

UC Santa Cruz

UC Santa Cruz Previously Published Works

Title

Species traits outweigh nested structure in driving the effects of realistic biodiversity loss on productivity

Permalink

<https://escholarship.org/uc/item/1100q9ct>

Journal

Ecology, 96(1)

ISSN

0012-9658

Authors

Wolf, Amelia A
Zavaleta, Erika S

Publication Date

2015

DOI

10.1890/14-0131.1

Peer reviewed

Species traits outweigh nested structure in driving the effects of realistic biodiversity loss on productivity

AMELIA A. WOLF¹ AND ERIKA S. ZAVALETA

University of California, Santa Cruz, California 95064 USA

Abstract. While most studies of the relationship between biodiversity and ecosystem functioning have examined randomized diversity losses, several recent experiments have employed nested, realistic designs and found that realistic species losses had larger consequences than random losses for ecosystem functioning. Progressive, realistic, biodiversity losses are generally strongly nested, but this nestedness is a potentially confounding effect. Here, we address whether nonrandom trait loss or degree of nestedness drives the relationship between diversity and productivity in a realistic biodiversity-loss experiment. We isolated the effect of nestedness through post hoc analyses of data from an experimental biodiversity manipulation in a California serpentine grassland. We found that the order in which plant traits are lost as diversity declines influences the diversity–productivity relationship more than the degree of nestedness does. Understanding the relationship between the expected order of species loss and functional traits is becoming increasingly important in the face of ongoing biodiversity loss worldwide. Our findings illustrate the importance of species composition and the order of species loss, rather than nestedness per se, for understanding the mechanisms underlying the effects of realistic species losses on ecosystem functioning.

Key words: *ecosystem function; functional groups; nestedness; plant traits; realistic biodiversity loss; serpentine grassland.*

INTRODUCTION

As species extinctions accelerate worldwide, understanding the effects of vanishing biodiversity on ecosystems is increasingly critical to mitigation and conservation efforts (Naeem et al. 2012). Many researchers have undertaken studies manipulating species numbers and examining the relationship between biodiversity and ecosystem functioning, most often with productivity as the outcome measured; the results of these studies have generally though not exclusively demonstrated a positive link between biodiversity and ecosystem functioning (Hooper et al. 2005, 2012, Cardinale et al. 2011). While these results have strongly advanced our understanding of these issues, the studies they stem from often lack a degree of realism necessary to apply the findings to conservation practice.

Some of the early experiments examining the relationship between biodiversity and ecosystem functioning (BD–EF) began by considering realistic, nested species loss. In these nested species-loss designs, lower diversity treatments were subsets of higher diversity treatments, and replicates within diversity levels contained the same mixture of species (Naeem et al. 1994, 1995). These experiments were criticized, however, as containing a

“hidden treatment” (Huston 1997) that did not allow for a robust assessment of the effects of species number on ecosystem functioning. In response to this criticism, and in order to focus on the question of diversity effects on functioning, the vast majority of subsequent BD–EF experiments turned exclusively to examining randomized species assemblages (Naeem et al. 2012). However, in understanding the effects of real biodiversity loss, randomized biodiversity loss experiments only tell part of the story: not all species are equally likely to experience decline or extinction. Rapid biodiversity losses at both local and global scales disproportionately involve species with particular values of traits such as size, trophic position, rarity, distribution, and degree of specialization (Fox 1987, McDonald and Brown 1992, Belyea and Lancaster 1999, Henle et al. 2004, Estes et al. 2011).

With many years of foundational groundwork laid by randomized BD–EF experiments (reviewed by Hooper et al. 2005, Cardinale et al. 2011, Naeem et al. 2012), some investigators have now returned to examining nested species loss in an effort to incorporate more realism into biodiversity research. In studies that explicitly compare randomized to realistic species losses, most find that the effect sizes of realistic, nested species losses are larger than those of randomized species losses (Solan et al. 2004, Bunker et al. 2005, Larsen et al. 2005, McIntyre et al. 2007, Bracken et al. 2008, Selmants et al. 2012, 2014; though see Srinivasan et al. 2007). The finding that realistic diversity losses often have larger

Manuscript received 21 January 2014; revised 13 June 2014; accepted 1 July 2014. Corresponding Editor: P. H. Templer.

¹ Present address: University of California, Los Angeles Institute of the Environment and Sustainability, Los Angeles, California 90095 USA. E-mail: amywolf@gmail.edu

effects on ecosystem functioning than randomized diversity losses has big implications for those interested in understanding the consequences of real-world diversity loss: specifically, that the foundational, combined results of randomized diversity-loss experiments may underestimate the effect size of real biodiversity loss (Duffy et al. 2009).

The higher effect sizes often demonstrated by realistic biodiversity experiments are generally attributed to the differential vulnerability of species based on traits and the importance of vulnerable traits to overall ecosystem functioning (Bunker et al. 2005, Duffy et al. 2009). Realistic loss orders are based on specific real-world drivers of diversity loss, and generally incorporate realism in two ways: first, they incorporate the vulnerability of each species in an assemblage to an identified driver of diversity loss (e.g., drought, fragmentation, invasion, etc.). That is, the species more vulnerable to a specific driver will be the species lost from a community first. Second, they incorporate the progressive nature of ongoing species loss. Progressive, realistic biodiversity losses through time are generally strongly nested, that is, each decreasing level of biodiversity represents a subset of the previous higher level of diversity. Nearly all experimental studies to date of realistic species losses have incorporated fully nested designs (e.g., Zavaleta and Hulvey 2004, 2007, Bunker et al. 2005, Isbell et al. 2008, Bracken and Low 2012, Selmants et al. 2012, 2014; but see Maherali and Klironomos 2007, Rinella et al. 2007, Bracken and Williams 2013). However, these realistic diversity-loss experiments encompass two undifferentiated treatments: (1) the nonrandom loss of vulnerable species and (2) the nested structure of species loss. While no study examining realistic species loss has tried to separate the effects of nestedness per se from the effects of nonrandom losses of vulnerable species, the nestedness of “realistic” experiments is a potentially confounding effect with major implications for the utility of realistic diversity loss studies. If nestedness alone can account for the higher effect sizes of realistic vs. randomized biodiversity loss on ecosystem functioning, these studies may be irrelevant for their intended purpose of informing conservation.

Why might the nested structure of species losses influence the effect of diversity on ecosystem functioning? We know from prior research that the ways in which BD–EF experiments are designed and species are chosen can influence experimental outcomes (Huston 1997, Doak et al. 1998). As more and more BD–EF experiments move toward understanding realistic, nested biodiversity loss, it is essential that we examine the potential biases introduced by a deterministic method of species selection (for realistic diversity loss) rather than a probabilistic one (for randomized diversity loss). It is plausible that nested structure could influence either selection or complementarity effects, the two main ways

in which diversity affects ecosystem functioning (Loreau and Hector 2001).

In randomized-loss BD–EF experiments, assemblages of varying richness are constructed by randomly drawing species from a pool (Tilman et al. 1997, Roscher et al. 2004). Any species can thus occur at any richness level and, across replicates, a given species typically occurs at all or most richness levels at least once. Mean functioning at every richness level therefore reflects the influences of all or most species in the experimental pool in a probabilistic way; no particular species are excluded from particular richness levels. This can be contrasted with the deterministic nature of nested loss orders, in which particular species drop out of assemblages as richness declines and do not return at lower richness levels. This could be viewed as an extreme sampling effect (Loreau and Hector 2001): except for the few that persist to low diversity levels, particular species identities do not contribute to every richness level, and species cannot drop out at high richness levels only to reappear at lower richness levels. This distinction is true for interactions between species as well; interactions between particular species are restricted to richness levels in which those species are present in nested loss orders, while all pairwise and higher-order interactions among species are possible at any richness level in a random loss order. In nested loss orders, there are fewer possible species identities and interspecific interactions that can contribute to the mean level of functioning at lower richness levels; if a strongly interacting species drops out of the loss order at a high diversity level, it cannot contribute to functioning at any lower richness level. Because of this, if particular species interactions (complementarity effects, *sensu* Loreau and Hector [2001]) strongly drive high ecosystem functioning, then nested structure in species loss could strengthen the positive BD–EF relationship relative to random species loss, independently of which species are actually lost.

Here we hypothesize that the structure of species losses from an ecological network may have effects on ecological processes independent of the species identities within that ecological network or loss order. While there is little previous work to inform this hypothesis in the BD–EF field, we can look a bit further afield for evidence to support or refute the hypothesis. A substantial literature has developed recently on network architecture within mutualist and trophic networks (Ulrich et al. 2009, Ulrich and Almeida-Neto 2012). Hypotheses stemming from this work posit that network architecture, independent of ecological processes occurring within a network, can influence community dynamics. This suggests that the configuration of species and their interactions within an ecosystem, regardless of the particular species present, may influence ecological processes. Findings within this field have been mixed, with several studies demonstrating that network architecture alone can influence competition, diversity, and community stability (Bastolla et al. 2009, Thebault and

Fontaine 2010), while other studies refute this causative relationship, showing that other ecological factors (such as the number of interactors) are the underpinning mechanisms affecting these ecological processes (James et al. 2012). If the static network architecture itself can influence ecological processes, it is plausible that the structure of changes to network architecture may also influence these processes.

In this study, we address the hypothesis that the nested structure of diversity loss alone can lead to larger effects of diversity loss on ecosystem functioning as compared to randomized (non-nested) diversity loss. We base our analyses on data from an experimental, field-based biodiversity manipulation in a serpentine grassland at Coyote Ridge, California in which we see higher effect sizes of realistic species loss when compared to randomized species loss (Selmants et al. 2012). Using these data, we disentangle the effects of nonrandom losses of vulnerable species from the effects of nested structure in biodiversity loss on the biodiversity-productivity relationship in an effort to better understand the results of realistic diversity-loss experiments. We use experimental data to create a set of highly nested, though non-realistic, loss orders and comparing them to non-nested loss orders constructed from the same data. We compare the degree of nestedness of each loss order with a metric of functional group vulnerability, that is, the order in which plant traits are lost as diversity declines, to assess their relative contributions to the effect of biodiversity on productivity.

METHODS

We examined the effects of nestedness on the relationship between productivity and plant species richness using data from an ongoing biodiversity-ecosystem functioning experiment at Coyote Ridge, near San Jose, California, USA. The experimental design and prior results are described in full in Selmants et al. (2012); briefly, the experiment aims to distinguish the effects of realistic species loss vs. random species loss on a variety of ecosystem functions, including productivity and invasibility. Plots contain 12, 8, 5, or 2 species, with species composition determined either at random (hereafter, field-based randomized treatment) or based on a drought-driven realistic loss order derived from 19-year species-composition data at the site (hereafter, field-based realistic treatment; see Selmants et al. [2012] for details on the long-term species composition data and determination of the field-based realistic species loss order). Within the two field-based loss treatments, each richness level is replicated nine times. There are nine plots that contain all 16 experimental species, three plots of each species growing in monoculture, and nine bare plots, for a total of 138 plots. There is also a soil-depth treatment (i.e., shallow, medium, and deep soil depths) incorporated into the study design, though this treatment will not figure into the data presented here. For this analysis, we used data only from 2011 when the soil-

depth treatment was not a significant predictor of productivity (ANOVA, $F_{2,79} = 1.79$; $P = 0.17$).

The field-based realistic treatment in this experiment is fully nested: plots at each diversity level contain a subset of the species in plots of the next-higher diversity level. Plots within the field-based randomized treatment, however, are not deliberately nested. Each plot within the field-based randomized treatment contains a unique group of species randomly drawn from the overall 16-species pool. Annual productivity measurements for all plots were conducted in April 2011. Aboveground biomass was harvested from one-third of each plot, representing an area of approximately 0.25 m². Harvested biomass was sorted to species, dried at 60°C, and weighed.

In order to assess the effects of nested structure on the relationship between diversity and productivity, we selected nested sets of plots, each set including one plot from each diversity-treatment level. Using the species assemblages of the field-based randomized plots of 2, 5, 8, and 12 species, we assigned plots to create 32 highly nested (though non-realistic) loss orders (hereafter, “constructed nested-loss orders”). These orders were constructed stepwise from high to low diversity: we found the best-nesting 8-species plot for each 12-species plot, the best 5-species plot for 8-species plot, and so on. If two 5- or 8-species plots of the same diversity nested equally well within a higher-diversity plot, two unique orders were created. Individual plots could therefore be assigned to more than one loss order, though at least two of the four plots had to differ for loss orders to be considered unique. If more than one two-species plot nested equally well within a loss order, those two-species plots were averaged.

In order to generate less-nested loss orders, random-loss orders (hereafter, “constructed random-loss orders”) were assembled from the same plots in the same abundance as they were used in creating the constructed nested-loss orders. (If a plot appeared twice in the constructed nested-loss orders, it also appeared twice in the constructed random-loss orders.) Plots were randomly selected to create these orders using the sample function in R 2.13.2 (R Development Core Team 2011).

While nestedness is often presented as a binary variable, sets of species can range continuously from highly nested to completely un-nested. There are many methods for assessing degree of nestedness within a set of plots; for this study, the degree of nestedness of the constructed loss orders was assessed as T (matrix temperature; Atmar and Patterson 1993, Rodriguez-Girones and Santamaria 2006). We chose T because of its focus on “surprise” presences or absences within a matrix and the weighting of surprises further from the isocline more strongly. Additionally, the problems that have been described for T (Almeida-Neto et al. 2008, Ulrich et al. 2009), notably its sensitivity to matrix size and shape, do not affect our calculations as our matrices do not vary in size. However, to assess the robustness of

our results to our selected nestedness metric, we also calculated NODF (nestedness based on overlap and decreasing fills) that is based on the degree of overlap between adjacent rows and columns (Ulrich et al. 2009). T decreases with increasing nestedness; NODF increases with increasing nestedness. These metrics were calculated in R 2.13.2 (R Development Core Team 2011) using the `nestedtemp` and `nestednodf` functions, respectively, in the `vegan` package (Oksanen et al. 2011). The `nestedtemp` function in R is based on the nested temperature metric first introduced by Atmar and Patterson (1993) and more explicitly described by Rodríguez-Gironés and Santamaria (2006), the latter of which describes the calculations used by the `nestedtemp` function (Oksanen et al. 2011). Nestedness temperature, T , is calculated as the sum of all “surprises” within a matrix, where a surprise is defined as the distance of an unexpected presence or absence from the isocline that divides expected presences and absences. A matrix with fewer surprises will have a lower value of T . The `nestedtemp` function in R iteratively packs the matrix—presences are packed to one corner of the matrix and absences are packed to the opposite corner—to achieve a low temperature, though, due to the iterative nature of this process, it does not always produce the same number (Oksanen et al. 2011). Therefore, we repeated the calculation of T 500 times for each constructed loss order, taking the average value of these runs as the mean T for each constructed loss order.

The slope of the productivity–diversity relationship was determined for each constructed loss order based on the productivity of the field-based 2-, 5-, 8-, and 12-species plots in each loss order as well as the average productivity of all nine 16-species plots. Because of the small number of points (five) per loss order and the lack of statistical power to differentiate between goodness-of-fit for different relationship shapes, these relationships were always assumed to be linear. This assumption is supported by the dataset from the larger experiment, where the significant relationship between diversity and productivity in the field-based realistic treatment is best fit as linear (Selmants et al. 2012). We refer to these slopes as diversity effect sizes for each constructed loss order, where a larger slope equals a larger effect of diversity on productivity. Anchoring each constructed loss order with a known productivity value for 16-species plots allows for this quantitative comparison of effect size based on slope across loss orders.

Each constructed nested-loss order can be viewed as a single replicate of an experimental (though not necessarily realistic) species-extinction order, with vulnerable species represented only in higher-diversity plots and more persistent species represented in lower-diversity plots. (These loss orders are strictly theoretical: they are not based on any real-world driver of extinction.) In order to quantify the vulnerability of certain traits when species are lost, we calculated functional group “vulner-

ability” ranks for each constructed nested-loss order. (These could not be calculated for the constructed random-loss orders because species do not drop out in a systematic fashion in those orders.) First, each species was ranked as described in the following paragraph; next, species ranks were summed by functional group to calculate functional group vulnerability ranks.

For each constructed nested-loss order, each species was given a rank based on where in the order it was lost (high rank means high vulnerability to extinction, species dropped out at high diversity; low rank means low vulnerability to extinction, species dropped out at low diversity). Each of the 16 experimental species were then classified by functional group as perennial, early-season annual, late-season annual, or nitrogen fixing. For each constructed nested-loss order, functional group vulnerability ranks were calculated as the sum of the rank of each species within that functional group. (For example, late-season rank would be calculated as the sum of the ranks of *Lessingia nemaclada* and *Hemizonia luzulifolia*. For a complete list of species and functional groups in this experiment, see Appendix: Table A1.)

Analysis

In order to confirm that the random-loss and nested-loss orders we constructed were different from each other, we used t tests to assess the difference in degree of nestedness between the constructed nested-loss orders and the constructed random-loss orders. The influence of nestedness on diversity effect size (the slope of the diversity-productivity relationship) was evaluated by linear regression with both nestedness and slope evaluated as continuous variables across nested and random-loss orders. The effects of individual functional group vulnerability ranks on diversity effect sizes were evaluated by linear regression across only the nested-loss orders.

We also constructed a pairwise correlation matrix between the different functional group vulnerability ranks to examine the degree of independence of these predictor variables. There will inherently be correlations between the functional group vulnerability ranks: when one functional group has a “low vulnerability rank” for a loss order, the other functional groups are statistically more likely to have a “high vulnerability rank.” Understanding and quantifying these correlations is essential to interpreting the predictive value of these functional group vulnerability ranks alone and, especially, in concert.

Last, we performed a stepwise regression to examine predictive models that incorporated functional group vulnerability rankings and nestedness temperature in concert, with diversity effect size as the response variable. We report the Akaike information criterion (AIC) values, P values, and R^2 values, as well as F scores and degrees of freedom, for these models. All analyses were performed in R 2.13.2 (R Development Core Team 2011).

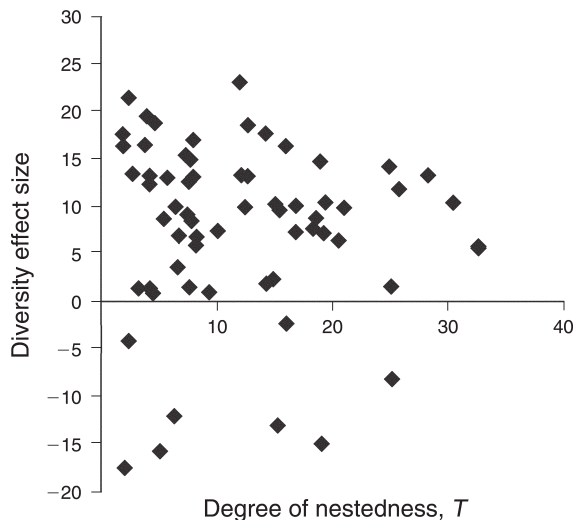


FIG. 1. Relationships between diversity effect size (i.e., the slope of the regression between diversity and productivity) and nestedness temperature (T) of a loss order. As T increases, the degree of nestedness of a loss order decreases. Each point represents a single loss order ($n = 64$ loss orders).

RESULTS

The degree of nestedness within a loss order did not have a significant effect on the relationship between diversity and productivity (Fig. 1). As expected, the constructed random-loss orders were significantly less nested (T was significantly higher) than the constructed nested-loss orders ($t = 11.3$, $P < 0.0001$). For the constructed nested-loss orders, T averaged 5.55, ranging from 1.88 to 10.1; for the constructed random-loss orders, T averaged 18.9, ranging from 8.21 to 32.6. Diversity effect size averaged 7.89 for nested-loss orders and 7.77 for random-loss orders. Results for nestedness as measured by NODF were similar to those for T : constructed random-loss orders were significantly less nested (NODF was significantly lower) than the constructed nested-loss orders ($t = 2.65$, $P = 0.02$). For nested-loss orders, NODF averaged 46.1, ranging from 35.9 to 51.5; for random-loss orders, NODF averaged 42.6, ranging from 29.2 to 52.9. Hereafter, degree of nestedness will be reported as T only.

While nestedness alone did not influence the diversity effect size, functional group vulnerability ranks did significantly predict diversity effect size. When early-season annuals dropped out low in a loss order (i.e., when early-season annuals had low vulnerability to experimental extinction), productivity declined more slowly as diversity declined (diversity effect size was low; Fig. 2a). However, when the N fixer dropped out low in a loss order, productivity declined more sharply as diversity declined, that is, led to higher diversity effect sizes (Fig. 2c). Vulnerability of perennials and late-season annuals were not significant predictors of diversity effect size (Figs. 2b and 2d).

Pairwise correlations between the functional group vulnerability ranks showed that early-season annual rank was significantly correlated to all three other (perennial, late-season annual, and N fixer) ranks. Correlation coefficients for these relationships were -0.32 , -0.42 , and -0.32 , respectively; P values for the relationships were 0.014, 0.0003, and <0.0001 , respectively. There were no other significant correlations between the functional group vulnerability rankings.

Based on the stepwise regression, vulnerability of the N fixer alone was the best explanatory model of the diversity effect size, productivity decreased most when legumes were lost last, though this model was statistically indistinguishable (based on AIC score and P values) from the model incorporating vulnerability ranks of both the N fixer and late-season annuals (Table 1). Based on P values, no significant models included T , the metric of nestedness temperature (Table 1), though a third model including T as well as vulnerability ranks of the N fixer and late-season annuals was within $2 \Delta\text{AIC}$ of the top-ranked model. Variables were stepped out in the order perennial rank $>$ early-season annual rank $>$ T $>$ late-season annual rank.

DISCUSSION

The degree of nestedness alone was not a significant predictor of the diversity–productivity relationship. In models incorporating both functional group vulnerability (the order in which functional groups are lost as diversity declines) and degree of nestedness, functional group vulnerability was the better predictor of the relationship between diversity and productivity. Across all of our constructed loss orders, the diversity effect size ranged widely, encompassing both positive and negative slopes of the relationship between diversity and productivity. These results emphasize the importance of the order in which species are lost on ecosystem functioning as diversity declines. Specifically, the vulnerability of certain functional groups to drivers of diversity loss largely determines how diversity affects productivity as the number of species dwindles.

These findings bear on how biodiversity–functioning relationships inform conservation and restoration in several ways. First, our results add to previous findings that species identity matters: our results suggest in particular that ecosystem functioning is a product of the species and functional groups that are present, not a product of the nested structure of species loss. Second, our findings indicate that the effects of realistic species losses are not due to nested species loss structure, and as such, that greater effect sizes of realistic species losses on ecosystem functioning are likely even in situations where species loss is not nested. While species loss is often assumed to be progressive and thus nested, this may not in reality always be the case, for example, when locally extinct species recolonize following extirpation or new species arrive following losses of other species. Our

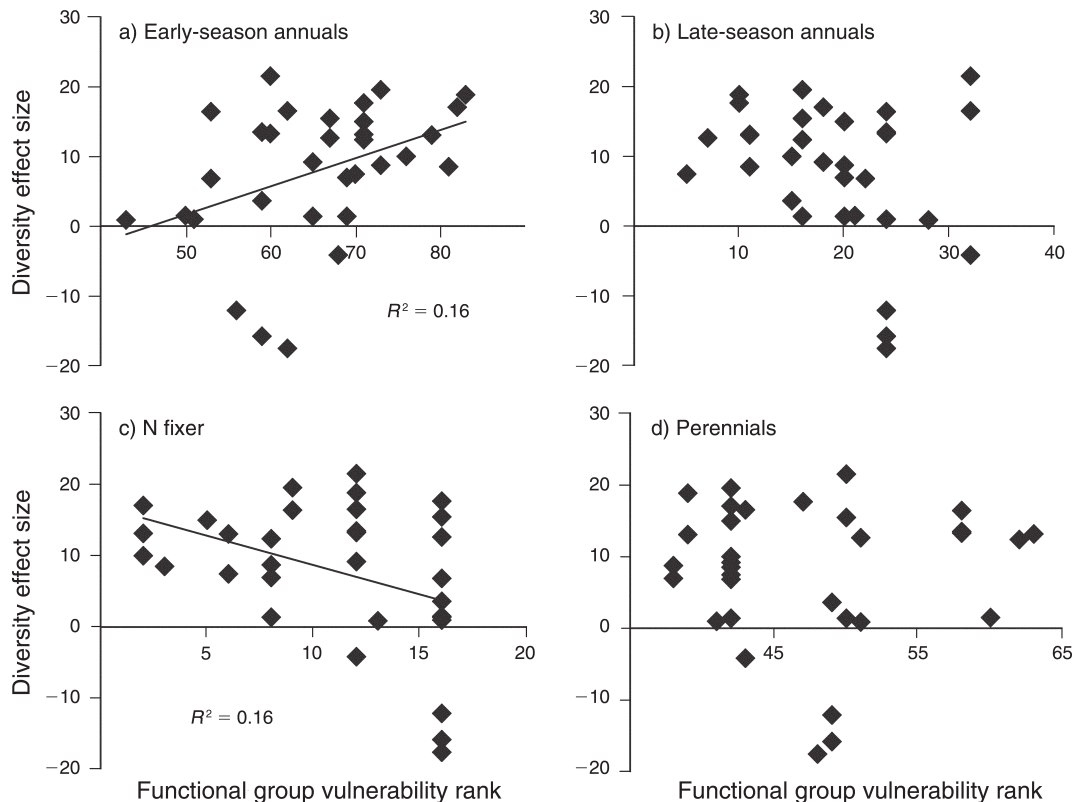


FIG. 2. Results of univariate models of the effects of functional group vulnerability ranks (the order in which functional groups are lost as diversity declines) on the diversity effect size (i.e., the slope of the regression between diversity and productivity). Each point represents a single, nested, loss order ($n = 32$ constructed nested-loss orders). Regression lines are only shown for significant relationships; the equations of the lines are (a) $y = 0.40x - 18.5$ ($P = 0.023$) and (c) $y = -0.82x + 16.85$ ($P = 0.023$).

study suggests that there can be large effects from net real-world species losses even if these losses are not nested.

Nestedness

The degree of nestedness of a loss order did not have a consistent effect on diversity effect size. While nestedness could theoretically influence diversity effect size, it appears that any effect is not directional across a wide range of species loss orders and thus that species and functional-group loss order are more influential in determining diversity effect size. This finding bolsters

previous studies of realistic biodiversity loss, supporting claims that high effect sizes in these experiments are due to the order in which species are lost rather than the nested structure of the losses. The deterministic nature of species selection in realistic biodiversity-loss experiments, as opposed to probabilistic species selection in randomized diversity-loss experiments, does not appear to independently affect the outcome of the relationship between diversity and productivity.

While nestedness does not appear to have a large independent effect, the inclusion of T in the stepwise regression with other variables produced a marginally

TABLE 1. Results of stepwise regression with nestedness temperature (T) and each of the four functional group vulnerability ranks (N fixer, late-season annual, early-season annual, and perennial) as predictor variables of the diversity effect size (i.e., slope of the productivity–diversity relationship).

Model parameters	F	df	R^2	P	AIC	Stepwise regression rank
N fixer	5.780	1, 30	0.1616	0.02259	143.84	1
N fixer, late-season annual	3.418	2, 29	0.1908	0.04645	144.70	2
N fixer, late-season annual, T	2.815	3, 28	0.2317	0.05735	145.05	3
N fixer, late-season annual, early-season annual, T	2.071	4, 27	0.2348	0.1125	146.92	4
N fixer, late-season annual, early-season annual, perennial, T	1.670	5, 26	0.2431	0.09756	148.57	5

Note: Functional group vulnerability ranks represent the order in which functional groups are lost as diversity declines. The stepwise regression ranks refer to the order in which the stepwise regression ranked the models in the table, with 1 being the best fit.

significant model, with N-fixer and late-season annual vulnerability ranks along with T as explanatory variables (Table 1). This suggests that T may add a small amount of explanatory power ($R^2 = 0.23$ for the model with three variables vs. $R^2 = 0.19$ for the two-variable model with N-fixer and late-season annual vulnerability ranks only). Based on this outcome, the lack of effect that we see due to nestedness should be confirmed in other systems before it is ruled out as unimportant to other studies.

Though we calculated values for NODF, we found that this metric did not capture nestedness in the expected way. For a highly nested matrix (e.g., for the deliberately nested loss order in the field-based “realistic” treatment of this biodiversity experiment, where each lower level of diversity represents an entirely overlapping subset of the higher diversity level), T delivered a score quite close to zero (0.5, on a scale from 0 to 100), as expected. However, NODF returned a value of 48 (on a scale from 100 to 0) for this same matrix, where a perfectly nested matrix should return an NODF value of 100. We believe this discrepancy stems at least in part from the fact that our nested design does not decrease by one species at a time (that is, from 16 to 15 to 14, etc., species), but rather declines by three to four species at a time (from 16 to 12 to 8, etc., species). In fact, this discrepancy seems to far overpower any decrement in nestedness score stemming from “surprises,” or non-nested species, in the matrix. Because NODF appears to be so sensitive to the number of species rather than the identity of those species, we view this metric with methodological skepticism as it relates to this study. Despite some of the known problems with T (Almeida-Neto et al. 2008, Ulrich et al. 2009), these problems are mostly irrelevant to this study (e.g., all matrices are the same size) and it better captures the variation in nestedness we find across our loss orders.

Species traits

While the degree of nestedness was not highly predictive of diversity effect size, the pattern of trait loss within a loss order was predictive of the effect of diversity on productivity. Based on univariate models, loss of both the N fixer and early-season annuals is important in determining the effect of diversity on productivity in this system. The importance of the N fixer is confirmed by the results of stepwise regression, which includes N fixer vulnerability rank in both significant models of diversity effect size. The early exclusion of the vulnerability of early-season annuals, a significant predictor as a lone variable, from the stepwise regression is likely due to the strong correlation between this variable and all three other functional group vulnerability ranks (N fixer, late-season annuals, and perennial).

The presence of N fixers has often been identified as a highly important explanatory variable in BD-EF studies (Hooper et al. 2005, Cadotte et al. 2009, Flynn et al.

2011). However, the direction of the N-fixer effect that we see here is generally opposite of the effect seen in other studies: when the N fixer in this experiment, *Acmispon wrangelianus*, is lost last (when the vulnerability of *A. wrangelianus* was low), we found productivity decreased more strongly with decreasing diversity (higher diversity effect size). In contrast, other studies have found that the presence of an N fixer *increases* productivity. The reason behind this discrepancy is not entirely clear, but there may be several contributing factors. First, *A. wrangelianus*, the only N fixer in this experiment, produces relatively little biomass per plant, thus contributing little to the overall productivity of any plot in which it grows. Despite its low aboveground biomass, as an N fixer, this species could lead to higher biomass in other species within the plots in which it grows; we have observed nodules on the roots of *A. wrangelianus* at our field site (A. Wolf, *personal observation*) and therefore know that the species does fix at least some N, though we do not have any measurements on the amount of N fixed (though see Selmann et al. [2014] for further discussion of N dynamics at the site). It is possible that very little N is fixed overall, or potentially that many of the other species in our experiment are unable to take much advantage of any fixed N derived from *A. wrangelianus*. Alternatively, the effect of any additional N derived from *A. wrangelianus* may be swamped by the relatively high rates of N deposition that occur at the field site as a result of prevailing winds and human activities of the adjacent San Francisco Bay area (Weiss 1999).

Typically, plot-level characteristics (e.g., species presence and composition) are used to assess the best predictors of biomass and productivity within a single plot in BD-EF experiments. In this study, developing a loss-order-level metric of species loss (functional group vulnerability ranks) was necessary in order to assess the relative predictive power of trait loss compared to degree of nestedness, an inherently loss-order-level metric. We use these two loss-order-level metrics, nestedness and functional group vulnerability ranks, to predict the *change* in productivity as measured by the slope of the line connecting five points rather than predicting the productivity of just a single plot. The relatively low R^2 values of our best models ($R^2 = 0.16$ – 0.23 ; Table 1) can partly be explained by the uncertainty encompassed by the calculation of the response variable (i.e., the slope of the productivity–diversity relationship), as well as uncertainty in determining the shape of the best-fit line for each regression. Additionally, qualitative functional-group classification, such as we used here, has been shown to be a coarser predictor of ecosystem functioning than quantitative trait metrics (Petchev and Gaston 2002), though acquiring values for the necessary large number of traits was beyond the scope of this project. While these factors are likely to account for a large portion of the unexplained error and more-precise predictions may be possible at the plot level, the

development of metrics to assess effect sizes over the scale of a full loss order advances our understanding of the effects of realistic, progressive and ongoing biodiversity losses. These types of loss-order-level metrics provide more predictive analytical power when assessing variables manifested across a set of plots (such as trait vulnerability) rather than those manifested at the plot level (such as species presence/absence). In BD–EF experiments with randomized species losses, a goal is for diversity to be the only loss-order-level variable to systematically change. As BD–EF experiments continue to move from testing random species loss to examining trait-based species loss, quantifying loss-order-level variation inherent to directional species loss will be essential to fully understanding the mechanisms behind the effects of diversity on ecosystem functioning.

While we found that the nested structure of a loss order did not have a strong effect on the relationship between biodiversity and ecosystem functioning, there were strong effects of traits and the vulnerability of functional groups on this relationship. In order to accurately characterize the effect of species loss on ecosystem functioning, we must not only understand the degree but also the order of biodiversity loss. As this study and others have shown, species identity and functional groups matter, underscoring the importance of understanding the order in which species and traits are lost from ecosystems. Studies of realistic, nested species loss are necessary to understanding ecosystem functioning in the face of biodiversity decline, but an essential first step in these types of experiments is to accurately determine species and functional group vulnerability to current and future drivers of diversity loss, as this a priori determination is likely to have a significant impact on the direction and magnitude of the experimental outcome.

ACKNOWLEDGMENTS

This work was funded by NSF grant DEB0918785. We thank E. Abelson, J. Pasari, the Zavaleta lab group, and four anonymous reviewers for help preparing and revising the manuscript. A. Wolf and E. Zavaleta designed the study and A. Wolf performed the research and analyses. A. Wolf wrote the first draft of the manuscript and E. Zavaleta contributed substantially to revisions.

LITERATURE CITED

- Almeida-Neto, M., P. Guimaraes, P. R. Guimaraes, Jr., R. D. Loyola, and W. Ulrich. 2008. A consistent metric for nestedness analysis in ecological systems: reconciling concept and measurement. *Oikos* 117:1227–1239.
- Atmar, W., and B. D. Patterson. 1993. The measure of order and disorder in the distribution of species in fragmented habitat. *Oecologia* 96:373–382.
- Bastolla, U., M. A. Fortuna, A. Pascual-Garcia, A. Ferrera, B. Luque, and J. Bascompte. 2009. The architecture of mutualistic networks minimizes competition and increases biodiversity. *Nature* 458:1018–1091.
- Belyea, L. R., and J. Lancaster. 1999. Assembly rules within a contingent ecology. *Oikos* 86:402–416.
- Bracken, M. E. S., S. E. Friberg, C. A. Gonzalez-Dorantes, and S. L. 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. E. S., and N. H. N. Low. 2012. Realistic losses of rare species disproportionately impact higher trophic levels. *Ecology Letters* 15:461–467.
- Bracken, M. E. S., and S. L. Williams. 2013. Realistic changes in seaweed biodiversity affect multiple ecosystem functions on a rocky shore. *Ecology* 94:1944–1954.
- Bunker, D. E., F. DeClerck, J. C. Bradford, R. K. Colwell, I. Perfecto, O. L. Phillips, M. Sankaran, and S. Naeem. 2005. Species loss and aboveground carbon storage in a tropical forest. *Science* 310:1029–1031.
- Cadotte, M. W., J. Cavender-Bares, D. Tilman, and T. H. Oakley. 2009. Using phylogenetic, functional and trait diversity to understand patterns of plant community productivity. *PLoS ONE* 4. <http://dx.doi.org/10.1371/journal.pone.0005695>
- 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.
- Doak, D. F., D. Bigger, E. K. Harding, M. A. Marvier, R. E. O'Malley, and D. Thomson. 1998. The statistical inevitability of stability-diversity relationships in community ecology. *American Naturalist* 151:264–276.
- 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—an ecological and economic perspective*. Oxford University Press, Oxford, UK.
- Estes, J. A., et al. 2011. Trophic downgrading of Planet Earth. *Science* 333:301–306.
- Flynn, D. F. B., N. Mirotchnick, M. Jain, M. I. Palmer, and S. Naeem. 2011. Functional and phylogenetic diversity as predictors of biodiversity–ecosystem-function relationships. *Ecology* 92:1573–1581.
- Fox, B. J. 1987. Species assembly and the evolution of community structure. *Evolutionary Ecology* 1:201–213.
- Henle, K., K. F. Davies, M. Kleyer, C. Margules, and J. Settele. 2004. Predictors of species sensitivity to fragmentation. *Biodiversity and Conservation* 13:207–251.
- Hooper, D. U., E. C. Adair, B. J. Cardinale, J. E. K. Byrnes, B. A. Hungate, K. L. Matulich, A. Gonzalez, J. E. Duffy, L. Gamfeldt, and M. I. O'Connor. 2012. A global synthesis reveals biodiversity loss as a major driver of ecosystem change. *Nature* 486:105–108.
- Hooper, D. U., et al. 2005. Effects of biodiversity on ecosystem functioning: a consensus of current knowledge. *Ecological Monographs* 75:3–35.
- 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.
- James, A., J. W. Pitchford, and M. J. Plank. 2012. Disentangling nestedness from models of ecological complexity. *Nature* 487:227–230.
- Larsen, T. H., N. M. Williams, and C. Kremen. 2005. Extinction order and altered community structure rapidly disrupt ecosystem functioning. *Ecology Letters* 8:538–547.
- Loreau, M., and A. Hector. 2001. Partitioning selection and complementarity in biodiversity experiments. *Nature* 412:72–76.
- Maherali, H., and J. N. Klironomos. 2007. Influence of phylogeny on fungal community assembly and ecosystem functioning. *Science* 316:1746–1748.

- McDonald, K. A., and J. H. Brown. 1992. Using montane mammals to model extinctions due to global change. *Conservation Biology* 6:409–415.
- McIntyre, P. B., L. E. Jones, A. S. Flecker, and M. J. Vanni. 2007. Fish extinctions alter nutrient recycling in tropical freshwaters. *Proceedings of the National Academy of Sciences USA* 104:4461–4466.
- Naeem, S., J. E. Duffy, and E. Zavaleta. 2012. The functions of biological diversity in an age of extinction. *Science* 336:1401–1406.
- Naeem, S., L. J. Thompson, S. P. Lawler, J. H. Lawton, and R. M. Woodfin. 1994. Declining biodiversity can alter the performance of ecosystems. *Nature* 368:734–737.
- Naeem, S., L. J. Thompson, S. P. Lawler, J. H. Lawton, and R. M. Woodfin. 1995. Empirical-evidence that declining species-diversity may alter the performance of terrestrial ecosystems. *Philosophical Transactions of the Royal Society B* 347:249–262.
- Oksanen, J., F. G. Blanchet, R. Kindt, P. Legendre, P. R. Minchin, R. B. O'Hara, G. L. Simpson, P. Solymos, M. H. H. Stevens, and H. Wagner. 2011. *vegan*: community ecology package. R package version 2.0-2. <http://CRAN.R-project.org/package=vegan>
- Petchey, O. L., and K. J. Gaston. 2002. Functional diversity (FD), species richness and community composition. *Ecology Letters* 5:402–411.
- R Development Core Team. 2011. *R: a language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. <http://www.R-project.org/>
- Rinella, M. J., M. L. Pokorny, and R. Rekaya. 2007. Grassland invader responses to realistic changes in native species richness. *Ecological Applications* 17:1824–1831.
- Rodriguez-Girones, M. A., and L. Santamaria. 2006. A new algorithm to calculate the nestedness temperature of presence-absence matrices. *Journal of Biogeography* 33:924–935.
- Roscher, C., J. Schumacher, J. Baade, W. Wilcke, G. Gleixner, W. W. Weisser, B. Schmid, and E. D. Schulze. 2004. The role of biodiversity for element cycling and trophic interactions: an experimental approach in a grassland community. *Basic and Applied Ecology* 5:107–121.
- 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.
- Selmants, P. C., E. S. Zavaleta, and A. A. Wolf. 2014. Realistic diversity loss and variation in soil depth independently affect community-level plant nitrogen use. *Ecology* 95:88–97.
- Solan, M., B. J. Cardinale, A. L. Downing, K. A. M. Engelhardt, J. L. Ruesink, and D. S. Srivastava. 2004. Extinction and ecosystem function in the marine benthos. *Science* 306:1177–1180.
- Srinivasan, U. T., J. A. Dunne, J. Harte, and N. D. Martinez. 2007. Response of complex food webs to realistic extinction sequences. *Ecology* 88:671–682.
- Thebault, E., and C. Fontaine. 2010. Stability of ecological communities and the architecture of mutualistic and trophic networks. *Science* 329:853–856.
- Tilman, D., J. Knops, D. Wedin, P. Reich, M. Ritchie, and E. Siemann. 1997. The influence of functional diversity and composition on ecosystem processes. *Science* 277:1300–1302.
- Ulrich, W., and M. Almeida-Neto. 2012. On the meanings of nestedness: back to the basics. *Ecography* 35:865–871.
- Ulrich, W., M. Almeida-Neto, and N. J. Gotelli. 2009. A consumer's guide to nestedness analysis. *Oikos* 118:3–17.
- Weiss, S. B. 1999. Cars, cows, and checkerspot butterflies: nitrogen deposition and management of nutrient-poor grasslands for a threatened species. *Conservation Biology* 13:1476–1486.
- Zavaleta, E. S., and K. B. Hulvey. 2004. Realistic species losses disproportionately reduce grassland resistance to biological invaders. *Science* 306:1175–1177.
- Zavaleta, E. S., and K. B. Hulvey. 2007. Realistic variation in species composition affects grassland production, resource use and invasion resistance. *Plant Ecology* 188:39–51.

SUPPLEMENTAL MATERIAL

Ecological Archives

The Appendix is available online: <http://dx.doi.org/10.1890/14-0131.1.sm>