

Realistic diversity loss and variation in soil depth independently affect community-level plant nitrogen use

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Abstract. Numerous experiments have demonstrated that diverse plant communities use nitrogen (N) more completely and efficiently, with implications for how species conservation efforts might influence N cycling and retention in terrestrial ecosystems. However, most such experiments have randomly manipulated species richness and minimized environmental heterogeneity, two design aspects that may reduce applicability to real ecosystems. Here we present results from an experiment directly comparing how realistic and randomized plant species losses affect plant N use across a gradient of soil depth in a native-dominated serpentine grassland in California. We found that the strength of the species richness effect on plant N use did not increase with soil depth in either the realistic or randomized species loss scenarios, indicating that the increased vertical heterogeneity conferred by deeper soils did not lead to greater complementarity among species in this ecosystem. Realistic species losses significantly reduced plant N uptake and altered N-use efficiency, while randomized species losses had no effect on plant N use. Increasing soil depth positively affected plant N uptake in both loss order scenarios but had a weaker effect on plant N use than did realistic species losses. Our results illustrate that realistic species losses can have functional consequences that differ distinctly from randomized losses, and that species diversity effects can be independent of and outweigh those of environmental heterogeneity on ecosystem functioning. Our findings also support the value of conservation efforts aimed at maintaining biodiversity to help buffer ecosystems against increasing anthropogenic N loading.

Key words: *biodiversity; ecosystem functioning; environmental heterogeneity; nonrandom diversity loss; nutrient cycling; resource use; species loss.*

INTRODUCTION

Accelerating declines in biodiversity at local and global scales have prompted concern that species losses will impair the functioning of natural ecosystems (Chapin et al. 2000, Butchart et al. 2010). Support for this “biodiversity–ecosystem–functioning” hypothesis appears abundant; numerous manipulative experimental studies over the past 20 years have demonstrated that species richness has a positive effect on ecosystem processes such as productivity and resource capture (as reviewed in Hooper et al. 2005, Cardinale et al. 2011). The strength of this evidence has led some to suggest, for example, that conservation of biodiversity might be an effective tool to reduce runoff and leaching of excess anthropogenic nitrogen (N) from watersheds (Hooper et al. 2005). Recently, however, a number of authors have raised concerns about whether results from highly controlled biodiversity–ecosystem–functioning (BEF) experiments are applicable to real-world conservation

and management efforts (Srivastava and Vellend 2005, Naeem 2008, Duffy et al. 2009).

One factor that may limit the relevance of most BEF experiments to real ecosystems is that nearly all BEF manipulations randomly assign species to each richness level (Srivastava and Vellend 2005, Duffy et al. 2009). Random loss designs were essential to the first generation of BEF experiments, because their goal was to isolate the effect of species richness on ecosystem functioning and avoid the confounding effects of species composition (Huston 1997, Duffy 2009). However, random loss designs necessarily assume that all species are equally susceptible to local extinction, which contrasts with the nonrandom diversity losses observed in natural ecosystems (Grime 2002, Raffaelli 2004, Zavaleta et al. 2009). Local extinctions are nonrandom because species vary in their susceptibility to particular environmental stressors, leading to nested patterns of species occurrences along gradients of diversity (Zavaleta and Hulvey 2004, Bracken et al. 2008) and nested species losses through time in declining communities (Zavaleta et al. 2009). Because species losses are biased toward certain types of species, the real-world consequences of human-caused biodiversity losses could differ systematically from the consequences of randomized biodiversity changes typical of most experimental BEF studies.

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Most experimental BEF studies also minimize or eliminate variation in environmental heterogeneity (Huston 1997, Grime 2002, Cardinale et al. 2011) to avoid the confounding effects of “hidden treatments” (Huston 1997). Yet increasing environmental variation can strengthen biodiversity effects on ecosystem functioning (Dimitrakopoulos and Schmid 2004, Griffin et al. 2009, Cardinale 2011), presumably because increasing biotope space provides more niche opportunities and allows species assemblages to exploit resources more completely (Colwell and Rangel 2009). Alternatively, an increase in biotope space may correspond with an overall increase in resource capital, potentially resulting in an independent effect of resource availability that equals or exceeds the effect of biodiversity on ecosystem functioning (Duffy 2009).

Although there is growing evidence that realistic species-loss scenarios have distinct functional consequences from randomized loss scenarios (Solan et al. 2004, Zavaleta and Hulvey 2004, Bunker et al. 2005, Bracken et al. 2008, Isbell et al. 2008, Selmants et al. 2012) and that environmental heterogeneity may affect the magnitude of biodiversity effects on ecosystem functioning (Dimitrakopoulos and Schmid 2004, Healy et al. 2008, Tylianakis et al. 2008, Griffin et al. 2009, Cardinale 2011), no study has yet combined these two more-realistic design elements in one experiment. In particular, this more realistic approach could greatly inform controls over ecosystem-level N uptake and storage, which are sensitive to the independent effects of species richness (Hooper and Vitousek 1998, Scherer-Lorenzen et al. 2003, van Ruijven and Berendse 2005, Fornara and Tilman 2009), species loss order (Bracken et al. 2008), and environmental heterogeneity (Reynolds et al. 1997, Gundale et al. 2006, Cardinale 2011). A better understanding of how these factors might interact to influence plant N assimilation is essential because plant uptake is a major driver of N cycling and storage in terrestrial ecosystems (Hooper et al. 2005) and can reduce excess N loading in streams and rivers, a leading cause of eutrophication and degraded water quality (Dodds 2006, Galloway et al. 2008).

Here we present the results of a field-based experiment directly comparing the response of community-level plant N use to realistic and randomized plant species losses across a gradient of soil depth in a native-dominated serpentine grassland of high conservation value in California, USA. Our realistic species loss scenario reflects plant species sensitivity to prolonged drought, which is particularly relevant given climate model predictions of increased periods of persistent drought across the United States over the next 20–50 years (Dai 2011). By incorporating systematic variation in soil depth into our design, we can examine for the first time whether increasing vertical heterogeneity of the rooting environment modifies the biodiversity–ecosystem-functioning relationship in a realistic species

loss order scenario. Our design also allows us to compare effect sizes of diversity, soil depth, and diversity–soil-depth interactions on ecosystem functioning in realistic and randomized species loss scenarios.

MATERIALS AND METHODS

Study site and experimental design

Coyote Ridge is a native-dominated grassland reserve in Santa Clara County, California, USA, that contains several plant species listed by the U.S. Fish and Wildlife Service as threatened or endangered. The study area is located on a 3000-ha serpentine outcrop in the central Coast Range of California and experiences the warm, dry summers and cool, wet winters typical of this Mediterranean climate. Mean monthly air temperature was $\sim 14.5^{\circ}\text{C}$ and total precipitation was 570 mm during the November 2009–April 2010 growing season based on data from an on-site weather station installed 18 March 2009.

Our experimental site was an 800-m² area on a relatively flat ridge top at 356 m elevation. This area was chosen for its large variation in soil depth within a small area after a comprehensive survey using a graduated cylindrical steel rod (1 m long, 1 cm diameter) to systematically measure soil depth (Selmants et al. 2012). We located three replicate blocks at each of three depths: shallow (10–15 cm), medium (15–30 cm), and deep (>30 cm). The experimental area was fenced above- and belowground to exclude cattle, gophers, pigs, and other large mammals and the area between blocks was mowed in April 2009 to minimize seed rain. Within each plot, we removed standing dead biomass by hand and plant perennating organs (i.e., bulbs, rhizomes) by excavation with a trowel down to a depth of 10 cm.

Each of the nine blocks contained plots randomly assigned to each of two loss orders (defined in detail in *Realistic and randomized loss orders*), one realistic and one random, each with 2, 5, 8, and 12 species. Each block also contained a plot with the full experimental species pool (16 species), a bare plot (no vegetation), and between five and seven monoculture plots such that each species was grown in monoculture in one shallow, one medium, and one deep soil block.

We collected native plant seeds from within 2 km of the experiment site at Coyote Ridge between April and September of 2009. We separated all seeds from attached fruiting material and stored them air dry at room temperature until planting. In addition to collecting seeds, we also excavated perennating organs of four perennial species (*Stipa pulchra*, *Chlorogalum pomeridianum*, *Calystegia subacaulis*, and *Muilla maritima*; see Table 1) from within 500 m of the experiment site and transplanted them into their assigned plots so that newly germinated individuals did not solely represent the functional contribution of these long-lived species. We transplanted perennating organs during the

TABLE 1. Species composition and target number of individuals per plot at each richness level in the realistic loss order treatment.

Species	Richness level (no. species)					Active season	Functional group
	2	5	8	12	16		
<i>Stipa pulchra</i>	324	158	72	47	45	late	perennial grass
<i>Chlorogalum pomeridianum</i>	48	24	9	7	7	late	perennial forb
<i>Calystegia subacaulis</i>		23	18	15	14	late	perennial forb
<i>Muilla maritima</i>		20	10	7	8	early	perennial forb
<i>Plantago erecta</i>		146	132	81	64	early	annual forb
<i>Lessingia nemaclada</i>			11	7	6	late	annual forb
<i>Festuca microstachys</i>			87	61	42	early	annual grass
<i>Microseris douglasii</i>			33	19	14	early	annual forb
<i>Lasthenia californica</i>				55	67	early	annual forb
<i>Hesperivax sparsiflora</i>				67	71	early	annual forb
<i>Eschscholzia californica</i>				5	4	early	perennial forb
<i>Calandrinia ciliata</i>				4	2	early	annual forb
<i>Layia gaillardiodes</i>					8	early	annual forb
<i>Acemispom wrangelianus</i>					9	early	annual N-fixer
<i>Cryptantha flaccida</i>					6	early	annual forb
<i>Hemizonia congesta</i>					7	late	annual forb
Total	372	372	372	372	372		

Notes: Plant species are listed in reverse order of their loss position (adapted from Selmants et al. [2012]), which corresponds (for the first 12 species) to the order in which nested subset analysis of 1991 occurrence data placed them to maximally nest the site-by-species matrix. The last four species listed were present in other years but entirely missing from the site in the 1991 end-drought year.

first two weeks of October 2009 and hand scattered seeds in each plot on 5 November 2009.

Realistic and randomized loss orders

For a loss order to be realistic, we argue that it must be both nested (i.e., that less species-rich assemblages are progressively nested subsets of more species-rich assemblages) and based on data indicating a likely order of species loss from species-rich to species-poor assemblages. We generated our realistic species loss order based on within- and across-year variation in plant species presence/absence using vegetation data collected annually at Coyote Ridge from 1991 to 2009 (Selmants et al. 2012). Within each year, we used nested subset analysis to analyze the meta-community for nested structure and identify the best-fit order of species losses from plots of progressively lower diversity (Atmar and Patterson 1993, Zavaleta and Hulvey 2004). We calculated nested temperature using the method of Rodriguez-Girones and Santamaria (2006). Nested temperature is an index of the order within an ecological system ranging from 0 to 100, with low temperature values indicating a high degree of nestedness (Atmar and Patterson 1993, Ulrich et al. 2009). We then generated null model communities to assess the nonrandomness of nestedness patterns (Miklòs and Podani 2004).

A meta-community is perfectly nested if species-poor microsites (quadrats) are subsets of progressively more species-rich microsites, and rare or uncommon species are present only in species rich microsites (Ulrich et al. 2009). In general, rare and uncommon species are at greater risk of local extinction due to their small population sizes (Pimm et al. 1995), and nonrandom, nested patterns of species occurrences along diversity gradients result from some species being more suscep-

tible to environmental stressors than others (Bracken and Nielsen 2004). Therefore, a highly nested distribution pattern indicates a predictable order of species loss as one moves from species-rich to species-poor microsites (Zavaleta and Hulvey 2004). By this definition, any realistic species loss order is necessarily specific to a particular environmental stressor or suite of stressors. Different stressors applied to the same meta-community would likely result in different species loss orders.

We based our realistic loss scenario on the nested pattern of species occurrences in 1991, the year with the highest degree of nestedness in the time series (nested temperature = 17.86, $P = 0.01$) and the year immediately following a three-year drought in which annual precipitation remained more than 40% below the 50-year mean (Selmants et al. 2012). Although it followed the most prolonged drought in 50 years at the site, 1991 had only slightly below-average precipitation (16% below the 50-year mean of 567 mm). In addition to the high degree of nestedness, 1991 was the year with the lowest species richness at both the quadrat and site scales in our 19-year data set. Four species present in at least 17 of the 19 years surveyed were absent in 1991: *L. gaillardiodes*, *A. wrangelianus*, *C. flaccida*, and *H. congesta* (see Table 1). We included these four species as the first group to drop out of our realistic loss order (16 species down to 12 species). The rest of the realistic loss order was based on ranks of the top 12 species from the 1991 nested subset analysis for which we could reliably collect seed. We set the minimum richness level at two species because there were no single-species quadrats observed in 1991 or any other year in our data set. The order of species loss in the realistic scenario (see Table 1) is broadly consistent with species sensitivity to drought in other grassland ecosystems, with annual forbs generally more drought

sensitive than annual grasses or perennials (Tilman and El Haddi 1992) and rare and uncommon species most susceptible to drought-induced local extirpation (Albertson and Weaver 1944).

To directly compare our realistic loss order to a random loss scenario, we used the same pool of 16 species (see Table 1) to generate a unique, non-nested randomized set of 2-, 5-, 8- and 12-species assemblages in each of the nine blocks. Species assemblages were constrained to ensure even representation of all 16 species across the nine replicate blocks by stratified random selection with replacement. In other words, a species could not be chosen for inclusion at a particular richness level within the random loss order for a second time until all other species had been chosen for inclusion at that richness level.

Biomass N content and N-use efficiency

We harvested aboveground plant biomass in each plot on two separate occasions, 22 March and 30 April 2010, to ensure that we captured peak biomass across variations in species composition among the plots. In total we harvested an area of 0.09 m² (approximately one-third of the plot area), 0.06 m² in March and 0.03 m² in April. Biomass was sorted by species in the laboratory, dried at 60°C for at least 48 h, and then weighed to determine species-specific biomass in each plot. Biomass from each plot (excluding nonnative species) was then recombined, finely ground in a Wiley mill, and analyzed for total N content on an Elementar vario MAX CNS analyzer (Elementar Analysensysteme, Hanau, Germany). The N content of aboveground biomass is expressed as grams of N per square meter. We calculated N-use efficiency as aboveground biomass produced per unit mass of N (van Ruijven and Berendse 2005, Fornara and Tilman 2009). For simplicity, we excluded nonnative species from our analysis because they were a minor component of total biomass in the majority of plots (Selmants et al. 2012), had low N concentration, and their inclusion did not alter trends or statistical significance of our analyses.

Data analysis

Because there was only one 16-species plot per block, we were unable to include the full range of species richness in a single analysis that incorporates both loss orders. To confirm that biomass N content and N-use efficiency differed in their responses to realistic and randomized species losses, we used analysis of covariance (ANCOVA) to compare the slopes of each loss order scenario using only data from the 2, 5, 8, and 12-species plots in each loss order. When the slopes differed significantly, we conducted separate analyses for each loss order to assess the effects of species richness (2–16 species), soil depth, and their interaction using a univariate general linear model (GLM) with soil depth as a fixed factor and species richness as a covariate (van Ruijven and Berendse 2005). Data were log-transformed

when necessary to satisfy assumptions of normality, linearity, and homoscedasticity. When the main effect of soil depth was significant and there was no interaction with species richness, we used Tukey's HSD as a post-hoc multiple comparisons test among the three depth categories.

Multiple observations at each species richness value allowed us to calculate lack-of-fit error, which is the difference between residual error from the model and pure error from the multiple replicates at each value of species richness. When lack-of-fit error is significantly greater than pure error, it indicates the functional form of the regressor is likely incorrect. Where there was no interaction between species richness and soil depth and the lack-of-fit test for species richness was significant ($P < 0.05$), we fit the untransformed data to linear, decelerating (log), saturating (hyperbolic), and unimodal (second-order polynomial) functions and then used Akaike information criteria corrected for small sample sizes (AIC_c) to judge the relative fits of these functions to the data (Burnham and Anderson 2002). We calculated partial eta squared (η_p^2) for each treatment (species richness, soil depth, and their interaction) in each loss order as an estimate of the effect size of each treatment. The effect size estimator η_p^2 describes the proportion of treatment plus error variance attributable to the treatment, calculated as the ratio of treatment sum of squares (SS_{trt}) to the sum of SS_{trt} and error sum of squares (SS_{err} ; Hughes and Stachowicz 2009).

We compared means of aboveground biomass N content and N-use efficiency of all species grown in monoculture across all soil depths to each richness level in both the realistic and randomized loss orders (there was no significant effect of soil depth on mean monoculture biomass; $F_{2,45} = 1.24$, $P = 0.30$). Differences were considered significant at $P < 0.05$ for all statistical tests. Data were analyzed using JMP version 9 (SAS Institute, Cary, North Carolina, USA).

RESULTS

Biomass nitrogen content

The slope of the relationship between species richness (2–12 species) and the N content of aboveground plant biomass differed significantly between the realistic and randomized species loss orders ($F_{1,68} = 11.83$, $P = 0.001$). When analyzed separately, there was no significant interactive effect of native species richness and soil depth on biomass N content in the realistic loss order ($F_{1,43} = 1.03$, $P = 0.37$), while there was a weak but statistically nonsignificant interactive effect of species richness and soil depth in the randomized loss order ($F_{2,42} = 3.03$, $P = 0.06$). Nitrogen content of aboveground biomass was a positive, log-linear function of species richness in the realistic loss order (Fig. 1a), but there was no relationship between species richness and biomass N content in the randomized loss order (Fig. 1b).

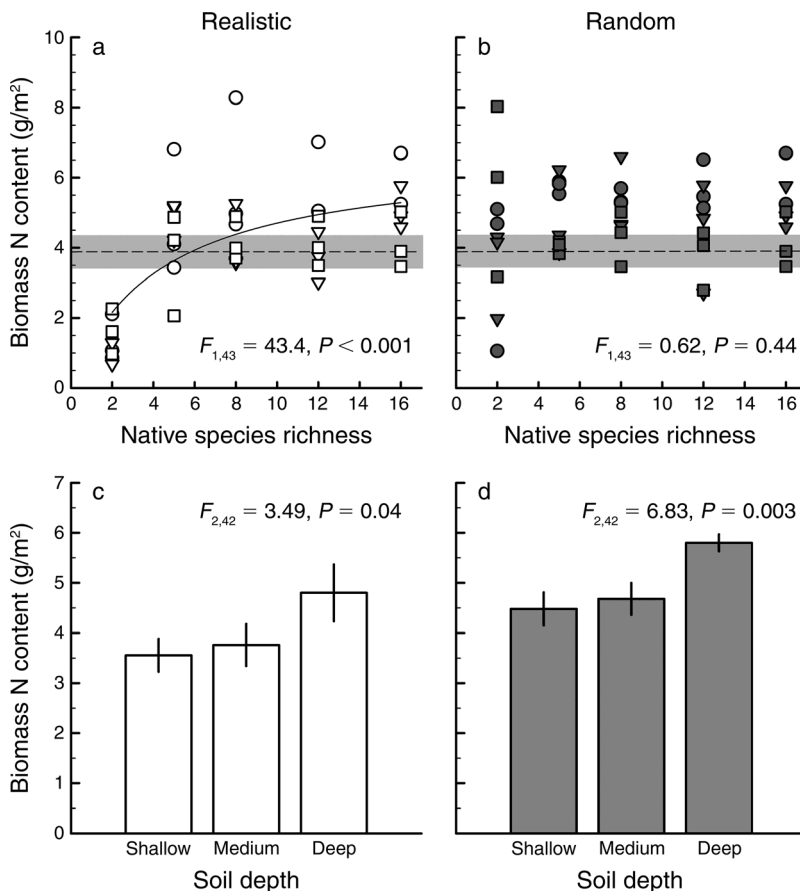


FIG. 1. Response of aboveground biomass N content to variation in (a, b) plant species richness and (c, d) soil depth in realistic and randomized native species loss order scenarios. In panels (a) and (b), square, triangular, and circular symbols represent shallow, intermediate, and deep soils, respectively. Horizontal dashed lines and gray shaded areas show mean \pm SE for all species grown in monoculture. In panels (c) and (d), error bars represent SE, and different lowercase letters above bars indicate significant differences ($P < 0.05$) based on Tukey's HSD test.

The least diverse polyculture in the realistic loss order (two species) had three-fold less N stored in aboveground biomass than the mean of all species grown in monoculture (Fig. 1a; 95% confidence interval of the difference, 0.52–4.70 g N/m²), while none of the randomized polycultures differed in biomass N from the average monoculture (Fig. 1b). Soil depth across both the realistic and randomized loss orders had a similar positive effect on biomass N content, increasing by an average of 1.24 g N/m² from shallow to deep plots in the realistic loss order and 1.32 g N/m² in the randomized loss order (Fig. 1c, d).

Nitrogen-use efficiency

The realistic and randomized species loss orders differed significantly in the relationship between species richness (2–12 species) and N-use efficiency ($F_{1,68} = 10.12, P = 0.002$). When analyzed separately, there was no interactive effect of species richness and soil depth on N-use efficiency in either the realistic ($F_{2,42} = 0.05, P = 0.95$) or randomized ($F_{2,42} = 0.002, P = 0.99$) loss orders.

Species richness had a positive linear effect on N-use efficiency (g biomass/g N) in the realistic loss order ($F_{1,43} = 10.2, P = 0.03$), but there was no effect of species richness on N-use efficiency in the randomized loss order (Fig. 2b). Soil depth did not affect N-use efficiency in either the realistic or randomized loss orders (Fig. 2c, d). Two-species realistic polycultures produced ~ 13 g less biomass per g N than the average monoculture (Fig. 2a; 95% CI of the difference, 1.85–23.59 g biomass/g N), while the 12-species realistic polycultures produced ~ 12 g more biomass per g N than the average monoculture (Fig. 2a; 95% CI of the difference, 0.96–22.9 g biomass/g N).

Although there was a statistically significant linear relationship between species richness and N-use efficiency, there was also a statistically significant lack of fit ($F_{1,43} = 8.54, P < 0.001$) and the linear relationship explained a relatively small proportion of the variance (adjusted $R^2 = 0.19, P = 0.03$). We compared linear, decelerating (log), saturating (hyperbolic), and unimodal (second-order polynomial) functions and found

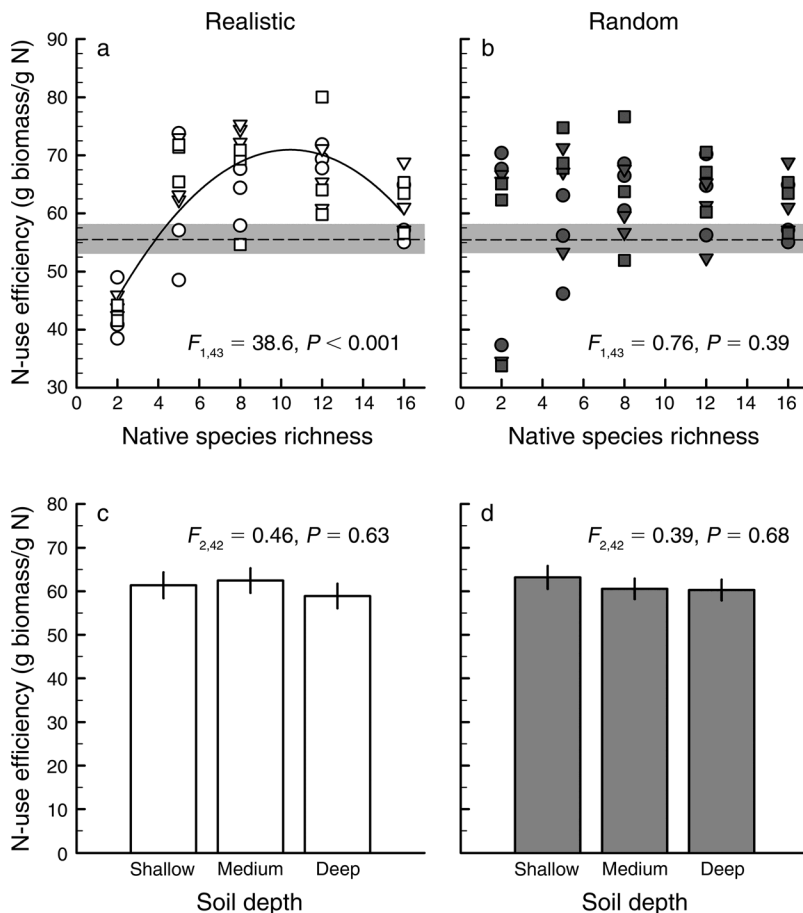


FIG. 2. Response of community-level plant N-use efficiency to variation in (a, b) plant species richness and (c, d) soil depth in realistic and randomized native species loss order scenarios. We calculated N-use efficiency as aboveground biomass produced per unit mass of N. Figure components are as described for Fig. 1.

that N-use efficiency as a unimodal function of realistic species loss had the highest relative likelihood (Akaike weight = 0.995) and explained the largest proportion of the variance (adjusted $R^2 = 0.63$; see Fig. 2a). Unimodal relationships between species richness and ecosystem functioning at the local scale are uncommon (Cardinale et al. 2011), which led us to hypothesize that early loss of *A. wrangelianus*, a N-fixing legume, strongly influenced the overall trend of N-use efficiency in the realistic loss order scenario. We tested this hypothesis by examining the relationship between fractional biomass of *A. wrangelianus* and community-level N-use efficiency in all realistic, random, and monoculture plots with *A. wrangelianus* as a component species ($n = 20$ plots). Linear regression analysis revealed that community-level plant N-use efficiency was a negative function of the fractional biomass of *A. wrangelianus* (Fig. 3).

Effect size of species richness and soil depth on plant N use

The relative importance of species richness, soil depth, and their interaction for biomass N content differed

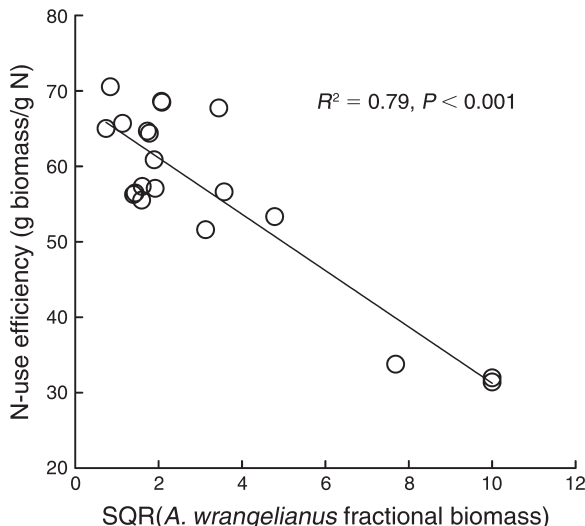


FIG. 3. Response of community-level plant N-use efficiency to the fractional biomass of *A. wrangelianus* (N-fixing legume; square-root [SQR] transformed). Analysis includes all realistic loss order, random loss order, and monoculture plots that contain *A. wrangelianus* ($n = 20$ plots).

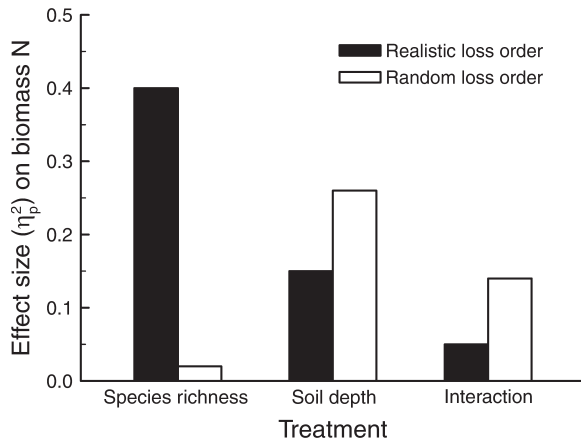


FIG. 4. Treatment effect sizes of native plant species richness, soil depth, and their interaction on aboveground plant biomass N content in realistic and randomized species loss order scenarios. Effect size is estimated by partial eta-squared (η_p^2); see *Methods* for a description of how this metric is calculated.

substantially between the realistic and randomized species loss order scenarios. In the realistic loss order, the effect magnitude of species richness on biomass N content was double that of soil depth (Fig. 4). In contrast, soil depth was the strongest predictor of biomass N content in the randomized species loss order followed by the interactive effect of species richness and soil depth. Species richness had the lowest effect magnitude in the randomized loss order.

DISCUSSION

Our results indicate the functional consequences of realistic (i.e., nonrandom, nested) changes in species richness differ substantially from those of random variation in richness. Specifically, we found that realistic plant species losses led to less complete use of available N and to an uncommon unimodal trend in N-use efficiency, but that randomized species losses had no effect on plant N use. While our results are consistent with other recent experiments and modeling efforts finding large effects of realistic species losses on ecosystem functioning (Smith and Knapp 2003, Solan et al. 2004, Zavaleta and Hulvey 2004, Bunker et al. 2005, Schlöpfer et al. 2005, Bracken et al. 2008, Selmants et al. 2012), our study is the first to directly compare how realistic and randomized plant species losses affect resource use under field-based conditions.

Ours is also the first study to compare how environmental heterogeneity moderates the relationship between species richness and ecosystem functioning in realistic and random species loss scenarios. Variation in soil depth did not alter the slope of the diversity–function relationship in either the realistic or random loss order, indicating that increased vertical heterogeneity and physical rooting space in deeper soils did not lead to greater complementarity of N use among plant

species in this ecosystem. Our results contradict those from a similar study by Dimitrakopoulos and Schmid (2004), who found diversity effects were strengthened in deeper soils, supporting their hypothesis that increasing physical space allows for greater complementarity among species. However, Dimitrakopoulos and Schmid (2004) kept soil nutrient concentration constant with soil depth in their microcosm experiment, thereby increasing the nutrient capital of deeper soils. As such, the species-richness–soil-depth interaction observed by Dimitrakopoulos and Schmid (2004) may have resulted from a fertilization effect and not from an increase in niche differentiation. In real-world ecosystems, N and other nutrients are concentrated in the upper portion of the soil profile (≤ 20 cm) and decline with depth (Jobbagy and Jackson 2001), which may explain why our results and those from other recent studies provide no evidence for an increase in vertical resource partitioning via species complementarity along gradients of soil depth (von Felten et al. 2009, 2012, Mommer et al. 2010).

Although there was no interactive effect of soil depth and species richness on plant N use in our experiment, soil depth had an independent positive effect on plant biomass N content in both the realistic and random species loss order scenarios. This is unsurprising, given that soil depth is highly correlated with productivity and resource availability in California serpentine grasslands in particular (Whittaker 1954, Davies et al. 2007, Moore and Elmendorf 2011) and grassland ecosystems in general (Baer et al. 2003, Dornbush and Wilsey 2010). However, realistic species losses had a much larger effect on plant N accumulation than did soil depth in our experiment. Moreover, the effect magnitude of soil depth was 1.5 times lower in the realistic loss order plots than in adjacent randomized loss order plots, suggesting realistic species losses may dampen the independent effect of soil depth on plant resource acquisition. A frequent criticism of BEF experiments has been that biodiversity effects are likely to be small when compared to other drivers of ecosystem processes (Duffy 2009). Our results demonstrate that the impact of realistic species losses on plant resource accumulation can greatly exceed that of soil depth, a major driver of ecosystem functioning in this and other grassland ecosystems.

Our results indicate that, at least in this ecosystem, it is the order in which different functional attributes of species are lost, not species loss in itself that drives declines in community-level N use. The species that first dropped out of the realistic loss order were primarily early-season annuals, while those that persisted to the end of the realistic loss order were late-season perennials (Table 1), a pattern reflecting observed community responses to prolonged drought (Tilman and El Haddi 1992). The perennial species that persist to the end of the realistic loss order are slow-growing, long-lived, stress tolerators (Hooper and Vitousek

1998, Dukes 2001), and although they tend to have relatively high concentrations of N in their above-ground tissues, monocultures and assemblages dominated by these perennial species used less N than monocultures and assemblages dominated by fast-growing, early-season annuals. These results illustrate how strong relationships between species' response traits (drought tolerance) and effect traits (completeness and efficiency of resource use) can amplify the effects of realistic species losses (Naeem and Wright 2003, Hooper et al. 2005, Zavaleta et al. 2009).

In addition to its effect on biomass N accumulation, realistic diversity loss also had a strong impact on community-level N-use efficiency. Nitrogen-use efficiency is often a positive linear or decelerating function of increasing species richness in randomized BEF experiments (Cardinale et al. 2011), presumably because increasing competition for light in more diverse assemblages, which are denser and more productive, leads to greater investment in high C:N stem tissue (as opposed to low C:N leaf tissue) to maximize plant height (van Ruijven and Berendse 2005, Fornara and Tilman 2009). In contrast to this typical trend, we found that N-use efficiency was a unimodal function of increasing diversity in the realistic loss order. We suggest that increasing light competition was driving a saturating relationship between N-use efficiency and species richness up to and including the 12-species realistic assemblages, but the inclusion of the N-fixing legume *A. wrangelianus* in the 16-species assemblages drove N-use efficiency down again. Two lines of evidence support the strong identity effect of *A. wrangelianus* on community-level N-use efficiency. First, *A. wrangelianus* had the lowest N-use efficiency (~31 g biomass/g N) of any species when grown in monoculture, and second, an increase in the relative abundance of *A. wrangelianus* leads to a steep decline in community-level N-use efficiency. If indeed the relationship between N-use efficiency and realistic species loss is driven by increasing light competition and presence of *A. wrangelianus*, then removing variation in light competition and randomly including *A. wrangelianus* across richness levels should result in no relationship between community-level N-use efficiency and species richness. We argue the randomized species loss order scenario provides this evidence, because constant aboveground biomass and canopy cover suggest a consistent light environment with increasing species richness (Selmants et al. 2012), *A. wrangelianus* is randomly distributed across all richness levels, and, as a result, N-use efficiency does not vary as function of randomized species richness.

Taken together, results from this experiment highlight the value of incorporating more realistic species loss scenarios into manipulative BEF experiments, especially if the goal is to understand the real-world consequences of declining biodiversity. One implication of this study is that conservation of plant diversity may help reduce N loading in streams and rivers by

sequestering excess anthropogenic N in terrestrial ecosystems. However, anthropogenic N deposition can itself reduce plant diversity (Suding et al. 2005) and so the relationship between species diversity and N loading may be bidirectional, such that biodiversity reduces N mobility but excess N loading reduces biodiversity (Cardinale 2011). Nevertheless, our direct comparative approach illustrates the importance of species identity effects on community-level resource use, and that the relative strength of diversity and resource availability effects on ecosystem functioning may depend on the order in which species are lost. We argue that the functional responses to realistic and randomized species losses differ because the realistic species loss scenario involves concentrated loss of particular traits rather than random loss of traits from across a spectrum. Because global species losses are also nonrandom with respect to traits (Zavaleta et al. 2009), our findings support the hypothesis that the effects on ecosystem processes of ongoing biodiversity losses in general are larger than those indicated by randomized biodiversity manipulations.

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LITERATURE CITED

- Albertson, F. W., and J. E. Weaver. 1944. Nature and degree of recovery of grassland from the great drought of 1933–1940. *Ecological Monographs* 14:393–479.
- Atmar, F. W., and B. D. Patterson. 1993. The measure of order and disorder in the distribution of species in fragmented habitat. *Oecologia* 96:373–382.
- Baer, S., J. Blair, and S. Collins. 2003. Soil resources regulate productivity and diversity in newly established tallgrass prairie. *Ecology* 84:724–735.
- Bracken, M., S. Friberg, C. Gonzalez-Dorantes, and S. Williams. 2008. Functional consequences of realistic biodiversity changes in a marine ecosystem. *Proceedings of the National Academy of Sciences USA* 105:924–928.
- Bracken, M., and K. Nielsen. 2004. Diversity of intertidal macroalgae increases with nitrogen loading by invertebrates. *Ecology* 85:2828–2836.
- Bunker, D., F. DeClerck, J. Bradford, R. Colwell, I. Perfecto, O. Phillips, M. Sankaran, and S. Naeem. 2005. Species loss and aboveground carbon storage in a tropical forest. *Science* 310:1029–1031.
- Burnham, K. P., and D. R. Anderson. 2002. *Model selection and multimodel inference: a practical information theoretic approach*. Second edition. Springer-Verlag, New York, New York, USA.
- Butchart, S. H. M., et al. 2010. Global biodiversity: indicators of recent declines. *Science* 328:1164–1168.
- Cardinale, B. J. 2011. Biodiversity improves water quality through niche partitioning. *Nature* 472:86–89.
- Cardinale, B. J., K. L. Matulich, D. U. Hooper, J. E. Byrnes, E. Duffy, L. Gamfeldt, P. Balvanera, M. I. O'Connor, and A.

- Gonzalez. 2011. The functional role of producer diversity in ecosystems. *American Journal of Botany* 98:572–592.
- Chapin, F., III, E. Zavaleta, V. Eviner, R. Naylor, P. Vitousek, H. Reynolds, D. Hooper, S. Lavorel, O. Sala, and S. Hobbie. 2000. Consequences of changing biodiversity. *Nature* 405: 234–242.
- Colwell, R., and T. Rangel. 2009. Hutchinson's duality: the once and future niche. *Proceedings of the National Academy of Sciences USA* 106:19651–19658.
- Dai, A. 2011. Drought under global warming: a review. *Wiley Interdisciplinary Reviews: Climate Change* 2:45–65.
- Davies, K. F., S. Harrison, H. D. Safford, and J. H. Viers. 2007. Productivity alters the scale dependence of the diversity-invasibility relationship. *Ecology* 88:1940–1947.
- Dimitrakopoulos, P. G., and B. Schmid. 2004. Biodiversity effects increase linearly with biotope space. *Ecology Letters* 7:574–583.
- Dodds, W. K. 2006. Eutrophication and trophic state in rivers and streams. *Limnology and Oceanography* 51:671–680.
- Dornbush, M. E., and B. J. Wilsey. 2010. Experimental manipulation of soil depth alters species richness and co-occurrence in restored tallgrass prairie. *Journal of Ecology* 98:117–125.
- Duffy, J. E. 2009. Why biodiversity is important to the functioning of real-world ecosystems. *Frontiers in Ecology and the Environment* 7:437–444.
- Duffy, J. E., D. S. Srivastava, J. McLaren, M. Sankaran, M. Solan, J. Griffin, M. Emmerson, and K. E. Jones. 2009. Forecasting decline in ecosystem services under realistic scenarios of extinction. Pages 60–77 in S. Naeem, D. E. Bunker, A. Hector, M. Loreau, and C. Perrings, editors. *Biodiversity, ecosystem functioning, and human wellbeing*. Oxford University Press, Oxford, UK.
- Dukes, J. 2001. Biodiversity and invasibility in grassland microcosms. *Oecologia* 126:563–568.
- Fornara, D., and D. Tilman. 2009. Ecological mechanisms associated with the positive diversity-productivity relationship in an N-limited grassland. *Ecology* 90:408–418.
- Galloway, J. N., A. R. Townsend, J. W. Erisman, M. Bekunda, Z. Cai, J. R. Freney, L. A. Martinelli, S. P. Seitzinger, and M. A. Sutton. 2008. Transformation of the nitrogen cycle: recent trends, questions, and potential solutions. *Science* 320: 889–892.
- Griffin, J. N., S. R. Jenkins, L. Gamfeldt, D. Jones, S. J. Hawkins, and R. C. Thompson. 2009. Spatial heterogeneity increases the importance of species richness for an ecosystem process. *Oikos* 118:1335–1342.
- Grime, J. 2002. Declining plant diversity: empty niches or functional shifts? *Journal of Vegetation Science* 13:457–460.
- Gundale, M., K. Metlen, C. Fiedler, and T. DeLuca. 2006. Nitrogen spatial heterogeneity influences diversity following restoration in a Ponderosa Pine Forest, Montana. *Ecological Applications* 16:479–489.
- Healy, C., N. J. Gotelli, and C. Potvin. 2008. Partitioning the effects of biodiversity and environmental heterogeneity for productivity and mortality in a tropical tree plantation. *Journal of Ecology* 96:903–913.
- Hooper, D., F. Chapin, III, J. Ewel, A. Hector, P. Inchausti, S. Lavorel, J. Lawton, D. Lodge, M. Loreau, and S. Naeem. 2005. Effects of biodiversity on ecosystem functioning: a consensus of current knowledge. *Ecological Monographs* 75: 3–35.
- Hooper, D., and P. Vitousek. 1998. Effects of plant composition and diversity on nutrient cycling. *Ecological Monographs* 68:121–149.
- Hughes, A. R., and J. J. Stachowicz. 2009. Ecological impacts of genotypic diversity in the clonal seagrass *Zostera marina*. *Ecology* 90:1412–1419.
- Huston, M. A. 1997. Hidden treatments in ecological experiments: re-evaluating the ecosystem function of biodiversity. *Oecologia* 110:449–460.
- Isbell, F. I., D. A. Losure, K. A. Yurkonis, and B. J. Wilsey. 2008. Diversity–productivity relationships in two ecologically realistic rarity–extinction scenarios. *Oikos* 117:996–1005.
- Jobby, E., and R. Jackson. 2001. The distribution of soil nutrients with depth: global patterns and the imprint of plants. *Biogeochemistry* 53:51–77.
- Miklós, I., and J. Podani. 2004. Randomization of presence–absence matrices: comments and new algorithms. *Ecology* 85: 86–92.
- Mommer, L., J. van Ruijven, H. De Caluwe, A. E. Smit-Tiekstra, C. A. M. Wagemaker, N. Joop Ouborg, G. M. Bögemann, G. M. Van Der Weerden, F. Berendse, and H. De Kroon. 2010. Unveiling below-ground species abundance in a biodiversity experiment: a test of vertical niche differentiation among grassland species. *Journal of Ecology* 98:1117–1127.
- Moore, K. A., and S. C. Elmendorf. 2011. Plant competition and facilitation in systems with strong environmental gradients. Pages 223–236 in S. Harrison and N. Rajakauna, editors. *Serpentine: the evolution and ecology of a model system*. University of California Press, Berkeley, California, USA.
- Naeem, S. 2008. Advancing realism in biodiversity research. *Trends in Ecology and Evolution* 23:414–416.
- Naeem, S., and J. Wright. 2003. Disentangling biodiversity effects on ecosystem functioning: deriving solutions to a seemingly insurmountable problem. *Ecology Letters* 6:567–579.
- Pimm, S. L., G. J. Russell, J. L. Gittleman, and T. M. Brooks. 1995. The future of biodiversity. *Science* 269:347–350.
- Raffaelli, D. 2004. How extinction patterns affect ecosystems. *Science* 306:1141–1142.
- Reynolds, H., B. Hungate, F. Chapin, III, and C. DAntonio. 1997. Soil heterogeneity and plant competition in an annual grassland. *Ecology* 78:2076–2090.
- Rodríguez-Gironés, M., and L. Santamaría. 2006. A new algorithm to calculate the nestedness temperature of presence–absence matrices. *Journal of Biogeography* 33:924–935.
- Scherer-Lorenzen, M., C. Palmberg, and A. Prinz. 2003. The role of plant diversity and composition for nitrate leaching in grasslands. *Ecology* 84:1539–1552.
- Schläpfer, F., A. Pfisterer, and B. Schmid. 2005. Non-random species extinction and plant production: implications for ecosystem functioning. *Journal of Applied Ecology* 42:13–24.
- Selmants, P. C., E. S. Zavaleta, J. R. Pasari, and D. L. Hernandez. 2012. Realistic plant species losses reduce invasion resistance in a California serpentine grassland. *Journal of Ecology* 100:723–731.
- Smith, M., and A. Knapp. 2003. Dominant species maintain ecosystem function with non-random species loss. *Ecology Letters* 6:509–517.
- Solan, M., B. Cardinale, A. Downing, K. Engelhardt, J. Ruesink, and D. Srivastava. 2004. Extinction and ecosystem function in the marine benthos. *Science* 306:1177–1180.
- Srivastava, D. S., and M. Vellend. 2005. Biodiversity–ecosystem function research: Is it relevant to conservation? *Annual Review of Ecology, Evolution, and Systematics* 36:267–294.
- Suding, K. N., S. L. Collins, L. Gough, C. Clark, E. E. Cleland, K. L. Gross, D. G. Milchunas, and S. Pennings. 2005. Functional- and abundance-based mechanisms explain diversity loss due to N fertilization. *Proceedings of the National Academy of Sciences USA* 102:4387–4392.
- Tilman, D., and A. El Haddi. 1992. Drought and biodiversity in grasslands. *Oecologia* 89:257–264.
- Tylianakis, J. M., T. A. Rand, A. Kahmen, A.-M. Klein, N. Buchmann, J. Perner, and T. Tschirntke. 2008. Resource heterogeneity moderates the biodiversity–function relationship in real world ecosystems. *PLoS Biology* 6:e122.
- Ulrich, W., M. Almeida-Neto, and N. J. Gotelli. 2009. A consumer's guide to nestedness analysis. *Oikos* 118:3–17.
- van Ruijven, J., and F. Berendse. 2005. Diversity–productivity relationships: initial effects, long-term patterns, and under-

- lying mechanisms. *Proceedings of the National Academy of Sciences USA* 102:695–700.
- von Felten, S., A. Hector, N. Buchmann, P. A. Niklaus, B. Schmid, and M. Scherer-Lorenzen. 2009. Belowground nitrogen partitioning in experimental grassland plant communities of varying species richness. *Ecology* 90:1389–1399.
- von Felten, S., P. A. Niklaus, M. Scherer-Lorenzen, A. Hector, and N. Buchmann. 2012. Do grassland plant communities profit from N partitioning by soil depth? *Ecology* 93:2386–2396.
- Whittaker, R. H. 1954. The ecology of serpentine soils. *Ecology* 34:258–288.
- Zavaleta, E. S., and K. B. Hulvey. 2004. Realistic species losses disproportionately reduce grassland resistance to biological invaders. *Science* 306:1175–1177.
- Zavaleta, E., J. Pasari, J. Moore, D. Hernández, K. Suttle, and C. Wilmers. 2009. Ecosystem responses to community disassembly. *Annals of the New York Academy of Sciences* 1162:311–333.