impact on Rao's Q ($p < 0.001$) but not on N-fixing plant cover ($p = 0.225$). Site and diversity treatment significantly influenced ($p < 0.001$) both variables, with significant interactions between diversity treatment and site ($p < 0.001$) also observed for both variables. Seasonal changes did not significantly affect either variable (Rao's Q $p = 0.82$; Plant Cover $p = 0.73$).

2.4.3. Principal Component Analysis

We conducted a principal component analysis (PCA) to ordinate the plots regarding the main drivers of variation in soil biogeochemistry across sites and climate and diversity treatments. Preliminary PCAs were run using a standardized data matrix including soil ANF, ammonium (NH_4^+), nitrate and nitrite (Total Ni = $NO_3^- + NO_2^-$), total C/N, available P, available Fe, pH, moisture concentration and WHC to select the critical variables for the final PCA.

3. Results

3.1. Plant Functional Diversity

Figure 2 illustrates the effects of climate treatments (Control, Drought, and Warming) on plant functional diversity (Rao's Q; panel a), N-fixing plant cover (panel b), and their relationships with ANF (panels c–d). Climate treatment had a significant effect on plant diversity ($p < 0.001$; Table S4 in Supporting Information S1), with warming generally increasing Rao's Q in the northern and central sites. However, climate treatment did not have a significant effect on N-fixing plant cover ($p = 0.225$), while both site and diversity treatment significantly influenced both variables ($p < 0.001$). It is worth noting that significant interactions between site and diversity treatment ($p < 0.001$) suggest that the response of N-fixing plant cover and functional diversity varied across regions. Seasonal effects were not significant for either Rao's Q ($p = 0.822$) or N-fixing plant cover ($p = 0.731$).

All sites were significantly different in terms of N-fixing plant cover (Figure 2b; Table S4 in Supporting Information S1). Low diversity plots did not have any N-fixing plant cover (Figure 2b). In high diversity plots, the

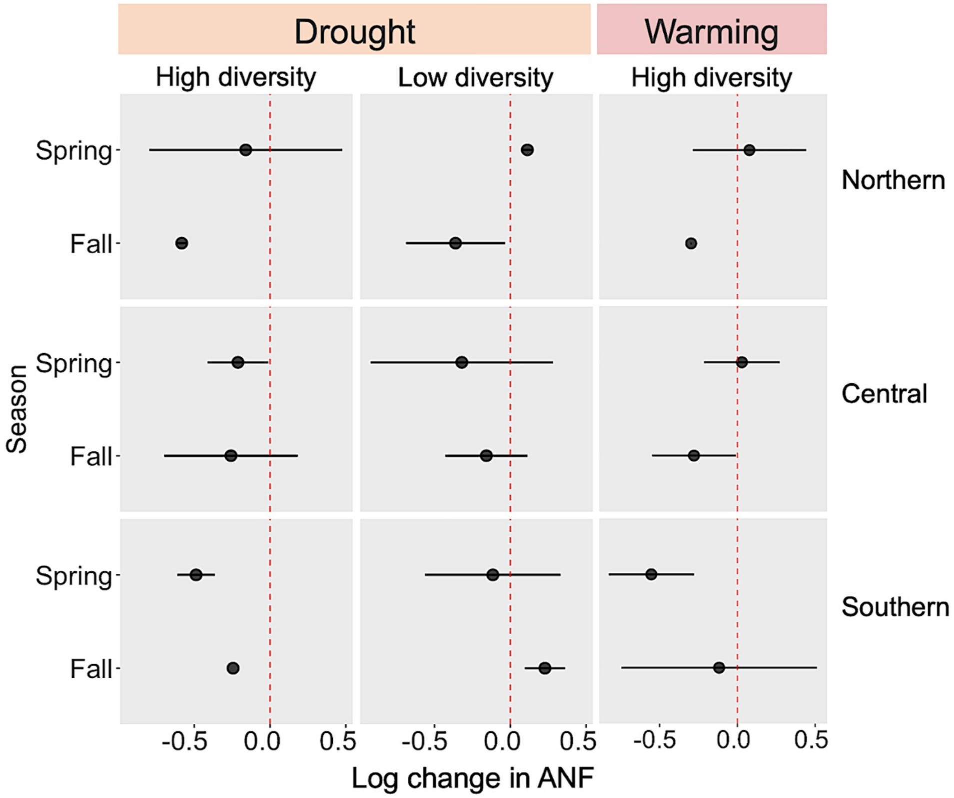

Figure 3. Drought and warming effects on asymbiotic N fixation (ANF) in pasture and restored prairie plots established in grasslands across three study sites located along a 520 km latitudinal gradient in the Pacific Northwest. ANF change is measured as mean $\log_{10}$ (ANF treatment/control) with 95% confidence interval. The vertical dashed lines indicate the control conditions. Points are means and horizontal lines indicate the 95% confidence interval.

northern site had the lowest N-fixing plant cover (1.29% ± 2.76) and the southern site had the highest (5.28% ± 6.53). Climate treatment and season of sampling did not change N-fixing plant cover, which increased significantly from 3.1% in the northern to 9.2% in the southernmost site. Restored prairie plots had twice as much plant diversity as pasture plots and there was a significant interaction between site and plot diversity (Table S4 in Supporting Information S1). The northern and central high diversity plots were the most diverse (Rao's $Q = 72.3 \pm 22.9$ and 71.7 ± 14.3; mean ± SD, respectively), whereas northern low diversity plots were the least diverse (Rao's $Q = 10.2 \pm 3.20$). Figures 2c and 2d suggest a compensatory mechanism between symbiotic and asymbiotic N fixation, with ANF rates increasing as N-fixing plant cover declines. Across all sites, higher ANF rates were observed at lower levels of Rao's Q and N-fixer cover, particularly under drought and warming treatments. This supports the hypothesis that ANF compensates for declines in SNF, potentially maintaining N inputs under climate stress. These results highlight the interactive effects of climate stress, site conditions, and plant diversity on N cycling dynamics in PNW grasslands.

3.2. Climate Effects on Soil and ANF

We detected ANF activity in 78% (94 out of 120) of the plots sampled across, sites, climate, and plant diversity treatments during the fall and spring seasons (Table S5 in Supporting Information S1). Average ANF rates varied substantially across measurements, ranging between 0.41 ng N g dry weight^{-1} hr^{-1} in the warming treatment of the southern site and 5.54 ng N g dry weight^{-1} hr^{-1} in the control plots of the central site. ANF effect sizes ranged from −42.3 ± 36.8% (mean ± SD) in the warming treatment of the southern site in the Spring and 25.9 ± 10.5% in the drought treatment of the southern site in the Fall (Figure 3).

Soil ANF response to drought increased along the latitudinal gradient in the Fall season (Figure 3). ANF rates were highest (~2-fold; Table S3 in Supporting Information S1) at the warmer and drier southernmost site, but the strongest response to climate stress was observed as a decrease in ANF at the cooler and wetter northernmost site.

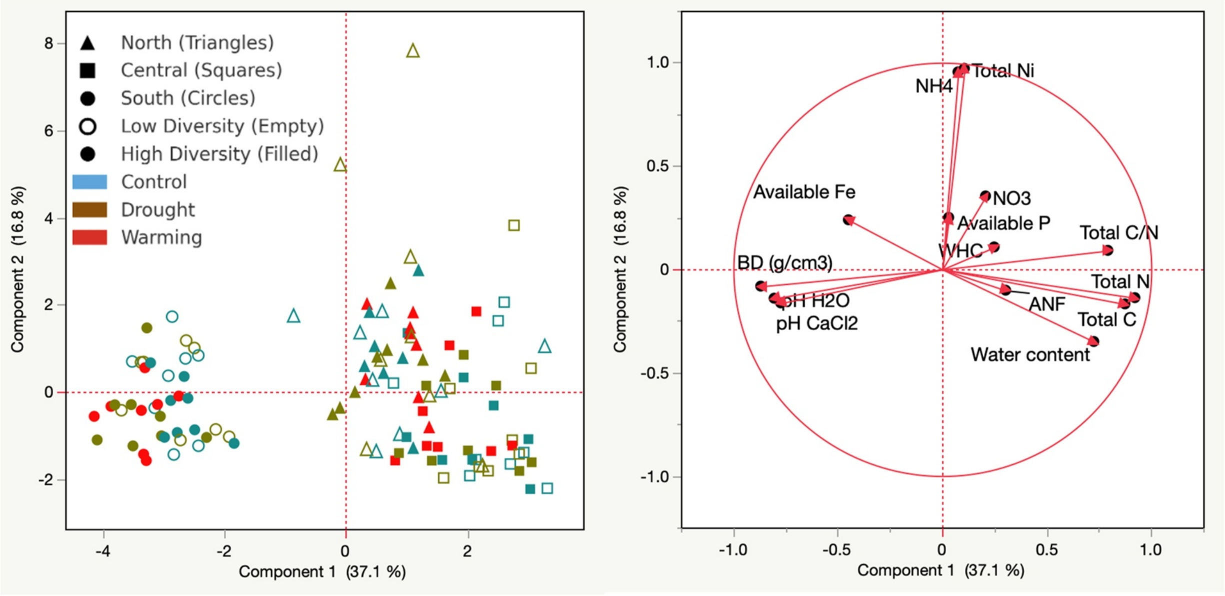

Figure 4. Principal component analysis illustrating the multivariate relationships between soil biogeochemistry variables including soil ANF (ng N g dry weight^{-1} hr^{-1}), NH_4^+ (μg g dry weight^{-1}), inorganic nitrogen (Total Ni = $NO_3^- + NO_2^-$ μg g dry weight^{-1}), total C/N (mass-based), available P (μg g dry weight^{-1}), available Fe (μg g dry weight^{-1}), pH and soil moisture expressed as water content (%) and water holding capacity. Each point represents the plot averages, in a total of 75 plots representing all sites and seasons. PC1 (x-axis) explained 40.4%, and PC2 (y-axis) explained 20% of the total variance in the soil biogeochemistry data. Arrows indicate the influence of each variable on the principal components, with the arrow associated with ANF highlighting correlations with other environmental factors in the data set (Table S6 in Supporting Information S1; Bomfim, 2025).

Among drought plots, we found a significant effect of plant diversity restoration treatment on log change in ANF (Kruskal-Wallis chi-square = 3.81, p-value = 0.048). ANF response to drought also varied depending on plant diversity, where low diversity pasture plots had more predictable response to drought than high diversity restored prairie plots. For instance, ANF in southern restored prairie plots was suppressed but no change was verified in central restored prairie plots. Among warming plots, there was no effect of site or season on log change in ANF. ANF response to warming varied by site, where rates increased with the drought severity gradient in the fall but decreased during the spring.

3.3. Soil Biogeochemical Responses and Effects on ANF

Multivariate ordination of the data (PCA; Figure 4) separates sites primarily based on soil fertility and physical structure, Component 1 (37.1%), with strong loadings from total N, C, C/N, and water content, all of which are positively associated with increased asymbiotic nitrogen fixation (ANF). Conversely, bulk density and pH (both H_2O and $CaCl_2$) are negatively associated with Component 1, suggesting that soils with better structure and higher pH support greater ANF activity. Component 2 (16.8%), reflects differences in plant diversity, separating plots based on inorganic N and available P and Fe, which are more strongly aligned with high-diversity sites. Although not statistically significant, N-fixing plant cover exhibits a general trend of exponential decline in ANF rates as legume cover increases (Figure S3 in Supporting Information S1, top panel), with the strength and direction of this relationship varying by treatment and season, as shown by differing log-log regression slopes (Figure S3 in Supporting Information S1, bottom panel). Across all sites, the correlation matrix (Table S6 in Supporting Information S1) further supports these patterns: ANF is positively correlated with water content (0.31), total N (0.29) and total C (0.25), and negatively correlated with bulk density (−0.20) and WHC (−0.18), indicating that N-fixing microbial communities respond to variation in both soil fertility and structure, with potential buffering by plant community composition and associated N cycling dynamics.

4. Discussion

We asked three questions about how climate affects plant communities and biogeochemical processes: (a) How does warming and drought affect plant functional groups and soils properties in grassland communities? (b) Are climatic effects mediated by plant functional diversity and soil biogeochemical features? (c) How does plant and soil responses vary across sites and seasons? We expected that warming and drought would lead to a decline in plant functional diversity, thereby increasing soil ANF inputs through a compensatory mechanism related to soil stoichiometry and N availability, with an expected increase in soil ANF due to an increase in diazotroph activity in warmer environments and warming treatments. Our findings provide evidence for a coupling between plant functional diversity and soil biogeochemistry, offering partial support for the compensatory hypothesis. Warming and drought treatments altered species composition and reduced legume abundance, which corresponded with shifts in N cycling and a detectable increase in soil ANF. However, our results also indicate that the positive effects of this compensatory mechanism were ultimately constrained by the negative impacts of drought on plant communities and soil moisture availability.

As expected, we found that the response of plant communities to experimentally imposed multiannual warming and drought included changes in species diversity and a corresponding change in biogeochemical processes including an increase in soil ANF. Our data suggest that ANF can buffer the negative effects of climatic stress on NPP and partially offset a decrease in legume cover (and presumably SNF inputs). However, warming plots had greater plant functional diversity on average while drought treatment did not affect plant functional diversity compared to control plots (Figure 2a). A previous study at the same sites showed that warming increased the cover of introduced annual species, causing subsequent declines in other functional groups and diversity (Reed, Bridgham, et al., 2021; Reed, Peterson. et al., 2021; Reed, Pfeifer-Meister, et al., 2021). The authors reported that competition for moisture and light or space, rather than N, were critical mechanisms of community change in these Mediterranean grasslands. Their findings suggest that future climate change will alter plant community composition and decrease diversity in PNW grasslands, driven by significant differences in intrinsic water- and nutrient-use efficiency of annual and perennial species (Dawson et al., 2022, 2024).

Climate-induced declines in grasslands productivity reflect variation in soil water and nutrients, a positive effect, and plant functional diversity, a negative effect (Reed et al., 2023). Our data provide evidence of a compensatory mechanism that could attenuate competition through shifts in SNF to ANF acquisition, which coincided with changes in certain plant functional groups. Specifically, warming and drought treatments were expected to drive a decline in plant functional diversity and legume cover, thereby altering the balance of N inputs to the soil. In ecosystems where SNF from legumes declines, compensatory increases in ANF could offset N losses, driven by shifts in soil stoichiometry and increased demand for biologically available N (Bomfim et al., 2020; Dawson et al., 2024). Indeed, diazotrophs can respond to declining SNF inputs by increasing N fixation activity, particularly in warmer environments where microbial metabolism and enzymatic processes are accelerated. Similar patterns have been observed also in colder environments, where microbial N fixation becomes more prominent in response to plant community shifts that reduce SNF inputs.

In our study, warmer sites tended to have higher ANF rates than cooler sites. This is expected both because of increased diazotroph activity under warmer conditions and because drought can interact with warming treatments exacerbating changes in plant and microbial communities, which can lead to changes in litter and microbial necromass stoichiometry (Malik & Bouskill, 2022), increasing N limitation and the relative importance of ANF as a compensatory mechanism for N fixation. More intense and prolonged droughts are expected to promote N cycling in some ecosystems via sustained N processing during periods of low plant uptake (Evans & Burke, 2013). However, severe droughts and warming in PNW grasslands can also favor exotic plants leading to larger changes in soil N pools by, for example, outcompeting N-fixing species and sequestering N in those already N-limited systems (Pérez Castro et al., 2020). In this context, herbaceous plants capable of fixing N can modulate the impacts of climate change on soil SNF and ANF.

The PCA biplot (Figure 4) further suggests a coupling between plant functional diversity, soil properties, and ANF. Component 1 reflects gradients in soil fertility and structure across the latitudinal gradient, with higher ANF rates associated with higher soil moisture, total C and N, and lower bulk density. Component 2 captures plant diversity effects, with high-diversity restored plots aligning with elevated inorganic N, P, and Fe availability, which were generally higher in control plots compared to drought and warming treatments. Total soil C and N contents were higher in restored prairie plots, with no significant effects of warming treatment during the period of observation. Soil P availability was also higher in control than in drought or warming plots, suggesting that reduced moisture availability may have constrained P accessibility. Although not statistically significant, our data suggests a general trend of decreasing ANF with increasing legume cover (Figure S3 in Supporting Information S1), implying a suppressive effect of symbiotic N fixers on free-living diazotrophs under climatic stress.

The observed clustering of high-diversity sites with increased ANF suggests that plant diversity may play a buffering role in maintaining microbial function under climatic stress. Greater functional diversity can contribute to improved soil conditions, including enhanced organic matter inputs and microclimate stabilization, which may facilitate the persistence of N-fixing microbial communities even under drought and warming pressures. As drought intensifies, the overall decline in plant productivity and shifts in soil structure limits this buffering effect, leading to the observed constraints on ANF. These findings highlight the interplay between plant community shifts and microbial N fixation, emphasizing that while ANF may serve as a compensatory mechanism for N cycling disruptions, its effectiveness is ultimately constrained by broader ecosystem stressors.

To date, there has been little attention given to N-fixing grassland species in the context of climate change. The most well-known N-fixing plant family is Fabaceae (legumes); however, researchers have also observed associations between Poaceae (grasses) and N_2-fixing bacteria, particularly in commercial crops such as sugar cane, wheat, and rice (Pankievicz et al., 2015). Even less is known about wild species and the commonality of such associations. Legumes can reduce respiration and microbial C:N in grasslands (compared to other forbs and grasses) and may lead to P limitation (Strecker et al., 2015). Because N_2 fixation is an enzymatic process, we expected to see a rate increase that corresponds with soil temperature until the enzyme denatures (Reed et al., 2011).

As we hypothesized, warming plots in the northern site had a lower soil ANF response rate during the cold rainy season (Figure 4); however, during the Fall, there was no difference at the other two, warmer, sites. The dependence of reaction rates on temperature, including the rates of enzymatic reactions, is described by the Arrhenius equation (Davidson & Janssens, 2006). According to this equation, the fractional increase in reaction rate is less for a temperature increase of one degree Celsius at higher temperatures than at lower temperatures. This suggests that the response of soil microbial respiration to temperature changes should be less pronounced in tropical climates than it is in temperate or boreal climates.

Much remains to be revealed about ANF responses to litter input and rhizosphere conditions and further research integrating plant functional traits and N flux modeling could provide deeper insights into the resilience potential of microbial and plant communities in the face of climatic changes. Controls on ANF in future studies may include C, N, and P availability as well as pH, temperature, and soil moisture and temperature (Smercina et al., 2019). Lower sampling soil temperature was correlated with reduced N fixation in cyanobacteria when incubated under constant temperature (Liengen & Olsen, 1997). Similarly, increased long-term incubation temperature significantly increased microbial respiration (Zogg et al., 1997). One might expect to see N-rich compatible solutes (e.g., proline and ectoine) dominate the drought response in N rich soils versus C-rich compatible solutes (e.g., trehalose) in N-poor soils. Those will likely have different metabolic costs for different species and functional groups, so that might be one explanation for the interaction between nutrient status and drought adaptation of plants and soil microbes.

5. Conclusions

Our findings reveal that the effects of warming and drought on plant communities are correlated with shifts in soil ANF and its biogeochemical controls, with these relationships varying considerably across the latitudinal gradient of Mediterranean PNW grasslands. Our data suggest that shifts in plant functional diversity coincide with changes in soil processes, particularly ANF and its associations with chemical properties and moisture availability. This compensatory dynamics suggest that while microbial processes can buffer some N cycle disruptions, their effect is ultimately constrained by broader environmental stressors, particularly moisture limitation. These results highlight the importance of incorporating plant-soil-microbe feedback into models of ecosystem function under climate change. Future research integrating plant functional traits, microbial community dynamics, and nutrient cycling is needed to improve predictions of ecosystem resilience in a rapidly changing climate.

Conflict of Interest

The authors declare no conflicts of interest relevant to this study.

Data Availability Statement

The data and code used to produce the figures and results presented are available in Bomfim (2025), with additional analyses published as supplementary files.

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