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Trait-based filtering mediates the effects of realistic biodiversity losses on ecosystem functioning

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Biodiversity losses are a major driver of global changes in ecosystem functioning. While most studies of the relationship between biodiversity and ecosystem functioning have examined randomized species losses, trait-based filtering associated with species-specific vulnerability to drivers of diversity loss can strongly influence how ecosystem functioning responds to declining biodiversity. Moreover, the responses of ecosystem functioning to diversity loss may be mediated by environmental variability interacting with the suite of traits remaining in depauperate communities. We do not yet understand how communities resulting from realistic diversity losses (filtered by response traits) influence ecosystem functioning (via effect traits of the remaining community), especially under variable environmental conditions. Here, we directly test how realistic and randomized plant diversity losses influence productivity and invasion resistance across multiple years in a California grassland. Compared with communities based on randomized diversity losses, communities resulting from realistic (drought-driven) species losses had higher invasion resistance under climatic conditions that matched the trait-based filtering they experienced. However, productivity declined more with realistic than with randomized species losses across all years, regardless of climatic conditions. Functional response traits aligned with effect traits for productivity but not for invasion resistance. Our findings illustrate that the effects of biodiversity losses depend not only on the identities of lost species but also on how the traits of remaining species interact with varying environmental conditions. Understanding the consequences of biodiversity change requires studies that evaluate trait-mediated effects of species losses and incorporate the increasingly variable climatic conditions that future communities are expected to experience.

realistic biodiversity loss | ecosystem function | productivity | environmental heterogeneity | invasion

As worldwide biodiversity losses continue, understanding their effects on ecosystem functioning is critical to conservation and mitigation efforts (1). The relationship between biodiversity loss and ecosystem functioning has been studied extensively over the last two decades (2–5); past research has demonstrated that reduced species richness generally decreases ecosystem functioning, with much of this research effort focused on understanding productivity responses (3, 4).

Most biodiversity–ecosystem functioning (BEF) studies (e.g., refs. 6 and 7) have employed randomized species loss scenarios, with the goal of isolating the effects of species diversity from species identity. Because real-world species losses are not random (8, 9), a growing number of studies have examined how ecosystem functioning responds to realistic biodiversity losses—directional, nested losses of species based on trait vulnerability to specific stressors such as temperature or precipitation changes. These studies have shown that the effect of realistic biodiversity loss on ecosystem functioning is often greater than that of randomized biodiversity loss (10–16). A general explanation for the finding that realistic species losses cause greater declines in ecosystem

functioning than randomized species losses is that entire functional groups are often lost with realistic diversity declines, leaving fewer niches occupied and leading to decreased complementarity and functional capacity (11, 12). However, the greater effect sizes of realistic biodiversity loss can also be viewed as a counterintuitive result: an alternate hypothesis is that the species remaining following a realistic reduction in diversity are those most suited to functioning highly in the new environment (17). For example, the species that remain following drought-induced diversity losses might be those with the highest functioning in drought conditions. This hypothesis predicts that realistic diversity losses would maintain or increase ecosystem functioning relative to randomized diversity losses. Distinguishing between these two hypotheses requires a more detailed understanding of how trait-based filtering impacts biodiversity losses and the subsequent effects on ecosystem functioning. While the BEF literature has long recognized that functional traits are a key aspect of understanding the effects of biodiversity loss, few studies have taken a trait-based approach, and several reviews have specifically called on researchers to examine the importance of functional effect and response traits on the outcomes of biodiversity loss (2, 18).

Whether ecosystem functioning increases or decreases with biodiversity loss depends strongly on the relationship between functional traits that are subjected to trait-based filtering (response traits) and traits that influence ecosystem functioning (effect traits;

Significance

We present multiyear results from a realistic biodiversity loss experiment, examining how two key ecosystem functions (productivity and invasion resistance) responded to randomized and realistic (drought-driven) species losses across years with high yearly climatic variation. We show that realistic low-diversity communities do not always have high functioning under the conditions that drove species loss, indicating a disconnect between functional response and effect traits. Our results also suggest that the functional stability of ecosystems experiencing realistic biodiversity declines could be lower than previous studies of randomized species losses have indicated. Understanding how functional traits interact with increasingly variable climatic conditions is critical to predicting ecosystem responses to the concurrent challenges of species loss and climate change.

ECOLOGY

refs. 2 and 19). If response and effect traits are strongly aligned, environmental changes and their effects on plant species could yield predictable effects on ecosystem functioning (20). For example, species responses to increased soil fertility (via response traits) should be positively correlated with productivity (via effect traits) (21). In contrast, weak relationships between response and effect traits reduce our ability to predict how environmental changes will impact the direction and magnitude of changes in ecosystem processes. For example, traits related to regeneration (e.g., dispersal and fecundity) strongly determine a species' response to environmental stress but are more weakly linked to ecosystem processes (20). Thus, the loss of an entire response group may have limited impacts on a given ecosystem process. Arid systems provide an example of a weak relationship between response and effect traits: in these systems, plant species use contrasting strategies to deal with water stress (e.g., stress avoidance versus tolerance; ref. 22), resulting in communities with a wide range of life forms (e.g., woody or herbaceous) and phenologies. This, in turn, results in a wide range of impacts on ecosystem processes via the timing and magnitude of resource acquisition and tissue senescence (reviewed in ref. 23). Experiments incorporating realistic species loss designs across multiple years with varying environmental conditions are necessary to shed light on how response and effect traits mediate the influence of species losses on ecosystem functioning.

We conducted a multiyear plant biodiversity manipulation experiment that compared a drought-based, realistic species loss scenario with randomized species losses. This study had two overarching goals. First, we aimed to understand how communities shaped by trait-based species losses function under varying environmental conditions. Do lower-diversity communities function better under the environmental conditions that led to diversity loss? Specifically, does filtering of response traits by drought lead to differences in ecosystem functioning under conditions of low versus high water availability? The answer to this question will help clarify how future realistic depauperate communities might function under future realistic climatic conditions. Second, we aimed to compare the functioning of communities subjected to trait-based filtering to

the functioning of communities that lost diversity at random. These results will help clarify how previous randomized biodiversity experiments might inform predictions of ecosystem functioning in real-world biodiversity loss scenarios. In addition, this comparison allowed us to isolate the relationship between response and effect traits by understanding how the loss of traits versus the loss of species numbers influences ecosystem functioning.

We examined the relationship between biodiversity and ecosystem functioning across a range of soil depths and across years that differed widely in precipitation. We examined two ecosystem functions—aboveground productivity (measured as peak aboveground biomass) and invasion resistance (measured as the inverse of peak exotic biomass)—selected for comparability with the existing body of BEF research and for their relevance to grassland ecosystem services. In addition, we quantified 12 functional traits for each of our experimental species. We hypothesized that if response and effect traits are aligned, communities formed by a realistic, drought-based diversity loss scenario should function at higher levels under water-limited conditions (low rainfall, shallower soil depth) than those generated by a randomized species loss scenario. We expected the inverse pattern if response and effect traits are not aligned.

This study was conducted in a native-dominated serpentine grassland near San Jose, California, an annual-dominated system in which spatial heterogeneity (e.g., soil depth) and interannual climatic variability (especially rainfall) strongly shape community and ecosystem dynamics (24). Previous studies in this ecosystem type have demonstrated that small-scale variation in soil depth strongly influences functions such as plant growth (25), with deeper soils generally thought to provide greater access to resources such as water and nutrients (26). Coastal California experiences high interannual variation in climate conditions, with increasingly variable conditions predicted in the coming years as a result of climate change (27, 28). This interannual climate variation, most notably in rainfall, strongly influences plant community dynamics including invasibility (24, 29), an increasing threat to the conservation of the endemic-rich serpentine flora.
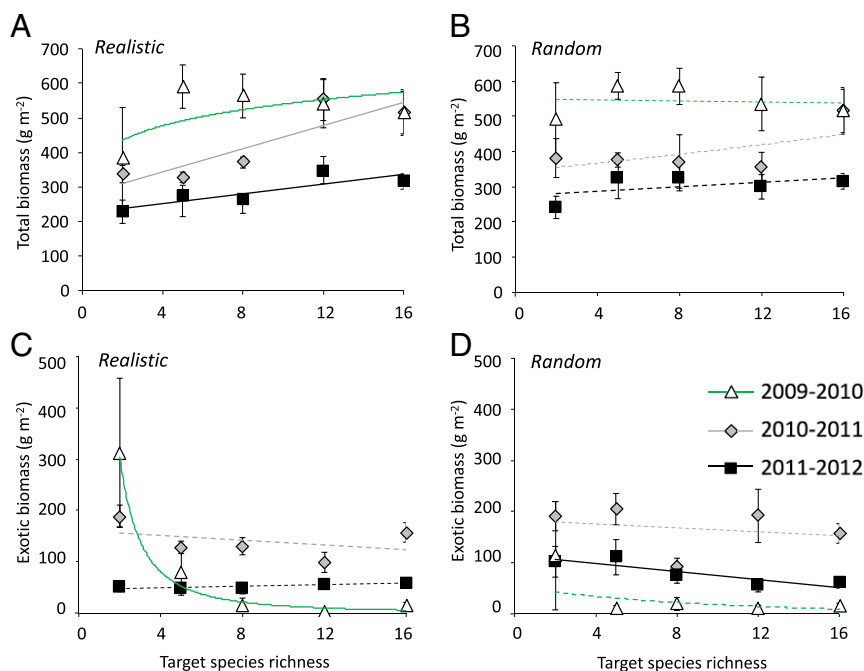

Fig. 1. Total or exotic biomass by target species richness across three growing years for (*A*) total biomass for plots in the realistic species loss scenario, (*B*) total biomass for plots in the random species loss scenario, (*C*) exotic biomass for plots in the realistic species loss scenario, and (*D*) exotic biomass for plots in the random species loss scenario. Values are means ± SE. Solid lines denote significant relationships, while dashed lines denote nonsignificant relationships.

We manipulated species richness, from 16 down to 2 species, in two ways. In one set of replicated plots, species composition at each species richness level was randomized; in another set of replicated plots, species composition in progressively less species-rich plots followed a realistic loss scenario based on long-term, drought-driven species loss observed over several decades at our field site (10).

Results

Cumulative rainfall during the growing season (October to April), as recorded by an on-site weather station, differed substantially among the three growing years included in our study (*SI Appendix,* Fig. S1). Total rainfall was 589 mm for 2009-2010 (118% of the 30-y average), 492 mm for 2010-2011 (90% of average), and 260 mm for 2011-2012 (41% of average).

Total peak aboveground biomass, a proxy for productivity, was significantly influenced by species richness ($F_{1, 200} = 13.33$, $P < 0.001$), soil depth ($F_{2, 6} = 5.08$, $P = 0.05$), and growing year ($F_{2, 200} = 39.68$, $P < 0.001$; Figs. 1 *A* and *B* and 2 *A* and *B*). Productivity increased with increasing species richness and with increasing soil depth. Across years, productivity was highest in 2009-2010 and lowest in 2011-2012. The effect of species richness on productivity was significantly different between the species loss scenarios (loss scenario by richness interaction, $F_{1, 200} = 7.81$, $P = 0.006$; Fig. 1*A* compared to Fig. 1*B*). Specifically, reductions in species richness led to a decline in productivity only in the realistic loss scenario. Based on extracted simple slopes within the realistic species loss scenario, productivity declined by 12.7 ± 3.3 g $\cdot$ m^{-2} per species lost or about 3% of the average productivity in the highest-richness plots; a 50% decline in species richness led to a 23% decline in productivity, on average. There was no significant reduction in productivity with species richness declines in the randomized loss scenario; based on simple slope extraction, productivity decline per species lost in the randomized loss scenario was not different from zero (0.86 ± 3.3 g $\cdot$ m^{-2}).

Invasion resistance was significantly influenced by species richness ($F_{1, 200} = 6.45$, $P = 0.01$) and growing year ($F_{2, 200} = 143.4$, $P \leq 0.001$; Figs. 1 *C* and *D* and 2 *C* and *D*). Exotic biomass increased as species richness declined and was highest in 2010-2011.

The effects of soil depth on exotic biomass were mediated by species richness (depth by richness interaction $F_{2, 200} = 3.70$, $P = 0.03$). In lower-diversity plots, exotic biomass was higher in deeper soils than in shallow soils. In high-diversity plots, by contrast, soil depth had little effect on exotic biomass. Lastly, there was a significant three-way interaction between species loss scenario, species richness, and growing year on invasion resistance ($F_{2, 200} = 5.41$, $P = 0.007$). In 2009-2010, invasion resistance decreased with decreasing richness in the realistic loss scenario ($F_{1, 29} = 5.72$, $P = 0.02$) but not in the random loss scenario ($F_{1, 28} = 0.003$, $P = 0.96$). In 2011-2012, the pattern reversed: invasion resistance decreased with decreasing richness in the random loss scenario ($F_{1, 27} = 6.70$, $P = 0.02$) but not in the realistic loss scenario ($F_{1,29} = 1.22$, $P = 0.28$; see green trendline versus black trendline in Fig. 1*C* versus Fig. 1*D*).

Species in the realistic, low-diversity treatments tended to have trait values consistent with drought tolerance and water acquisition (high values for root to shoot biomass ratio, specific root length, root depth, leaf mass per unit area [LMA], and seed mass) at the expense of traits associated with carbon assimilation (low values for photosynthetic rate, leaf nitrogen [N] concentration, and light-use efficiency; *SI Appendix,* Table S2). The first axis of the principal component analysis (PC1) aligned with growth rate strategies (*SI Appendix,* Fig. S5A). Specifically, species with high PC1 scores had traits associated with rapid growth rates, including high values for leaf N concentration, light-use efficiency, and photosynthetic rate. The second principal component (PC2) was associated with root to shoot biomass ratio, LMA, and seed mass. Drought-tolerant species (those that persisted at progressively lower richness levels in our realistic loss scenario) tended to have high PC2 scores, corresponding with greater investment in seed resources, leaf structure, and root biomass (*SI Appendix,* Fig. S5B).

To assess the role of PC1 and PC2 as functional response traits, we examined how community-weighted mean PC1 (cwmPC1) and community-weighted mean PC2 (cwmPC2) changed with species richness in the two different species loss scenarios. While cwmPC1 varied by species richness similarly across the two loss scenarios (species richness effect $F_{1, 212} = 10.96$, $P = 0.001$; Fig. 3 *A* and *B*), cwmPC2 showed strong differences across the loss scenarios
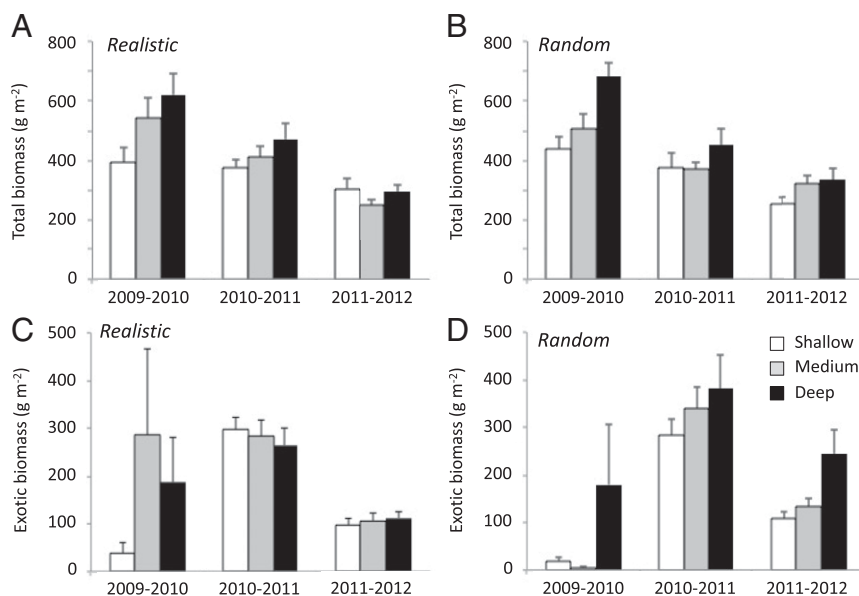

Fig. 2. Total or exotic biomass by soil depth treatment across three growing years for (*A*) total biomass for plots in the realistic species loss scenario, (*B*) total biomass for plots in the random species loss scenario, (*C*) exotic biomass for plots in the realistic species loss scenario, and (*D*) exotic biomass for plots in the random species loss scenario. Values are means ± SE.

Wolf et al.
Trait-based filtering mediates the effects of realistic biodiversity losses on ecosystem functioning

PNAS | 3 of 7
https://doi.org/10.1073/pnas.2022757118

(Fig. 3 *C* and *D*). Community-weighted PC2 strongly decreased with increasing species richness in the realistic loss scenario only (species richness by loss scenario interaction, $F_{1, 212} = 33.07$, $P < 0.001$; Fig. 3 *C* and *D*), supporting the role of PC2 as representing a suite of response traits for drought-driven species loss.

To assess the role of PC1 and PC2 as functional effect traits, we examined how cwmPC1 and cwmPC2 correlated with ecosystem functioning (productivity and invasion resistance). Total aboveground biomass was correlated with both cwmPC1 and cwmPC2 (Fig. 4 *A* and *B*). The correlation shifted across growing years for cwmPC1 (growing year by cwmPC1 interaction, $F_{1, 212} = 15.82$, $P < 0.001$), while cwmPC2 was negatively correlated with total aboveground biomass in all growing years ($F_{1,212} = 31.22$, $P < 0.001$). Invasion resistance, however, was uncorrelated with cwmPC1 in two growing years (growing year by cwmPC1 interaction, $F_{1, 212} = 4.13$, $P = 0.04$; Fig. 4*C*) and uncorrelated with cwmPC2 in all growing years (Fig. 4*D*). Thus, PC2 reflects a suite of functional response traits to drought and reflects a suite of functional effect traits inversely correlated with one ecosystem function, productivity, but uncorrelated with another ecosystem function, invasion resistance.

Discussion

Species assemblages filtered by a certain driver (in this case, drought) did not always generate higher ecosystem functioning under the environmental conditions that caused species loss. Response and effect traits in our realistic, drought-driven species loss scenario were aligned for productivity but not for invasion resistance. That is, the traits that were filtered by drought were the same traits that influenced biomass but were not the same traits that influenced invasion resistance. Across all years, realistic species loss consistently led to larger declines in productivity than randomized species loss, regardless of climatic conditions. The response of invasion resistance, however, changed across years: realistic, drought-driven diversity losses led to higher invasion

resistance under drought conditions but reduced invasion resistance in wetter years. Under lower-water conditions (shallow soil or low precipitation), species assemblages with traits indicative of drought tolerance were less productive than random plant assemblages. This underscores the importance of testing biodiversity loss effects on multiple relevant functions (30, 31) across a variety of environmental conditions (2).

The finding that realistic species losses reduced productivity more than randomized species losses in all three contrasting years appears to be driven by the consistent inverse relationship between a suite of drought tolerance traits (cwmPC2) and productivity, even across years with very different climatic and rainfall conditions. Response traits and effect traits for productivity appear to be aligned, with drought-resistance traits leading to consistently lower productivity. There was a larger effect of realistic species loss on productivity than of random species loss, suggesting that the large body of randomized loss BEF experiments may underestimate the loss of ecosystem functioning. Based on a meta-analysis of mostly randomized loss BEF experiments (4), a 50% decline in species richness reduced biomass production by 13% on average, while a 50% richness decline across three years in our realistic species loss scenario led to an average 23% reduction in biomass productivity.

In contrast to the consistency of our productivity results, we found high interannual variability of invasion resistance responses to the different species loss scenarios. In the highest rainfall year (2009-2010), realistic species losses reduced invasion resistance, while randomized species losses did not affect invasion resistance. This pattern reversed in the lowest rainfall year (2011-2012), with realistic species losses having no effect on invasion, while randomized species losses led to reduced invasion resistance. Our trait data demonstrate that functional response and effect traits are not aligned for this ecosystem function. Invasion resistance was almost entirely uncorrelated with any of our measured traits, suggesting that unmeasured traits or higher-order interactions
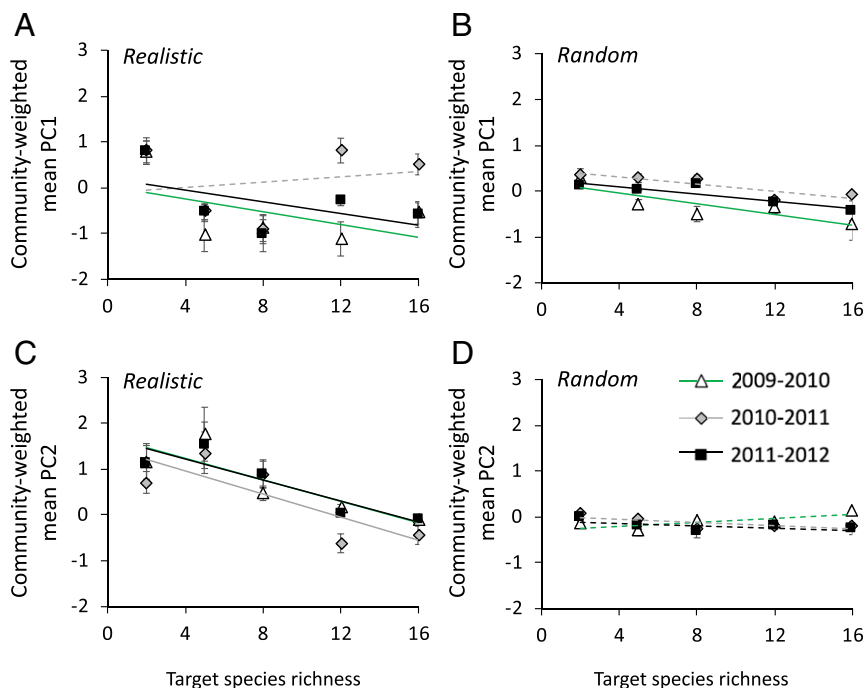

Fig. 3. Analysis of the PC1 and PC2 suite of traits as functional response traits to the realistic or randomized diversity loss scenarios. Figures show the correlation between (*A*) target species richness and cwmPC1 in the realistic loss scenario, (*B*) target species richness and cwmPC1 in the random loss scenario, (*C*) target species richness and cwmPC2 in the realistic loss scenario, and (*D*) target species richness and cwmPC2 in the random loss scenario. Values are means ± SE. Solid lines denote significant relationships, while dashed lines denote nonsignificant relationships.
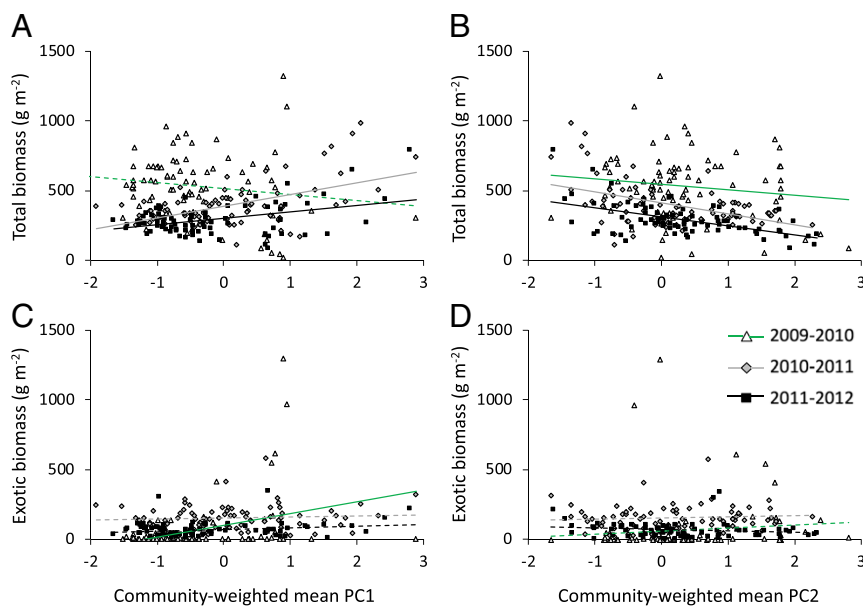
Fig. 4. Analysis of the PC1 and PC2 suite of traits as functional effect traits in determining ecosystem functioning. Figures show the correlation between (*A*) cwmPC1 and total biomass, (*B*) cwmPC2 and total biomass, (*C*) cwmPC1 and exotic biomass, and (*D*) cwmPC2 and exotic biomass. Values are means ± SE. Solid lines denote significant relationships, while dashed lines denote nonsignificant relationships.

between traits, species, and environmental conditions influence invasion resistance. Indeed, the relationship between bare ground and invasion resistance reversed between wet and dry years. In 2009-2010, the highest rainfall year, invasive plants produced significantly more biomass in plots with more bare ground (10), while in 2011-2012, the lowest rainfall year, this pattern reversed and invasive plants produced significantly less biomass in plots with more bare ground (*SI Appendix*, Fig. S8). This suggests that the determinants of invasion success in this system shift with changes in climactic conditions, a dynamic that was not captured by our trait analyses.

The effects of spatial environmental heterogeneity have rarely been examined in previous BEF studies (10, 16, 26). Our results demonstrate that the effects of soil depth, which influences nutrient and water availability (26), are not consistent across years and are mediated by climatic conditions. Additionally, soil depth affected the realistic and randomized species loss scenarios differently, again illustrating that the composition of the plant community influences the effects of variable resource availability on ecosystem functioning. Contrary to previous findings (26), we found no effect of soil depth on the relationship between species richness and productivity. However, diversity effects on invasion resistance were stronger in plots with deeper soil, such that low-diversity plots with deeper soil had substantially lower resistance to invasion. This was likely due to invasive species taking advantage of the increased resource availability in deeper plots.

There has long been interest in understanding how diversity affects ecosystem stability over time (2). Previous randomized species loss experiments (32) found that higher-diversity communities had higher stability over a range of years with varying climatic conditions. While we lack sufficient experimental duration to quantify the effects of diversity on the stability of ecosystem functioning, our results suggest a trend. For invasion resistance, but not productivity, realistic species losses appeared to cause wider interannual variation in functioning than did randomized losses. This raises the possibility that biodiversity–stability effects observed in previous studies would be more pronounced under realistic species losses. Additionally, a meta-analysis (33) found that increasing stress has a stronger negative effect on ecosystem

functioning in low-diversity communities resulting from randomized species loss. In contrast, we found no relationship between the environmental stress imposed by rainfall limitation and the effect of diversity on productivity. Our results for invasion resistance also suggest that reduced water stress (higher water availability) can increase the negative effects of realistic species losses on ecosystem functioning. This is consistent with invasion theory, which posits that opportunistic invasions are more likely under high-resource conditions when particular niches are left unfilled (34, 35). The trait-based filtering that leads to realistic, lower-diversity communities is likely to lead to such vacant niches.

Rainfall is likely to be the main driver of differences among experimental years in this study, though it is possible that another factor or combination of factors contributed to interannual variation. Rainfall is widely recognized as a primary factor limiting productivity in this and other Mediterranean ecosystems worldwide (24, 29, 36, 37). In addition, our on-site measurements of potential evapotranspiration, insolation, and growing degree days have lower variation across years than rainfall (*SI Appendix*, Figs. S1–S4), though we cannot rule out the influence of these or other abiotic factors on productivity or invasion resistance. Additionally, experimental artifacts may contribute to interannual differences observed in ecosystem functioning (38). However, our plant community consisted of annuals seeded in each year and mature herbaceous perennials, while previous studies (38) demonstrating a maturation effect have largely attributed it to longer-lived woody species and community change over time. The absence of a directional relationship between experimental year and either absolute or proportional perennial biomass (*SI Appendix*, Figs. S6 and S7) suggests that the experimental artifacts of plant communities "maturing" over the course of the experiment were minimal.

Increasing climatic variability is a hallmark of global change predictions. If biodiversity loss due to drought proceeds as we have inferred, the resulting plant communities will experience increasingly variable climatic conditions, including both high and low rainfall periods. By examining multiple aspects of environmental variation (soil depth and interannual climatic variability) in concert with drought-driven and randomized species losses, this study contextualizes our understanding of realistic species loss. While

Wolf et al.
Trait-based filtering mediates the effects of realistic biodiversity losses on ecosystem functioning

PNAS | 5 of 7
https://doi.org/10.1073/pnas.2022757118

some previous studies have found that realistic species losses increase BEF effect sizes relative to randomized species losses, we illustrate how environmental context can affect the magnitude and direction of ecosystem responses to realistic species loss. Realistic, low-diversity communities do not always support high ecosystem functioning under the conditions that caused species loss and demonstrate a disconnect between functional response and effect traits. The degree of alignment between response and effect traits is a critical factor in predicting the effects of biodiversity loss on ecosystem functioning. Assessing these effects, across a range of key ecosystem functions and climatic conditions, is necessary for predicting the future consequences of multiple, concurrent global changes.

Materials and Methods

Study Site. This experiment was conducted at Coyote Ridge, a serpentine grassland near San Jose, California (37°15′ N, 121°45′ W). The climate is typical of Mediterranean ecosystems, with an October to April growing season during which an average of 541 mm rain falls. A weather station at the experimental site recorded rainfall, temperature, incoming solar radiation, and potential evapotranspiration during the three experimental growing years: 2009-2010 (10), 2010-2011, and 2011-2012. Further details of the experimental design can be found in the *SI Appendix* and in Selmants et al. (10).

Experimental Design. Within an 800-m^2 fenced area, individual experimental plots (circular, 58 cm diameter) were arranged into nine blocks. Soil depth for each block was characterized as shallow (10 to 15 cm), medium (15 to 30 cm), or deep (>30 cm); three replicate blocks represented each of the three soil depths. Within each block, plots were randomly assigned a planted species diversity based on one of two loss scenarios, realistic or random, consisting of 2, 5, 8, or 12 species. Each block contained one representative of each diversity by loss scenario combination, consisting of eight plots. In addition, each block contained one plot containing the full set of 16 experimental species [species names follow Baldwin et al. (39); Table 1]. All plots had an equal number of target individuals per plot; the abundance of each species varied by diversity level and was based on observed field abundances of the different experimental species (*SI Appendix*, Table S1). The realistic loss scenario used in this experiment (Table 1) is based on nestedness analysis of a 19-y dataset quantifying plant species presence and absence on Coyote Ridge and is based on drought-based diversity loss (*SI Appendix*).

Seeds for each of the 16 target species were collected within 2 km of the experimental site from April through September preceding each experimental growing year. We also excavated and transplanted perennating organs from four perennial species (*Stipa pulchra*, *Chlorogalum pomeridianum*, *Muilla maritima*, and *Calystegia subacaulis*) from within 500 m of the experimental

site in order to allow full functional contributions of these perennial species to the experiment. Perennating organs were transplanted in late October of each year; seeds were scattered on plots in early November, with the specific date each year corresponding to the predicted onset of the first soaking rain. Beginning when seedlings were identifiable to species, nontarget native species were weeded from each plot; invasive species (*Bromus hordeaceus* and *Festuca perennis*) were not weeded out of plots.

Peak aboveground biomass (which in this ecosystem corresponds to aboveground net primary productivity because all aboveground tissue dies back at the end of each growing season) was assessed in April of each year. Between one-sixth and one-third of each plot was harvested each year. All plants within the harvest area were clipped at soil level and sorted to species. Harvested biomass was dried and massed to determine species-specific biomass by plot. Native biomass was determined as the sum of all native species harvested within a plot; exotic biomass was determined as the sum of the two exotic species found in the plots. Total aboveground biomass was defined as the sum of native and exotic biomass, standardized by harvested area (g · m^{-2}).

Functional Trait Survey. Functional traits were measured from January to April 2010 on plants growing in monoculture at the experimental site. We measured traits corresponding to carbon capture strategy (photosynthetic capacity and LMA), water and nitrogen acquisition and use (water-use efficiency, photosynthetic nitrogen-use efficiency, leaf nitrogen concentration, root depth, specific root length, and root to shoot biomass ratio), light acquisition and use (light-use efficiency and height), and seed mass (which is often positively correlated with seedling performance in low resource environments; reviewed in ref. 40). Physiological and chemical analyses were performed on recently mature leaves from five individuals per species. Details about the measurements can be found in Funk and Wolf (41). Principal components analysis was used to explore major axes of plant function across species. cwmPC1 and cwmPC2 were calculated using species-specific biomass measurements for the target (noninvasive) species in each plot individually. Species scores for PC1 and PC2 were regressed against richness level to examine which suites of traits were more prevalent in species selected for low-diversity treatments under realistic species loss predictions.

Data Analysis. To analyze the effects of our experimental treatments on ecosystem functioning across years, we ran mixed-effects models with loss scenario (realistic or random), species richness (2 to 16 species), soil depth (shallow, medium, or deep), and growing year (2009-2010, 2010-2011, and 2011-2012) as the fixed main effects as well as all possible interactions between those variables. The response variable was either total aboveground biomass or exotic biomass, and block was included as a random effect. To examine the predictive power of cwmPC1 and cwmPC2 on ecosystem functioning across years, we ran a very similar mixed-effects model, substituting

Table 1. List of species used in this experiment and the lowest diversity mixed-species plots in which each species was included in the realistic loss order

Species	Lowest realistic diversity level	Growth form
Stipa pulchra	2	Perennial grass
Chlorogalum pomeridianum	2	Perennial forb
Calystegia subacaulis	5	Perennial forb
Muilla maritima	5	Perennial forb
Plantago erecta	5	Annual forb
Lessingia nemaclada	8	Annual forb
Festuca microstachys	8	Annual grass
Microseris douglasii	8	Annual forb
Lasthenia californica	12	Annual forb
Hesperevax sparsiflora	12	Annual forb
Eschscholzia californica	12	Perennial forb
Calandrinia ciliata	12	Annual forb
Layia gaillardioides	16	Annual forb
Acmispon wrangelianus	16	Annual forb (N-fixing)
Cryptantha flaccida	16	Annual forb
Hemizonia congesta	16	Annual forb

In the realistic loss order used in this experiment, species in the lowest diversity plots persist under drought conditions, while species in the highest-diversity plots disappear under drought conditions. Each species was also grown in monoculture. Species names follow Baldwin et al. (39).

cwmPC1 or cwmPC2 for species richness. Again, the response variable was either total aboveground biomass or exotic biomass. To investigate the role of PC1 and PC2 as response traits, we ran mixed-effects models with loss scenario, species richness, soil depth, and growing year as the fixed main effects as well as all possible interactions between those variables; block was included as a random effect, and cwmPC1 and cwmPC2 were the response variables. Because there was only one 16-species plot per block, each 16-species plot was randomly assigned to either the random or the realistic species loss scenario, such that $n = 4$ for the random 16-species plots and $n = 5$ for the realistic 16-species plots. All other loss scenario/richness combinations were represented by nine plots each. To meet assumptions of normality and homoscedasticity, total biomass was square-root transformed, and exotic biomass was $\ln(x + 1)$ transformed for mixed-effects models.

When we found significant two- or three-way interactions between predictor variables on total biomass or exotic biomass, we further examined effects separately by growing year, loss scenario, or depth with linear mixed-effects models. We extracted simple slopes to examine the influence of one effect while holding others constant; these are reported as mean effect estimate ± SE. When we found significant interactions between cwmPC1 and year for predicting ecosystem functions, we examined those relationships separately by year.

All analyses were performed in R 3.6.0 (42) with an alpha value of $P < 0.05$ for all significance tests. Mixed-effects models were run using the nlme package (43), simple slopes were extracted using the reghelper package (44), and the principal components analysis was completed with the princomp function. Data shown in figures are not transformed and represent mean values ± SE. A Grubb's test for outliers was performed on total biomass and exotic biomass by growing year; this test did indicate that there was one significant outlier per year for exotic biomass. All tests were rerun excluding the outliers, and results did not qualitatively change. We expect these data points fall within the range of possible biological variation at our field site and have no reason to suspect these outliers were caused by methodological or experimental errors, so we have opted to leave the data points in for the final analyses presented here.

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ECOLOGY

Wolf et al.
Trait-based filtering mediates the effects of realistic biodiversity losses on ecosystem functioning

PNAS | 7 of 7
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