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Plant removal across an elevational gradient marginally reduces rates, substantially reduces variation in mineralization

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Abstract

The loss of aboveground plant diversity alters belowground ecosystem function; yet, the mechanisms underpinning this relationship and the degree to which plant community structure and climate mediate the effects of plant species loss remain unclear. Here, we explored how plant species loss through experimental removal shaped belowground function in ecosystems characterized by different climatic regimes and edaphic properties. We measured plant community composition as well as potential carbon (C) and nitrogen (N) mineralization and microbial extracellular enzyme activity in soils collected from four unique plant removal experiments located along an elevational gradient in Colorado, USA. We found that regardless of the identity of the removed species or the climate at each site plant removal decreased the absolute variation in potential N-mineralization rates and marginally reduced the magnitude of N-mineralization rates. While plant species removal also marginally reduced C-mineralization rates, C-mineralization, unlike N-mineralization, displayed sensitivity to the climatic and edaphic differences among sites, where C-mineralization was greatest at the high elevation site that receives the most precipitation annually and contains the largest soil total C pools. Plant removal had little impact on soil enzyme activity. Removal effects were not contingent on the amount of biomass removed annually, and shifts in mineralization rates occurred despite only marginal shifts in plant community structure following plant species removal. Our results present a surprisingly simple and consistent pattern of belowground response to the loss of dominant plant species across an elevational gradient with different climatic and edaphic properties, suggesting a

common response of belowground ecosystem function to plant species loss regardless of which plant species are lost or the broader climatic context.

Keywords: biodiversity loss, carbon mineralization, elevational gradient, nitrogen mineralization, plant-soil linkages, plant removal

Introduction

Loss of plant species from communities will impact the structure and function of ecosystems as interactions among plant species, evolutionary dynamics such as competition and facilitation that shape plant communities, and plant-soil linkages shift in response to diminished biodiversity (Pugnaire *et al.*, 2019). Ecologists often explore the impact of plant species loss on ecosystem function by building correlations between ecosystem functions and aspects of plant community structure such as dominant plant functional type, functional traits, species relative abundances, and the diversity of the remaining plant community (Symstad *et al.*, 1998; Lyons and Schwartz, 2001; Gilman *et al.*, 2010; Ockendon *et al.*, 2014). However, the properties of communities or ecosystems that determine ecosystem sensitivity to changes in plant communities or to abiotic changes remain unclear (Klanderud, 2005; Gilman *et al.,* 2010; Wardle *et al.*, 2011; Adler, 2012). Furthermore, we know little about the sensitivity of belowground soil processes to changes in species interactions relative to aboveground ecosystem functions (Zak *et al.*, 2003). Despite ample research defining the relationship between species richness and ecosystem function in experimental and observational frameworks (Tilman *et al.*, 1996; Grace *et al.*, 2016), we also know that the loss of individual species from a plant community can have a different impact on the biodiversity–ecosystem function relationship than is captured by studies that assess this relationship in the direction of increasing diversity across a biodiversity gradient (Naeem *et al.*, 1995; Fox and Kerr, 2011; Kardol *et al.*, 2018). Plant removal experiments, especially when conducted across an environmental gradient, can be used as a tool to address many of these problems that arise when attempting to quantify the impact of losing a plant species on ecosystem function (Sundqvist *et al.,* 2013).

Some plant species have a disproportionately large impact on soil processes due to their dominance (Smith and Knapp, 2003; Avolio *et al.,* 2019), the uniqueness of their traits in a community, or their longevity on the landscape, i.e. long-lived perennial species. The loss of these influential plant species from a community should have a greater impact on ecosystem

processes than the loss of a plant species with a less pronounced presence in the community (Tilman *et al.*, 1997; Chapin *et al.*, 1998; McLaren and Turkington, 2010). In short, the identity of the plant species that is lost from an ecosystem should matter when predicting the impact of species loss on belowground processes (Wardle *et al.*, 1999; Johnson *et al.*, 2008). In a 2003 review, Díaz *et al.* proposed three mechanisms as pathways by which plant species removal impacts ecosystem function as the loss of a single plant species shifts species interactions: 1) Ecosystems could respond to the loss of the specific functional role filled by the removed species, 2) changes to ecosystem function could be a response to the re-assembly of the remaining community members following the loss of a species, and 3) changes to ecosystem function could be a response to the disturbance of the removal treatment itself through loss of aboveground biomass. By incorporating treatments that account for each of these mechanisms of plant species removal impact, removal experiments are uniquely positioned as an experimental framework to discern the pathways by which removing a single plant species can impact belowground ecosystem function.

Environmental conditions such as growing season temperature and precipitation modulate the relationship between above- and below-ground ecosystem components, meaning that the loss of the same plant species could have a starkly different impact on belowground processes in ecosystems characterized by different climates (Klanderud and Totland, 2005; Brooker, 2006; Bardgett *et al.*, 2013). Changes in climate as well as the associated environmental stress alter both the direction and magnitude of plant-soil feedbacks (van der Putten *et al.*, 2016; Baert *et al.*, 2018). The nature of this feedback effect is highly variable and depends on the context of both present environmental conditions and the legacy of historical climate (Kaisermann *et al.*, 2017). The soil microbial communities involved in carbon (C) and nitrogen (N) mineralization, the nutrient recycling processes in terrestrial ecosystems, may also be limited by different abiotic factors than is the aboveground community. Thus, global change drivers may decouple above- and below-ground linkages as plant and microbial communities respond differently to environmental change (Wardle *et al.*, 2013; Classen *et al.*, 2015).

Taken together, the effect of losing a plant species from a community on belowground ecosystem processes likely depends on both the functional identity of the plant species removed,

climatic context, and the degree to which plant community structure shifts following the species loss (Díaz *et al.*, 2003; McLaren and Turkington, 2010; Wardle *et al.*, 2011; Pugnaire *et al.*, 2019). However, the influence of the combination of factors described above on the relationship between plant species removal and belowground ecosystem processes has yet to be empirically tested using field experiments. Therefore, to understand the biotic and abiotic variables that shape the impact of species loss on potential C- and N-mineralization, we sampled a series of plant removal experiments established at different sites across an elevational gradient. We predicted that the effect of removing a dominant plant species would vary across the environmental gradient, with the greatest decrease in belowground mineralization rates following plant removal occurring at the lowest elevational site where moisture limitation, i.e. environmental stress, is the highest (García-Palacios *et al.*, 2018; Pugnaire *et al.*, 2019). We also hypothesized that the impact of plant species removal on belowground processes would scale with the amount of biomass removed from each plot (Díaz *et al.*, 2003; McLaren and Turkington, 2015). Our results lend insight to how the loss of species from communities might alter important nutrient cycles in climatically different ecosystems through the restructuring of plant communities and ecosystem function.

Materials and Methods

To investigate the impact of removing a plant species on C- and N-mineralization, we sampled four existing, independent plant removal experiments located at four locations along an elevational gradient near the Rocky Mountain Biological Laboratory, Gothic, Colorado, USA. Each of the four experiments removed different focal plant species, and the experiments have been running from five to seventeen years. A dominant plant species was removed at each site along the elevational gradient, but because there is nearly complete turnover of the plant community across the elevational gradient, the identity of the removed species was different at each elevation, confounding the functional type of the removed species as well as environmental conditions across the elevational gradient.

At the low elevation site, located at 2740 m asl (38.71 N, -106.82 W), we removed the dominant plant species *Wyethia x magna* (Asteraceae), a perennial forb that is a stable hybrid of *Wyethia amplexicaulis* and *W. arizonica* (Weaver, 1915), by clipping aboveground biomass to the soil

surface annually for five years prior to sampling. Plots at this site were 2.0 m × 2.0 m in area (n = 8). Soils at the low elevation site were classified as Mayoworth loam. At the mid-low elevation site, located at 2890 m asl (38.95 N, -106.98 W), we maintained a seventeen-year removal of *Linaria vulgaris* (Plantaginaceae), a perennial, invasive forb with an extensive horizontal root system, in 2.0 m × 2.0 m (n = 6) (Wilke and Irwin, 2010). Plants were removed from the mid-low elevation site by gently pulling on the base of the plant to remove the plant and a small portion of the *L. vulgaris* root mat. Soils at the mid-low elevation site were classified as Tine sandy loam and Bassel sandy loam, depending on the slope of each plot. At the mid-high elevation site, we removed *Festuca thurberi* (Poaceae), a perennial grass that forms shallow but dense root systems, by clipping to the soil surface annually for seven years prior to sampling. Removal treatments at the mid-high elevation site (2904 m asl, 38.94 N, -106.99 W) were applied in 1.5 m × 1.5 m (n = 4) (Read *et al.*, 2018, Henning *et al.*, 2019), with soils at this site classified as Leaps silty clay loam. In the *W. magna* and *F. thurberi* removals at the mid-high and low elevation sites, a small amount of glyphosate herbicide was applied to the remaining base of the stems after the aboveground biomass was removed to attempt to kill belowground biomass. Herbicide was only applied to stem bases at these sites for the first two years of the removal treatments, and because of the meticulous way in which it was applied to the base of the remaining stems following removal using a paintbrush, we are confident that herbicide application had negligible effects on the fitness of the other plant species. At the high elevation site (3460 m asl, 38.99 N, -107.06 W), we removed the dominant species *Juncus drummondii* (Juncaceae), a perennial rush that grows in thick bunches, by clipping aboveground biomass to the soil surface annually for five years before sampling. Plots at this site covered an area of 2.0 m × 2.0 m (n = 8). Soils at the high elevation site were classified as Moran-Rubble land complex, characterized by extremely gravelly loam/sandy loam. Roots of the removed plants species were left intact at all sites that used clipping to removed aboveground biomass in removal treatments (low, mid-high, and high elevation sites) in order to minimize disturbance caused by the removal treatment for the remaining plant species and the soil community. While *L. vulgaris* individuals were removed by tugging gently on the base of the stem, removing some root biomass in the process, the majority of the root runners that extend horizontally just beneath the soil surface remained behind in the soil.

In addition to the control (i.e. no plant biomass removed from plots with the focal species naturally present) and individual species removal treatments present at all sites, the mid-low and mid-high elevation sites had an additional random biomass removal treatment where biomass from random plant species was removed to reflect the amount of biomass that was removed annually from the treatments in which a specific plant species was removed. This treatment was intended to isolate the disturbance effect that plant removal has on the remaining plant community through loss of aboveground biomass. Finally, a natural control treatment, i.e. plots where the focal plant species was naturally absent, existed at the mid-low elevation site. Table 1 summarizes site-level experimental design, climate data for the 2018 growing season, relative abundance of the focal species at each site, and the average amount of biomass removed annually from removal treatment plots at each site.

During the peak of the 2018 growing season, when aboveground biomass was greatest at each site, we conducted plant community surveys by visually assessing the percentage of the plot area covered by each plant species. Rare species were recorded as covering < 1 %. At the low, mid-high, and high elevation sites, data were collected annually on the amount of plant biomass removed from each plot that received a removal treatment by collecting removed biomass in paper bags and drying the plant biomass for 48 h at 60 °C. We calculated the average amount of biomass removed annually from each plot by pooling the total amount of biomass removed over the entire length of the experiment at each site, and dividing this cumulative number by the length of each experiment in years (Table 1). On July 23rd-25th, 2018, we took two separate soil cores (< 15 cm deep), one for the potential mineralization incubation and one for extracellular enzyme assays, from random locations within each plot. Soil samples were refrigerated and transported to the University of Vermont, Burlington, VT USA for processing. We sieved each sample to remove rocks and large plant material (> 2 mm) and measured gravimetric water content (g H_2O g^{-1} dry soil) by drying 10 g of field-moist soil in an oven at 105 °C for 48 hours (Robertson *et al.*, 1999). We measured soil organic matter content using the loss on ignition method (SOM-LOI) by combusting oven-dried soil samples (105 °C) at 550 °C for six hours, and then measuring SOM-LOI (g C kg^{-1} dry soil) by quantifying the mass difference between oven-dried and combusted soil samples (Hoogsteen *et al.*, 2015).

To measure the effect of plant removal on potential C- and N- mineralization in soils under standardized environmental conditions, we conducted a 30-day laboratory incubation of soils exposed to ideal moisture and temperature conditions (30% volumetric water content, 20 °C) (Robertson *et al.*, 1999). 30% volumetric water content was the maximum soil moisture content recorded during the growing season at these four sites in the two years preceding this study, so this moisture threshold was chosen to represent 'ideal' but realistic conditions under which we could investigate the full capacity of the microbial community in sampled soils to mineralize C and N. We divided field-moist soil samples from each plot into paired 10 g subsamples. One subsample was extracted immediately using a 2.0 M KCl solution to measure extractable NH_4^+ and NO_3^-. We incubated the second subsample in the dark for 30 days in 1 L clear, glass jars fitted with rubber septa in the metal lid. We measured the CO_2 evolved in the headspace of the jars via direct injection by using a syringe to sample the air in each sealed jar and injecting 7 mL of air into a LI-COR 7810 trace gas analyzer (LI-COR Instruments, USA) at six time points (days 1, 2, 4, 8, 16, and 30 of the incubation) to track potential C-mineralization (μg C g^{-1} dry soil day^{-1}). At the completion of the incubation, we extracted the incubated soil subsample with 2 M KCl to again measure extractable NO_3^- and NH_4^+ concentration. We measured the concentration of NO_3^- and NH_4^+ in the extractions colorimetrically (Doane and Horwath, 2003) using a Synergy HT microplate fluorimeter/spectrophotometer (Synergy HT, Biotek Inc., Winooski, VT, USA). We then calculated total N-mineralization rates by subtracting the sum of NO_3^- and NH_4^+ (i.e. inorganic N) in the initial subsample from the sum of extractable inorganic N in the final subsample and dividing the amount of inorganic N produced during the reaction by the length of the incubation in days (mg N kg^{-1} dry soil day^{-1}).

In addition to a laboratory incubation of soils to measure N- and C-mineralization rates, we measured the activities of six different soil extracellular enzymes to understand how plant removal impacts potential microbial activity. Using the protocol established by Saiya-Cork *et al.* (2002), we assayed the carbon degrading enzymes α-glucosidase (AG), β-glucosidase (BG), Cellobiohydrolase (CBH), and β-Xylosidase (XYL), nitrogen acquiring enzyme β-N-acetylglucosaminidase (NAG), and phosphorus acquiring enzyme Acid phosphatase (PHOS). All assays were performed by incubating enzymes in a soil slurry prepared with a buffered solution (pH 5.0 sodium acetate buffer) at an ideal temperature (20.0 °C) with non-limiting amounts of

245 substrate. Following incubation, potential enzyme activities were quantified using a Synergy HT
246 microplate spectrophotometer (Biotek Inc., USA).
247
248 To analyze shifts in plant community structure in response to the plant removal treatments across
249 the elevational gradient, we performed non-metric multidimensional scaling (NMDS) on the
250 plant community survey data in each plot using the 'metaMDS' function in the vegan:
251 Community Ecology Package in R (Oksanen *et al.*, 2019) with distance between plant
252 communities in plots within the same treatment calculated according to the modified Gower
253 ('altGower') method (Anderson *et al.*, 2006). We performed a permutational multivariate
254 analysis of variance using the 'adonis' function in the vegan package with two thousand
255 permutations to quantify the extent to which the removal treatment explains dissimilarity
256 between communities. To calculate dissimilarity among plant communities at each site and with
257 the removal treatments, we chose to use the modified Gower method (Anderson *et al.*, 2006)
258 ('altGower' in the 'vegdist' function in the vegan package). The modified Gower method for
259 calculating dissimilarity between communities is most appropriate for our study because it
260 explicitly weights an order-of-magnitude change in abundance equivalent to a change in species
261 composition. This feature is important because this analysis relies on our ability to detect
262 changes in abundance driven by our removal treatments despite high turnover in species
263 composition across the elevational gradient that would overwhelm changes in abundance in most
264 other dissimilarity indices that consider abundance (Anderson *et al.*, 2006). Finally, we used the
265 'betadisper' function to evaluate the homogeneity of variances in community structure across
266 treatment groups to understand how the removal treatments, and separately, site, affect the
267 variation in plant community structure. This test calculates the average linear distance between
268 individual plots within a treatment group and the within-group centroid which represents the
269 median community structure for that group.
270
271 To measure the impact of plant removal on potential C- and N-mineralization, potential soil
272 enzyme activity, and SOM-LOI content, we performed ANOVAs using the 'Anova' function in
273 the car package to measure the extent to which elevation, removal treatment (dominant species
274 removal vs. non-removal), and the interaction between these two variables explained variation in
275 C- or N-mineralization (Fox and Weisberg, 2019). To account for heteroscedasticity in potential

N-mineralization rates among removal treatment and site groups, we used a White-adjustment for corrected standard errors within the 'Anova' function (White, 1980). Additionally, to understand if the effect of species removal on mineralization rates was mediated by simply the removal of aboveground biomass, we again conducted ANOVAs to analyze variation in potential mineralization rates across removal treatments using only the mid-low and mid-high elevations where random biomass removal treatments were applied. To further understand whether potential mineralization rates are influenced by the amount of biomass removed from each plot, we analyzed linear relationships between average biomass removed annually from each plot and the residual variation in C- and N-mineralization after accounting for the effects of removal treatments and site. Finally, we conducted an ANOVA using only the mid-low elevation plots which included a treatment where the focal species, *L.* vulgaris, was naturally absent to analyze the similarity between mineralization rates in focal species removal plots and mineralization rates in plots where the focal species was absent. A high degree of similarity between potential mineralization rates in removal treatment plots and focal species absent plots would lend support to one of the hypotheses proposed by Díaz *et al.* (2003), that the impact of removing a species from a system is mediated by the loss of functions or influence uniquely attributed to that plant species. To understand how site and removal treatment impact variation among potential C- and N-mineralization rates, we also performed modified Levene's tests (applying an ANOVA test to the absolute deviation of each observation from the group median) using the 'levene.test' function in the lawstat package (Brown and Forsythe, 1974; Gastwirth *et al.*, 2019). All statistical analyses were performed in RStudio (R version 3.6.3 "Holding the Windsock") (Rstudio Team, 2016).

Results

Plant species removal marginally decreased the magnitude of potential N-mineralization rates by 27% ($F_{1,43} = 3.32$, $p = 0.075$) and significantly decreased the variation in potential N-mineralization ($p = 0.016$), regardless of plant species removed or climate at each elevation (Fig. 1a). Neither site ($F_{3,43} = 0.84$, $p = 0.481$) nor the interaction between site and removal treatment ($F_{3,43} = 0.35$, $p = 0.791$) significantly affected N-mineralization. Variation in potential N-mineralization rates within a single site was relatively homogenous across the elevational gradient ($p = 0.462$). Conversely, potential C-mineralization rates varied significantly across the

elevational gradient ($F_{3,42}$ = 5.31, p = 0.003), with notably higher C-mineralization rates at the high elevation site confirmed by a Tukey HSD test (Fig. 1b). Plant species removal tended to decrease potential C-mineralization rates by 9% ($F_{1,42}$ = 3.22, p = 0.08), whereas the interaction of site and plant removal treatment had no discernible impact on potential C-mineralization rates ($F_{3,42}$ = 0.57, p = 0.637). Rates of C-mineralization over the course of the incubation showed consistent patterns across all treatments and sites, with CO_2 efflux peaking during the first days of the incubation and stabilizing at a lower mineralization rate after the first week of the incubation (Appendix S1: Fig. S1). SOM content, measured via LOI, reflected the same pattern as potential C-mineralization rates, where SOM-LOI was greatest at the high elevation site ($F_{3,42}$ = 3.93, p = 0.015) with no detectable response to removal treatments ($F_{1,42}$ = 1.00, p = 0.322) nor an interaction between removal treatments and site ($F_{3,42}$ = 0.52, p = 0.669) (Appendix S1: Fig. S2). We did not find an effect of site or removal treatment on the variation in potential C-mineralization rates within a treatment group at each site (site p = 0.349, removal treatment: p = 0.112).

While the activity of all soil extracellular enzymes differed significantly across the elevational gradient (AG: p = 0.002, BG: p < 0.001, CBH: p = 0.007, XYL: p = 0.010, NAG: p < 0.001, PHOS: p < 0.001), we did not detect a response of potential soil enzyme activity to the plant removal treatments (AG: p = 0.232, BG: p = 0.113, CBH: p = 0.241, NAG: p = 0.533, PHOS: p = 0.220), with the exception of XYL activity that decreased marginally with plant removal ($F_{1,36}$ = 3.65 p = 0.064) (Appendix S1: Fig. S3). Activity of NAG, a nitrogen degrading enzyme, and XYL, a carbon degrading enzyme involved in the breakdown of hemicellulose, both showed significant differences in response to the interaction between site and removal treatment (NAG: $F_{3,41}$ = 3.55, p = 0.023; XYL: $F_{3,36}$ = 4.33 p = 0.010).

Plant species removal at all sites had only a marginal impact on plant community structure ($F_{1,43}$ = 1.81, p = 0.060), but community structure shifted significantly across the elevational gradient, largely driven by species turnover ($F_{3,43}$ = 7.76, p < 0.001; Fig. 2). We did not detect a significant interactive effect between plant removal treatment and site on plant community structure ($F_{3,43}$ = 0.054, p = 0.125). Overall, site, removal treatment, and the interaction of these two parameters explained 40% of the variation in plant community structure across all plots.

Furthermore, neither site nor removal treatment altered the dispersion or within-group variation in plant community structure (site: $F_{3,47} = 1.23$, $p = 0.310$; removal treatment: $F_{1,49} = 0.10$ $p = 0.750$; Appendix S1: Fig. S4).

Our comparison of plant community structure and potential soil mineralization rates in random biomass removal plots vs. focal plant species removal plots verified that the effect of removal treatments does not stem from biomass removal alone. However, we could not detect a distinct effect of plant removal on plant community structure or potential mineralization rates in data from this subset of field sites. When analyzing the plant community structure at the two sites that included random biomass removal treatments (mid-high and mid-low elevation sites), we found only a marginally significant difference in plant community dissimilarity across removal treatments ($F_{2,23} = 1.45$, $p = 0.083$) and did not find a significant change in the distribution of variation in plant community structure in response to either of the removal treatments ($F_{2,26} = 0.135$, $p = 0.875$). Likewise, potential C-mineralization ($F_{2,22} = 0.68$, $p = 0.519$) and potential N-mineralization ($F_{2,23} = 0.98$, $p = 0.389$) were seemingly unaffected by removal treatments, and mineralization rates in random biomass removal plots were indistinguishable from the rates in control plots (C-mineralization: $p = 0.750$, N-mineralization: $p = 0.667$) and species removal plots (C-mineralization: $p = 0.928$, N-mineralization: $p = 0.912$). Linear regressions correlating the amount of biomass removed annually with the residual variation in N- and C-mineralization rates after accounting for the effects of site and removal treatment further supported our finding that the amount of aboveground biomass removed was not significantly correlated with belowground mineralization rates in these meadow ecosystems (C-mineralization: $p = 0.588$, N-mineralization: $p = 0.455$). These results indicate that the mechanism driving changes in belowground ecosystem function in response to plant removal treatments stems beyond loss of aboveground biomass.

By isolating the mid-low elevation site "natural removal control" plots where the focal species, *L. vulgaris*, was naturally absent from plots, our results showed that none of our removal treatments had a discernible effect on any plant community properties or ecosystem function rates as measured above (community dissimilarity: $F_{3,18} = 0.86$, $p = 0.735$; community dispersion: $F_{3,18} = 0.48$, $p = 0.703$; potential C-mineralization: $F_{3,17} = 1.41$, $p = 0.275$; potential

N-mineralization: $F_{3,18} = 0.83$, $p = 0.494$). Furthermore, potential C-mineralization and N-mineralization in the plots where *L. vulgaris* was naturally absent were indistinguishable from potential mineralization rates in control plots (C-mineralization: $p = 0.906$, N-mineralization: $p = 0.993$) and *L. vulgaris* removal plots (C-mineralization: $p = 0.9997$, N-mineralization: $p = 0.905$), indicating that the effect of removing the dominant species cannot be attributed to the loss of the specific influence of this species on belowground processes.

Discussion

By incubating soils sampled from plant species removal experiments spread across an elevational gradient, our results show that species removal consistently decreases the variation in potential N-mineralization rates, with the magnitude of N-mineralization rates in plant removal plots being marginally lower. These results were consistent across the entire elevational gradient, with no differences in potential N-mineralization rates among sites, a finding that stands in contrast to our hypothesis that the effect of dominant plant species removal would be mediated by variation in climate and environmental stress across the elevational gradient. While potential C-mineralization rates also decreased subtly with removal treatments, we found significantly higher mineralization rates at the high elevation site where soil moisture across the growing season is relatively higher. This result likely reflects SOM content across the elevational gradient, where potential C-mineralization rates are highest at the high elevation site where SOM content was significantly greater than at all other sites (Appendix S1: Fig. S2). We also found that variation in soil potential N-mineralization rates was significantly lower in the plant species removal treatment, indicating convergence in potential N-mineralization with the loss of a plant species regardless of its identity. Interestingly, we observed these changes in belowground processes in response to plant species removal despite little to no change in overall plant species composition and potential extracellular enzyme activity with the removal of a focal species in each ecosystem.

Why might there be an interactive effect of elevation and removal on XYL and NAG enzyme activities? We suspect that shifts in NAG activity in response to removal might be sensitive to the changes in edaphic properties like soil total C and soil moisture that vary across the elevational gradient. The NAG activity patterns revealed here likely mirror our N-mineralization

results because this enzyme is a key agent in the nitrogen mineralization process. Additionally, the interactive effects of site and removal treatment might be linked to the functional identity of the plant species removed at each site. XYL enzymes hydrolyze specific linkages in β-(1,4)-xylose compounds found in the cell walls of plant species. As the cell walls of monocot species substitute a different xylan compound as the structural backbones of cell walls, the biomass of dicot species contains more of the β-(1,4)-xylose XYL substrate (Hatfield *et al.,* 2017). Therefore, removal of a monocot (as at the high and mid-high elevational sites) vs. the removal of a dicot (mid-low and low elevational sites) could have contrasting effects on the synthesis and activity of extracellular enzymes like XYL that aid in the decomposition of lignocellulosic biomass.

Previous work in these meadow ecosystems revealed fairly strong resistance to change in both above- and belowground communities to *F. thurberi* removal, where plant community composition, community-weighted plant functional traits, and fungal colonization of plant roots showed no clear response to removal of the dominant species or nitrogen fertilization (Read *et al.*, 2018; Henning *et al.*, 2019). Alpine ecosystems experience frequent disturbances at a variety of scales, from burrowing mammals to avalanches and landslides, possibly conditioning these ecosystems and the plant and soil communities that inhabit montane meadows to a relatively large degree of disturbance, resulting in resilience to more minor disturbances plant species removal. These results suggest that plant species loss impacts belowground processes by some other mechanism beyond the loss of the specific influence of the removed plant species on belowground nutrient cycling.

By comparing the response of potential N-mineralization to removal of four distinct species along an elevational gradient, we can discern between the mechanisms by which plant removal might impact ecosystem function proposed by Díaz *et al.* (2003). Because removal decreased potential N-mineralization at all field sites, regardless of the functional identity of the plant species removed, our results suggest that this ecosystem response is not due to the loss of the influence of a particular species, eliminating the first mechanism as a plausible explanation. When considering the "natural removal control" plots at the mid-low elevation, we find that any response of N-mineralization rates to the removal of this species was not due to the loss of a

specific function performed by the removed species, *L. vulgaris*, because N-mineralization rates did not differ between plots where *L. vulgaris* was present and plots where *L. vulgaris* was naturally absent. The results from our analysis of shifts in the structure of the plant communities in each experiment in response to removals (Fig. 1a) showed that the removal had only a marginal effect on plant community structure, making it unlikely that community re-assembly following removal could be driving the shift in ecosystem function found here. Finally, potential N-mineralization rates did not differ significantly between control plots and random biomass removal plots, indicating that disturbance is also unlikely to be the dominant driver of shifts in potential N-mineralization rates in response to removal. Our experimental design therefore allows us to speculatively rule out all three of the mechanisms that drive removal effects as proposed by Díaz *et al.* (2003), so we turn to other more indirect mechanisms that might drive variation in mineralization rates following species removal.

Plant removal may have several indirect impacts on belowground microbial processes that could explain our results. Removals across all experiments were conducted by either clipping aboveground biomass of the removed species to ground level, or by gently pulling on aboveground biomass to break shoots from the extensive network of belowground roots, as in the *L. vulgaris* removal. This method of removal leaves much of the belowground biomass of the removed plant behind, potentially constituting a significant pool of N that is immobilized in plant root litter. Several studies estimate that root mean residence time is approximately four years for fine roots in ecosystems characterized by a -1.8 to 2.7 °C range in mean annual temperature (Gill and Jackson, 2000; Leifeld *et al.*, 2015), and root turnover in grasslands globally is limited by precipitation (Wang *et al.*, 2019). Estimates of fine root decomposition in arid grasslands with precipitation regimes similar to the climate of this study system indicate that as much as 60% of root biomass remains after 4-10 years of decomposition, immobilizing 60% of the N content of root litter at the onset of decomposition (Parton *et al.* 2007). Delay in root decomposition following the onset of plant removal could lead to a short-term decrease in the size of the soil N pool that is available to microbes and plants until the roots of removed plants are decomposed. Our data lend some support to this explanation when N-mineralization rates are analyzed within the context of each individual experiment across the elevational gradient. Results from the mid-low elevation site, where *L. vulgaris* has been actively removed for seventeen years, show no

462 effect of removal–or any of other treatments–on potential N-mineralization rates, possibly
463 indicating that the legacy of the removal treatment has faded as roots have decomposed
464 following removal to release N immobilized in belowground *L. vulgaris* root litter. In contrast,
465 the effect of species removal on soil N-mineralization rates remains across all three younger
466 experiments. Immobilization of N in belowground root litter might therefore be a mechanism by
467 which loss of species locally could reduce ecosystem N cycling in the short-term.
468
469 Species removal likely affects ecosystem function more generally by reducing ecosystem
470 resilience to climatic extremes (Tilman and Downing, 1994). This response to species removals
471 may be a belowground manifestation of an effect that Kardol *et al.* (2018) found in a long-term
472 plant removal experiment where plant species loss led to greater temporal variability in
473 aboveground plant biomass. Perhaps plant species loss leads to similar variability belowground
474 where properties such as soil microbial community composition, microbial biomass, or microbial
475 activity and carbon use efficiency in communities that have lost plant species are especially
476 vulnerable to climatic extremes, driving marked shifts in belowground processes that are not
477 seen in more diverse and resilient communities. Heightened temporal variability of above- and/or
478 below-ground ecosystem components in response to species loss may also negatively impact
479 ecosystem resilience (Oliver *et al.,* 2015). As 2018 was an especially dry summer in our study
480 area, loss of the ability of plant and microbial communities to function in spite of climatic events
481 like a severe drought may explain lower mineralization rates in plant removal plots.
482
483 In conclusion, we found that removing the dominant plant species consistently reduces the
484 variation in soil N- mineralization rates, while marginally decreasing the magnitude of C- and N-
485 mineralization rates, in alpine meadows. We find these changes to belowground function in
486 response despite only subtle shifts in aboveground plant community structure and no clear
487 changes in extracellular enzyme activity in response to plant removal. While our results are
488 limited in their ability to pinpoint a clear mechanism by which species loss affects potential soil
489 mineralization rates, the results do offer an unexpectedly simple pattern describing the overall
490 effect of species loss on soil N and C cycling that holds across an elevational gradient. Moreover,
491 our study offers insight into how loss of aboveground plant species might indirectly impact

492 belowground processes with implications for ecosystem function in a future world characterized
493 by global change.
494
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512
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514 Additional supporting information may be found online at: [link to be added in production]
515
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519
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647 **Table 1.** Focal species removed, experimental design, and climate variables for each of the four field experiments included in this
648 study.

Site	Length of Experiment (yr)	Plant Species Removed	Replication (n)	Elevation (m asl)	Mean Growing Season Temp. (C)	Growing Season Precipitation (mm)	Mean Growing Season Soil Moisture (VWC)	Relative Abundance of Focal Species	Average Biomass Removal Rate (dry g y^{-1})	Average Soil Total C (g kg^{-1} dry soil)
Low Elevation	5	*Wyethia x magna*	8	2740	15.6	61.4	3.61%	19.9%	173.02	111.6
Mid-Low Elevation	17	*Linaria vulgaris*	6	2890	12.1	98.3	9.67%	19.8%	–	109.4
Mid-High Elevation	7	*Festuca thurberi*	4	2904	—	—	—	29.5%	29.51	104.2
High Elevation	5	*Juncus drummondii*	8	3460	12.2	127.6	7.71%	19.1%	52.49	153.5

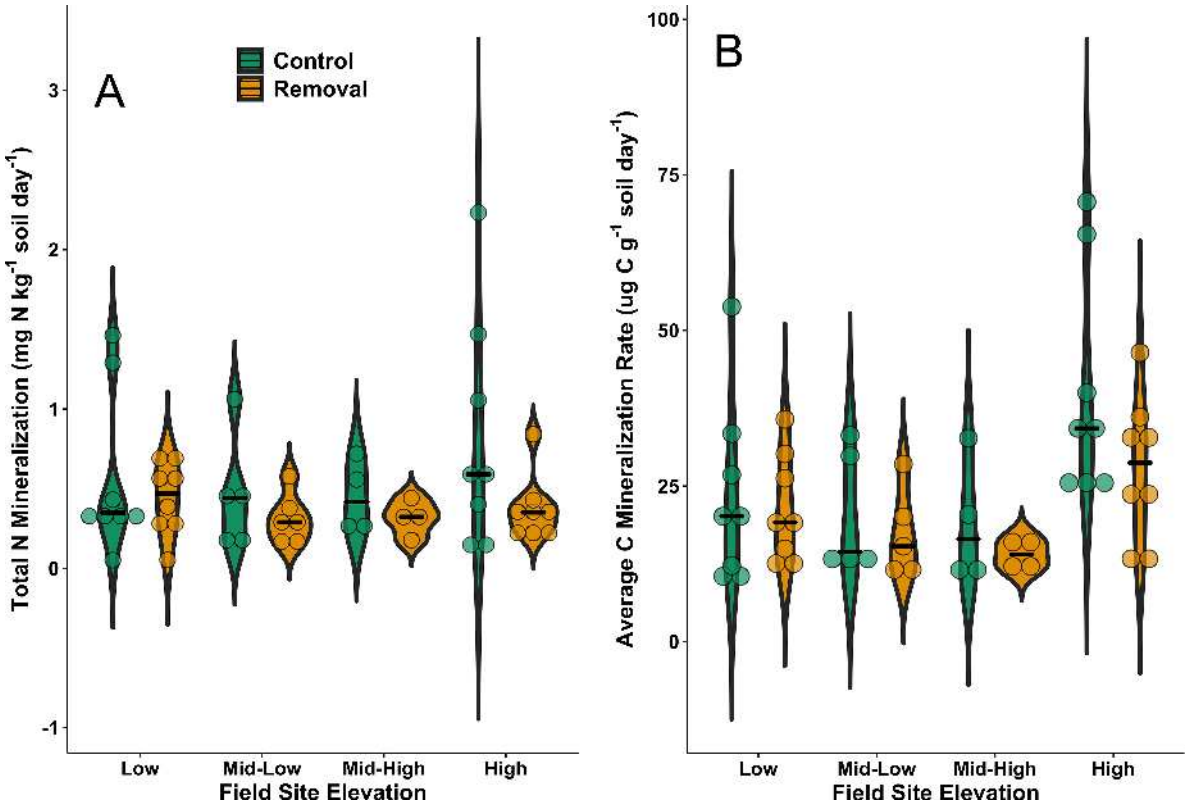
649

650 Notes. Both mid-elevation sites are located in close proximity to one another and therefore share climate data. Collection of growing
651 season climate data for all sites began on June 1st, 2018, and ended on August 31st, 2018, with the exception of the high-elevation field
652 site where the weather station was installed on June 27th at the beginning of the growing season. The relative abundance of the focal
653 species targeted in removal treatments at each site was calculated by dividing the percent of the plot area covered by the focal species
654 in control plots by the total area covered by all plant species in each control plot. Data quantifying the amount of biomass removed
655 annually from removal treatments at the mid-low elevation site were not collected. Climate data for the mid-elevation sites are made
656 available through the Rocky Mountain Biological Laboratory (RMBL, see Acknowledgements).
657

Figure Captions

Figure 1: A) Plant removal decreased the variation in and, marginally, the magnitude of N-mineralization rates. Site and the interaction between elevation and removal treatment had no discernible effect. B) C-mineralization rates varied significantly among sites and were marginally impacted by plant species removal while the interaction between site and species removal treatment did not significantly impact C-mineralization rates. Crossbars indicate group medians.

Figure 2: An NMDS ordination of plant community structure in response to plant removal treatments at four different sites along an elevational gradient. Polygons encompass communities in all plots within a specific removal treatment at each site. Teal polygons indicate control treatments, gold polygons encompass communities that received the plant removal treatment, blue polygons indicate the random biomass removal treatment, and the gray polygon at the mid-low elevation site indicates a natural removal control treatment where the focal plant species, *Linaria vulgaris*, naturally did not occur. Community structure varied by site but only varied marginally with removal treatments and was not significantly impacted by an interaction between removal treatment and site.



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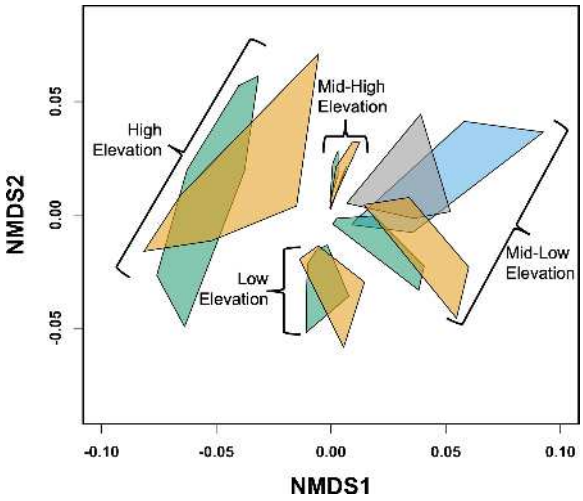

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