

Testing the mechanisms of diversity-dependent overyielding in a grass species

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Abstract. Plant diversity enhances many ecosystem processes, including productivity, but these effects have been studied almost exclusively at the taxonomic scale of species. We explore the effect of intraspecific diversity on the productivity of a widespread and dominant grassland species using accessions collected from populations throughout its range. We found that increasing population/ecotype diversity of *Pseudoroegneria spicata* increased productivity to a similar degree as that reported for species diversity. However, we did not find evidence that overyielding was related to either resource depletion or to pathogenic soil fungi, two causes of overyielding in species-diverse communities. Instead, larger accessions overyielded at low diversity at the expense of smaller accessions, and small accessions overyielded through complementarity at all levels of diversity. Furthermore, overyielding was stronger for accessions from mesic environments, suggesting that local adaptation might predictably influence how plants respond to increases in diversity. This suggests that mass-based competition or other cryptic accession-specific processes had complex but important effects on overyielding. Our results indicate that the effects of diversity within a species can be substantial but that overyielding by intraspecifically diverse populations may not be through the same processes thought to cause overyielding in species diverse communities.

Key words: biodiversity; complementarity; dominance effects; nitrogen use efficiency; overyielding; plant–plant interactions; productivity; *Pseudoroegneria spicata*; resource use; selection effects; soil fungi.

INTRODUCTION

Species diversity can have strong positive effects on ecosystem function (Tilman et al. 1996, Knops et al. 1999, Hooper et al. 2005, Balvanera et al. 2006). Species and functional group diversity can increase the productivity of plant communities, with species-rich communities yielding on average 70% more than monocultures (Cardinale et al. 2007). Studies of such diversity-dependent overyielding have focused primarily on the diversity of species (Hooper et al. 2005, Balvanera et al. 2006) or functional groups (Díaz and Cabido 2001), as these generally define the key operational units in communities (McGill et al. 2006). However, a large but poorly understood extent of Earth's biological diversity resides within species (Garnier et al. 2004, Albert et al. 2010b). Virtually all species vary genetically within populations and across regional distributions, and many natural populations consist of functionally distinct, locally adapted ecotypes (Clausen et al. 1941, Albert et al. 2010a). Functional variation within a species appears to be high enough to have significant effects on a variety of ecosystem processes, including productivity (Cianciaruso et al. 2009). For example, Crutsinger et al. (2006) found that the productivity of

Solidago altissima stands increased with genotypic richness on a scale equivalent to changes caused by interspecific diversity in other systems. Cook-Patton et al. (2011) found that *Oenothera biennis* overyielded in response to increased intraspecific diversity. In a study of *Ammophila breviligulata*, neither increases in intraspecific richness nor interspecific richness alone caused overyielding, however considered together both forms of diversity were important for determining the productivity *A. breviligulata* populations (Crawford and Rudgers 2012). In contrast, Fridley and Grime (2010) found that variation in the genetic diversity of *Festuca ovina* did not affect productivity, but the effects of both genetic and species diversity on other processes were stronger when soil depth, and therefore resource availability, was greater. Latzel and colleagues (2013) found that epigenetic diversity of genetically similar *Arabidopsis thaliana* strains was associated with 20% increases in productivity. Overyielding in *Arabidopsis* has been associated with soil feedback, although the mechanism of this feedback remains unknown (Bukowski and Petermann 2014). Thus there is growing evidence that intraspecific diversity can have important effects on productivity, but compared to our knowledge of the mechanisms that cause species diverse communities to overyield, we know little about the mechanisms that cause overyielding of intraspecifically diverse populations.

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Interspecific diversity-dependent overyielding is most commonly ascribed to complementary variation among species, particularly in how species utilize space and resources. Increased diversity and reduced niche overlap in species-rich mixtures is thought to lead to more efficient resource use and improved performance. Direct experimental support for this hypothesis is lacking (but see Bracken and Stachowicz 2006), but increased nutrient uptake by diverse assemblages of species is common and has been interpreted as evidence that diversity-dependent overyielding is often a resource-driven process (Tilman et al. 1996, Hooper and Vitousek 1997, 1998, Bracken and Stachowicz 2006; but see Huston 1997, Petchey 2003). Specialized interactions with pathogens or herbivores can also cause overyielding, because inhibition caused by specialized herbivores (Carson and Root 2000) and pathogens (Maron et al. 2011, Schnitzer et al. 2011, Kulmatiski et al. 2012) is weaker in more diverse communities. Such effects result principally from variation among community members rather than from particular interactions between neighbors, and they have been termed complementarity effects. Competition can also produce overyielding by particular species, as strong competitors will tend to overyield in mixtures and weak competitors will tend to underyield (Huston 1997). Such dominance of an individual species is called the ecological selection effect (Loreau and Hector 2001), the selection probability effect (Huston 1997), or the dominance effect (Fox 2005). We use the latter term because the processes that cause the dominance effect are not clearly analogous to selection (Fox 2005). Size-dependent competitive ability is the most commonly cited cause of ecological dominance effects, partly because it can be easily measured in experimental manipulations of community diversity (Loreau and Hector 2001, Fox 2005) and partly because size is known to have important effects on competitive ability.

We investigated the processes responsible for overyielding in experimental assemblages of one species, the bunchgrass *Pseudoroegneria spicata* (see Plate 1), in which experimentally constructed assemblages varied in the number of accessions represented. These accessions represent 12 different *Pseudoroegneria* populations from throughout the native North American range of *Pseudoroegneria*. Manipulating population, or ecotypic, variation rather than within-population variation also allowed us to expose the maximum functional variation in *Pseudoroegneria* to detect causes of overyielding, which was the primary goal of our study. It also enabled us to observe how home climate influenced overyielding responses. We investigated accession-specific responses further by treating half our plots with a fungicide intended to suppress pathogenic fungi, while leaving mycorrhizal fungi intact (Maron et al. 2011). Specifically, we sought to determine (1) whether intraspecific diversity affects the productivity of *Pseudoroegneria*, (2) whether overyielding is due to size-dependent or size-

independent effects, (3) whether overyielding is due to accession-specific responses associated with patterns of local adaptation, and (4) whether changes in productivity are related to resource use or interactions with soil fungi.

METHODS

We obtained seeds of *Pseudoroegneria* from 12 sites throughout western North America. Seeds were field collected in Montana or acquired from true-bred lines managed by the USDA Plant Germplasm Introduction and Testing Research Station in Pullman, Washington, USA. The one exception was a wild-selected cultivar from southeastern Washington, Goldar, which we purchased because of problems with seed viability of some of the naturally collected accessions. For each seed locality, we obtained PRISM climate data compiled by the Centre for Forest Conservation Genetics at the University of British Columbia (data available online).⁴ Climate data were reduced by unrotated principle components analysis (see Appendix: Table A1) using package psych (Revelle 2013) in R. Factors with low communalities (<0.500) were removed for final analysis. Although three components passed the Kaiser criterion (Eigenvalue ≥ 1), only two were retained to simplify interpretation and analysis. Increased values of the first component (temperature) corresponded to warmer conditions, and increased values of the second component (aridity) corresponded to drier conditions.

Seeds from each accession were grown for two months in starter pots in a greenhouse, then transplanted into 0.96×0.64 m common garden plots with 24 plants/plot at Fort Missoula, Missoula, Montana, USA, in March of 2010. Plots had four levels of richness (1, 3, 8, or 12 accessions per plot). Fungicide was applied in April and June of 2010 and 2011, consisting of 1 g/m^2 of Cleary 3336 WP (active ingredient, thiophanate-methyl; a systematic broad-spectrum fungicide) and $117.3 \text{ }\mu\text{L}$ Ridomil Gold EC (active ingredient, metalxyl; a soil drench preventative fungicide) following Maron et al. (2011), who found strong effects of fungicide on species-diverse plots growing in the same garden. Fungicide was not found to have significant short term effects on plant available nutrients in that study. Unsprayed plots were watered to compensate for water added during the fungicide treatment. Otherwise, no water was added once transplants had established. For the 3-, 8-, and 12-accession plots, there were six paired replicates of the control and six of the fungicide. Six of the 12 accessions were planted in monoculture, with 1–2 replicates of the control and fungicide treatment. When analyses required knowledge of monoculture yield, we restricted analyses to the six accessions planted in monoculture. We also extrapolated estimates of monoculture yield from regressions fitted on data from the 3-, 8-, and 12-

⁴ http://www.genetics.forestry.ubc.ca/cfcg/ClimateWNA_web/

accession plots for each accession. Both approaches yielded quantitatively and qualitatively similar results (see Appendix: Fig. A1), and we report the results of both together throughout this manuscript.

Accessions were grown for two full growing seasons (March 2010–November 2011). Accounting for water added during fungicide application and controls, the common garden conditions were close to the average of the home climates of the different accessions, but slightly cooler and wetter (not more than 0.25 SD from the average value for all variables except annual mean temperature). In November 2011, we harvested, dried, and weighed the aboveground biomass from each plot. Dried plant material was pooled for each plot, ground, and analyzed for elemental carbon and nitrogen at the University of Montana Environmental Biogeochemistry Laboratory, Missoula, Montana, USA. Immediately after harvest, three 15 cm depth soil cores were taken from each plot and from six locations between plots and extracted overnight in 2 mol/L KCl buffer and colorimetrically analyzed for ammonium and nitrate.

In order to determine whether home climate influenced how accessions responded to increased richness, we used model-averaging. This approach can be used to analyze complex models that are susceptible to overfitting when full models are tested and bias when stepwise elimination is used to reduce complexity (Burnham and Anderson 2002). Model-averaging uses sample-size corrected AIC scores (AIC_c) to compare the information quality of models containing every allowed subset of predictor variables. It then averages parameter estimates from a specified top subset of models (based on AIC_c score; we used $\Delta AIC_c \leq 10$), weighted according to the information quality of the model. Parameters absent from a given model are set to zero. These parameter estimates are equal in sign and scale to those from conventional linear models and can be interpreted in the same way. This approach is gaining increasing traction in the ecological sciences due to its ability to provide accurate and unbiased parameter estimates for models with an unavoidably large number of terms (Johnson and Omland 2004).

We used natural log-transformed average per-plant biomass and relative overyielding (ΔRY) as response variables. We calculated relative overyielding following Loreau and Hector (2001). This represents the relative increase in yield of an accession grown in polyculture compared to monoculture. Positive values of ΔRY indicate overyielding and negative values indicate underyielding. Because this metric is standardized according to monoculture yield and relative abundance in a plot, it can be compared across accessions and richness treatments. In each of these models, richness levels (3, 8, and 12 accessions) and treatment (fungicide vs. control) were treated as fixed factors with polynomial contrasts specified a priori. Monocultures were not included because while all accessions were grown in equal abundance in polyculture, not all accessions were

grown in monoculture. Home temperature and aridity were used as covariates in both models and monoculture yield was used as a covariate in models of ΔRY . Aridity was excluded from models using only the six accessions with known monoculture yield, because it correlated strongly with monoculture yield in these accessions. Plot was entered as a random factor. Second-order interactions between two factors and between one factor and one covariate were tested. Candidate models were fitted using the lmer function from package lme4 in R (Bates et al. 2014), and model-averaging was done using R package MuMIn (Barton 2013). Results from Goldar were excluded a priori from analyses involving climate to avoid biasing the results by including a yield-selected cultivar from a particularly wet climate. All response variables and continuous predictors were standardized ($\bar{x} = 0$, $s = 0.5$) to facilitate parameter comparison and ensure that averaging performed correctly (Grueber et al. 2011). The average biomass or relative overyielding of each accession in each plot constituted a sample.

To test whether overyielding was due to size-based competition, we partitioned overall biodiversity effects (BE) into three independent components: the size-independent effect (SIE), the size-dependent dominance effect (SDDE), and size-dependent complementarity effect (SDCE) effects of biodiversity after Fox (2005). Fox (2005) referred to these as trait-dependent and trait-independent effects because they can be generalized to many traits, but we refer to them as size-dependent and size-independent effects because size was the trait we used to partition diversity effects. SIE quantifies average overyielding among accessions that is independent of the monoculture size of the accession. This is the same as Loreau and Hector's (2001) complementarity effect (CE), but because it can be influenced by processes other than complementarity (Petchey 2003, Fox 2005), we call it the size-independent effect rather than the complementarity effect. SDDE quantifies size-dependent dominance effects that incur costs to neighbors. In other words, a positive SDDE means that large accessions overyield at the expense of small accessions. In contrast, SDCE quantifies size-dependent complementarity effects that do not incur a cost to neighbors. When SDCE is positive, large accessions overyield and small accessions are unaffected. When SDCE is negative, small accessions overyield but large accessions are unaffected. Thus, the relative importance of SDDE and SDCE describe the extent to which size-dependent overyielding is a zero-sum game. We investigated the effects of richness on SIE, SDDE, and SDCE using a series of fully factorial Type III ANOVAs with richness and treatment as fixed factors and SIE_a , SIE_p , $SDDE_a$, $SDDE_p$, $SDCE_a$, and $SDCE_p$ as response variables, where a is actual and p is projected. These were done using the car package (Fox and Weisberg 2002). Again a polynomial contrast for richness was specified a priori.

To investigate how soil and plant nitrogen content correlated with size-independent overyielding, we used

TABLE 1. ANOVA analyses of the effects of richness and fungicide treatment on yield, SIE (size-independent effects), SDDE (size-dependent dominance effects), and SDCE (size-dependent complementarity effects).

Yield or effect	Fungicide (df = 1, 30/32)	Richness (df = 2, 32/30)	Linear (df = 32/30)	Quadratic (df = 32/30)
Yield				
<i>B</i>	-13.94	...	70.96	-19.12
<i>F</i> or <i>t</i>	0.155	2.874	2.315	-0.624
<i>P</i>	0.696	0.071	0.027	0.537
SIE				
Actual				
<i>B</i>	9.77	...	99.05	-125.90
<i>F</i> or <i>t</i>	0.050	8.520	2.567	-3.367
<i>P</i>	0.825	0.01	0.016	0.002
Projected				
<i>B</i>	-2.91	...	97.3	-59.5
<i>F</i> or <i>t</i>	0.008	7.869	3.384	-2.071
<i>P</i>	0.931	0.02	0.002	0.047
SDDE				
Actual				
<i>B</i>	-74.63	...	-83.13	131.93
<i>F</i> or <i>t</i>	1.988	5.562	-1.779	2.913
<i>P</i>	0.169	0.009	0.085	0.007
Projected				
<i>B</i>	-8.41	...	-31.41	14.151
<i>F</i> or <i>t</i>	0.391	4.378	-2.698	1.216
<i>P</i>	0.536	0.021	0.011	0.233
SDCE				
Actual				
<i>B</i>	38.42	...	42.27	-58.20
<i>F</i> or <i>t</i>	2.473	5.519	1.960	-2.785
<i>P</i>	0.126	0.009	0.059	0.009
Projected				
<i>B</i>	-3.023	...	4.381	-4.383
<i>F</i> or <i>t</i>	0.240	0.672	0.820	-0.820
<i>P</i>	0.628	0.518	0.418	0.418

Notes: Results from the six accessions with known monoculture yields (actual; df = 30) are printed alongside results using estimated monoculture yields for eleven accessions (projected; df = 32). Estimated effect sizes are given (*B*) where appropriate, along with test statistics (*F* for omnibus tests, *t* for contrasts) and *P* values. Ellipses indicate no data are available.

Pearson correlations. Selected correlations between overyielding partitions, soil NH_4^+ ($\mu\text{g/g}$ soil), soil NO_3^- ($\mu\text{g/g}$ soil), and plant elemental N (% dm) were evaluated. We also performed a MANOVA analysis with soil NH_4^+ , soil NO_3^- , and plant total N as response variables, and richness and fungicide treatment as fixed factors, in order to investigate how patterns of nitrogen use responded to experimental treatments, using the Manova function in package car (Fox and Weisberg 2002). All analyses were performed in R version 3.0.1 (R Development Core Team 2012).

RESULTS

The 8- and 12-accession plots were 20–25% more productive than the monocultures due to complex effects of richness on both per-plant biomass and relative overyielding (Table 1, Fig. 1). The effects of richness on both ln-biomass and ΔRY were evident in models using both projected data and for only accessions with known

monoculture yield. In general, these effects were caused by underyielding of the 3-accession plots and equivalent overyielding of both the 8- and 12-accession plots (Fig. 2). Home aridity, but not temperature, had effects in the best models involving all accessions, but it was replaced by temperature in models involving only the accessions with known monoculture yields (Table 2). We note that aridity was not entered into models involving accessions with known monoculture yields due to a high correlation between aridity and monoculture yield for these accessions. In general, accessions from either warmer or higher rainfall climates were larger and more likely to overyield (see Appendix: Fig. A2).

Monoculture yield had positive effects on biomass but negative effects on overyielding (Table 2). In other words, accessions that were large in monoculture were also large in polyculture, but they benefitted less from growing in polyculture than did smaller accessions. For accessions with known monoculture yields, monoculture

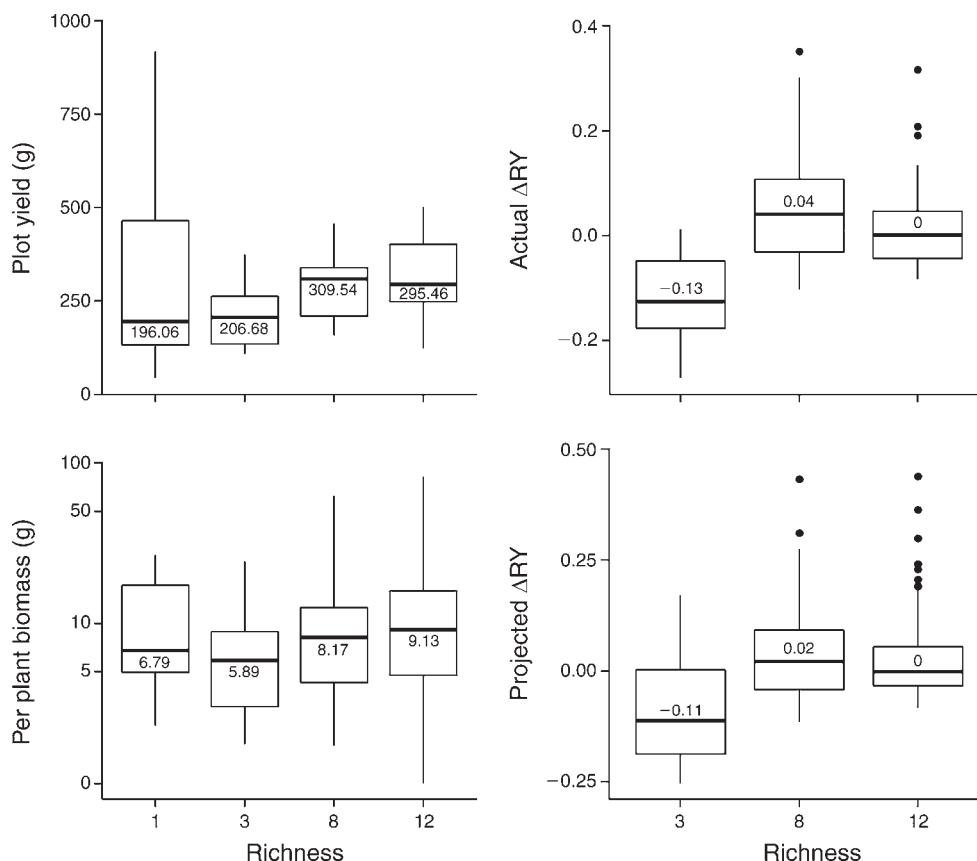


FIG. 1. Boxplots showing total yield, per plant biomass, relative overyielding for accessions with known monoculture yields (actual ΔRY), and relative overyielding for all accessions using estimated monoculture yields (projected ΔRY), at each level of richness. Boxes describe the median (thick bar and value), 25% and 75% quartiles (box edges), and 5% and 95% quantiles (whiskers). Outliers are shown as dots. Median values are shown for each plot. Monoculture yields are shown for reference but cannot be directly compared to polyculture yields because accessions are not equally represented in monoculture vs. polyculture.

yield interacted with richness to influence overyielding. Partitioning of biodiversity effects corroborated these results (Table 1). Large accessions had a small dominance (i.e., zero-sum) advantage over small accessions at low richness and a small disadvantage at high richness (SDDE; Table 1, Fig. 3). However, small accessions benefitted from weak complementarity effects at all levels of richness (SDCE; Table 1, Fig. 3). The sum of these effects, i.e., the net size-dependent effect, was not significantly different from zero, and it was not affected by richness ($P \geq 0.092$). Thus while there were nuanced interactions between size and overyielding, these cancelled each other out. As a result, most of the overyielding detected in this experiment was due to size-independent complementarity effects (SIE; Table 1, Fig. 3). These could have been true complementarity effects or they could be trait-dependent effects caused by unknown traits that were not correlated with size.

The best models identified by multimodel inference tended to have strong, but not unequivocal support (with weights being between 0.334 and 0.774; Table 3). In general, support was stronger for top models fitted on

all accessions than those fitted on accessions with known monoculture yields. Variables present in the top model were usually present in most or all other models with $\Delta AIC_c \leq 10$, with other variables being found only rarely and sporadically in the poorer models. As a result, variable importance was generally either high or very low, providing a clear picture of the relationship between predictor and response variables. These predictors also had statistically significant effects in the top models (Table 3), however we caution against basing inference on these results (Symonds and Moussalli 2010).

Both net biodiversity effects and SIE were stronger in plots with higher soil nitrate concentrations (BE_a , $r = 0.347$, $P = 0.048$; BE_p , $r = 0.327$, $P = 0.055$; SIE_a , $r = 0.368$, $P = 0.035$; SIE_p , $r = 0.342$, $P = 0.045$; see Appendix: Table A2), and accessions in overyielding plots had lower N concentrations in their aboveground tissue (BE_a , $r = -0.370$, $P = 0.031$; BE_p , $r = -0.313$, $P = 0.063$). Also, MANOVA indicated that soil available ammonium, soil available nitrate, and plant [N] were not affected by fungicide treatment or plot richness, either

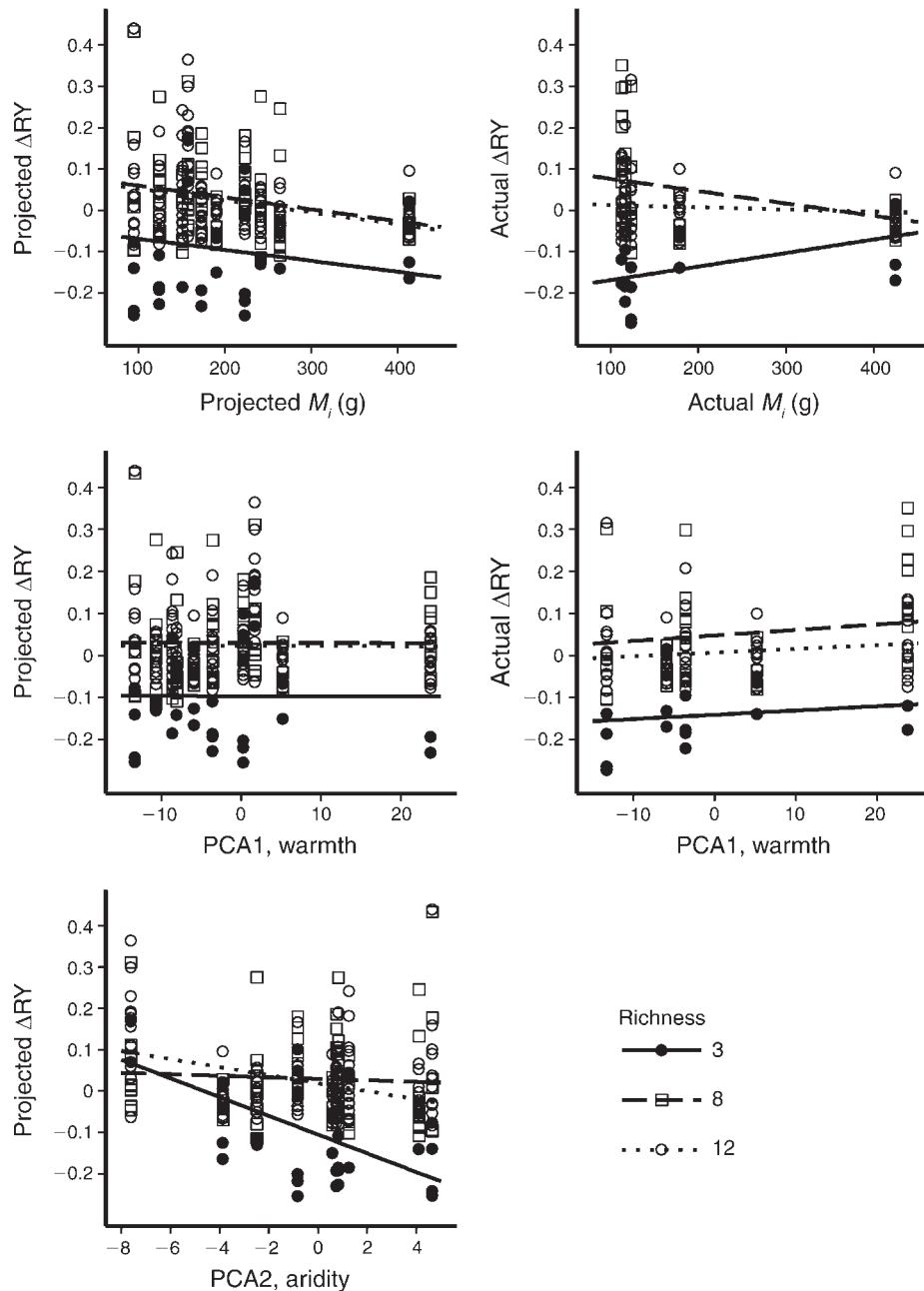


FIG. 2. Effects of monoculture yield (M_i), home climate temperature (PCA1), and home climate aridity (PCA2) on relative overyielding (ΔRY). Points show average ΔRY of each accession in each plot, with shape indicating plot richness (solid circles, three accessions; open squares, eight accessions; open circles, 12 accessions). Lines show linear relationships between predictor and response variables, estimated by full model averaging, with line type indicating plot richness (solid, three accessions; dashed, eight accessions; dotted, 12 accessions). The left column shows models fitted to all accessions, and the right shows models fitted only to accessions with measured monoculture yields.

together (see Appendix: Table A3; $P \geq 0.332$) or when analyzed for univariate effects ($P \geq 0.212$). Planted plots had 25% lower nitrate concentrations than unplanted bare soil ($t_{57} = -1.996$, $P = 0.051$). Fungicide treatment did not have significant effects in any aspect of our experiment.

DISCUSSION

The productivity of artificially assembled *Pseudoroegneria spicata* populations increased with ecotypic richness; populations containing 12 accession yielded roughly 25% more biomass than monocultures (Fig. 1). These yield increases were not caused by sampling

TABLE 2. Results of averaged models predicting ln-transformed aboveground biomass and relative overyielding.

Model and parameter	Projected		Actual	
	Importance	β	Importance	β
Log-per plant biomass				
Intercept	1.00	-0.039	1.00	-0.021
Richness (L)	0.28	-0.059	0.25	0.045
Richness (Q)	0.28	-0.038	0.25	-0.054
Fungicide	0.08	0.001	0.11	0.006
Monoculture yield	1.00	0.275	1.00	0.531
Temperature	0.04	0.000	0.59	0.113
Precipitation	1.00	-0.265	NE	NE
Monoculture yield \times richness (L)	a	a	0.08	-0.015
Monoculture yield \times richness (Q)	a	a	0.08	0.030
Precipitation \times richness (L)	a	a	NE	NE
Precipitation \times richness (Q)	a	a	NE	NE
Overyielding (Δ RY)				
Intercept	1.00	-0.102	1.00	-0.128
Richness (L)	1.00	0.385	1.00	0.457
Richness (Q)	1.00	-0.250	1.00	-0.412
Fungicide	0.06	0.000	0.08	0.005
Monoculture yield	0.99	-0.224	0.77	-0.001
Temperature	0.07	-0.005	0.63	0.122
Precipitation	1.00	-0.371	NE	NE
Monoculture yield \times richness (L)	0.05	-0.012	a	a
Monoculture yield \times richness (Q)	0.05	0.005	a	a
Temperature \times richness (L)	a	a	0.13	-0.010
Temperature \times richness (Q)	a	a	0.13	-0.033
Precipitation \times richness (L)	0.94	0.299	NE	NE
Precipitation \times richness (Q)	0.94	-0.381	NE	NE

Notes: Analyses done on all accessions (projected) and on those with known monoculture yields (actual) are shown; Q represents quadratic and L represents linear. Importance describes the weighted proportion of top models containing each factor. Standardized parameter estimates (β) are also shown. Parameters with significance ≥ 0.5 are bold; those with significance ≤ 0.01 are not shown or labeled absent. We use absence to distinguish from parameters that were not entered. Parameters were not entered into the full model due to collinearity with other predictors. Top models from each analysis are shown in Table 3. Random effects of plot were modeled but are not shown. NE is not entered, a is absence.

during population assembly, but were instead caused by overyielding of individual accessions at high richness. Several other recent studies have also shown that strong overyielding can result from within-species diversity (Reusch et al. 2005, Crutsinger et al. 2006, Milla et al. 2009, Kotowska et al. 2010, Cook-Patton et al. 2011) and that such overyielding can be similar in scale to yield increases associated with high interspecific diversity (Crutsinger et al. 2006, Cook-Patton et al. 2011, Crawford and Rudgers 2012; but see Fridley and Grime 2010). Our results contribute to this growing body of evidence and provide new insight into the mechanistic causes of overyielding of intraspecific diversity.

One of these insights derives from mathematical partitioning of the overall effect of biodiversity, which shows that size-dependent effects had small, complex effects on yield (Table 1, Fig. 3). At low levels of ecotypic diversity, we found positive size-dependent dominance effects (SDDE), meaning that large accessions overyielded at the expense of smaller accessions. We suggest that in the 3-accession plots the frequency of encounters between large and small accessions were high, allowing large accessions to exert substantial

competitive dominance. As diversity increased, SDDE weakened, perhaps because interactions between accessions became more diffuse. In contrast to dominance effects, size-dependent complementarity effects (SDCE) were weakly negative at all levels of diversity, meaning that in general small accessions overyielded with no cost to the larger accessions. It is unclear what processes could lead to preferential overyielding of small accessions, although weakly negative size-dependent effects are common within species (Reusch et al. 2005, Cook-Patton et al. 2011; but see Roscher et al. 2007) and among species (Cardinale et al. 2007). Furthermore, using the same *Pseudoroegneria* accessions used in this study, it has recently been shown that neighbor recognition may play a role in the differential responses of *Pseudoroegneria* accessions to neighbors from the same vs. different accessions (Yang et al. 2015), although it is unknown whether or how this would lead to preferential overyielding of small accessions.

Another important insight is that the processes that cause size-independent overyielding in species-diverse communities did not appear important in the overyielding of ecotypically diverse *Pseudoroegneria* popu-

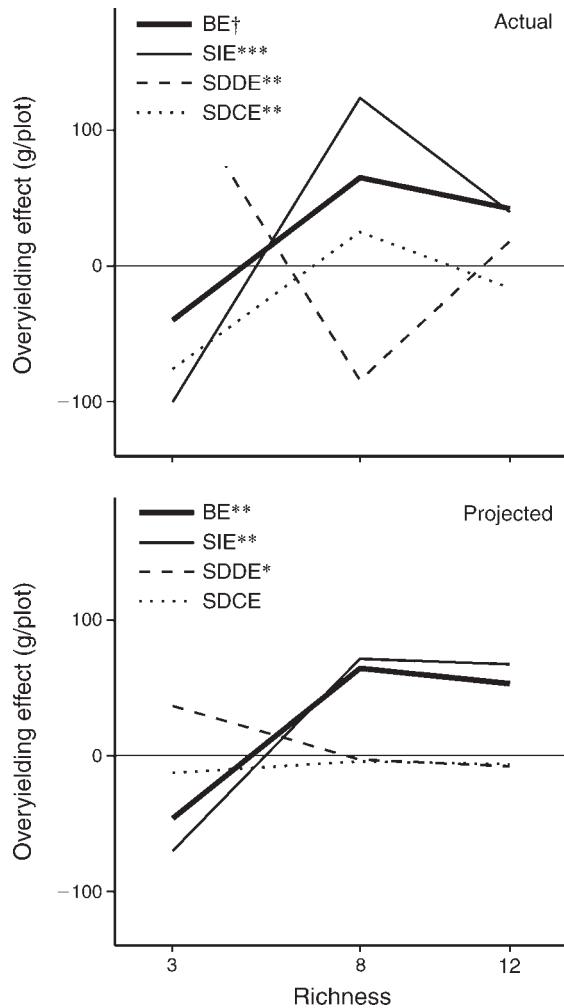


FIGURE 3. Relationships between richness and overyielding for accessions with known monoculture yields (actual) and for all accessions (projected). The total biodiversity effect in each polyculture plot (BE; thick line) is partitioned into three components: size-independent overyielding (SIE; thin line), size-dependent overyielding (SDDE; dashed line), and size-independent overyielding (SDC; dotted line). Significant effects of richness are indicated.

* $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$; † $P = 0.098$.

lations, despite the importance of size-independent (i.e., complementary) overyielding in this study (Fig. 3). Resource complementarity is by far the most commonly cited cause of size-independent overyielding in species diverse communities (see reviews by Díaz and Cabido 2001, Loreau and Hector 2001, Cardinale et al. 2007), and negative correlations between soil nitrogen and species richness have been taken as evidence for complementary resource partitioning (Tilman et al. 1996, Hooper and Vitousek 1997). However, plant-available nitrogen in soil did not decrease in our overyielding plots. Instead, we observed that soil nitrogen was higher and plant nitrogen was lower in



PLATE 1. *Pseudoroegneria spicata* is a large, perennial bunchgrass and a dominant species in the montane grasslands of the western U.S. Photo credit: D. Z. Atwater.

overyielding plots. Low nitrogen concentrations in overyielding plants have been attributed to increased nitrogen use efficiency or increased allocation to nitrogen poor tissue (e.g., stem tissue; van Ruijven and Berendse 2005). We did not manipulate nitrogen availability nor did we follow changes in nitrogen concentrations through the growing season, thus we cannot propose causal relationships between accession performance and soil and plant nitrogen concentrations. Nonetheless, our results are not consistent with the hypothesis that size-independent overyielding was due to increased uptake of a nutrient thought to be limiting. These results imply that resource partitioning may not be responsible for the complementary overyielding done by genetically diverse populations.

Soil pathogens can also have important size-independent effects on yield, primarily by suppressing growth in communities with few species (Maron et al. 2011, Schnitzer et al. 2011). However, we found no evidence that soil fungi influenced overyielding of *Pseudoroegneria*, despite the fact that fungicide applied in the same manner had strong effects on overyielding of experimentally assembled communities of different

TABLE 3. Top models (those with the lowest Akaike's information criterion corrected for sample sizes, AIC_c).

Models	Actual				Projected			
	β	df	<i>t</i>	<i>P</i>	β	df	<i>t</i>	<i>P</i>
Biomass								
Monoculture yield	0.55 ± 0.07	89.5	7.357	<0.001	0.27 ± 0.06	200.4	4.928	<0.001
Temperature	0.19 ± 0.07	88.9	2.617	0.010	a	a	a	a
Precipitation	NI	NI	NI	NI	-0.27 ± 0.06	201.2	-4.786	<0.001
AIC _c	140.0	279.5
Weight	0.423	0.625
Next best model AIC _c	141.1	281.7
No. models Δ AIC _c ≤ 2	1	0
Relative Overyielding (Δ RY)								
Richness (L)	0.46 ± 0.09	43.9	5.341	<0.001	0.38 ± 0.07	49.3	5.190	<0.001
Richness (Q)	-0.41 ± 0.08	39.5	-4.974	<0.001	-0.25 ± 0.07	38.1	-3.607	<0.001
Monoculture yield	0.01 ± 0.08	93.4	0.095	0.925	-0.23 ± 0.06	202.0	-3.986	<0.001
Temperature	0.18 ± 0.07	87.6	2.501	0.014	a	a	a	a
Precipitation	NI	NI	NI	NI	-0.38 ± 0.07	218.6	-5.452	<0.001
Monoculture yield × richness (L)	-0.37 ± 0.12	90.1	-2.992	0.004	a	a	a	a
Monoculture yield × richness (Q)	0.50 ± 0.14	99.7	3.622	<0.001	a	a	a	a
Precipitation × richness (L)	NI	NI	NI	NI	0.32 ± 0.13	220.8	2.563	0.011
Precipitation × richness (Q)	NI	NI	NI	NI	-0.41 ± 0.10	215.0	-3.957	<0.001
AIC _c	138.7	289.4
Weight	0.334	0.774
Next best model AIC _c	139.0	294.3
No. models Δ AIC _c ≤ 2	1	0

Notes: Models with weight closer to one have the most support. These data are provided for reference only. Although support for the top models is strong, it is not unequivocal. We recommend that inferences be based on the results of model averaging shown in Table 2. Analyses done on all accessions (projected) and on those with known monoculture yields (actual) are shown. Standardized parameter estimates (β) are also shown as mean ± SE along with estimated df, *t*, and *P* values. Random effects of plot were modeled but not shown. NI is not included, a is absence, and ellipses are no data.

species in the same experimental garden (Maron et al. 2011). *Pseudoroegneria* was not a study species used by Maron et al. (2011), and it is possible that it is less susceptible to self-limitation caused by fungal pathogens than other species. It is also possible that fungal pathogens of *Pseudoroegneria* did not distinguish among the accessions used in this study and so were insensitive to intraspecific variation.

Not all *Pseudoroegneria* accessions responded in the same way to increased richness. Our analyses indicated that wet-environment accessions overyielded more than dry-environment accessions (Table 2, Fig. 2). It is possible that adaptation to more productive habitats gave wet-environment accessions a size-independent competitive advantage over accessions from dry climates or that they experienced stronger plastic responses to ecological release or neighbor unfamiliarity at high richness. This discovery is important because it suggests that plant genotypes or species may react differently to increased biodiversity in a predictable, but size-independent manner. Thus, size may not be the only quantifiable trait that influences overyielding, and some overyielding ascribed to complementarity effects may instead result from predictable trait-mediated reactions to increased diversity.

We manipulated functional diversity by assembling *Pseudoroegneria* accessions collected from throughout the USA, meaning that overyielding resulted from interactions among genotypes that would not be

expected to co-occur in nature. We did this to maximize functional variation (Milla et al. 2009) and also to investigate how home climate influenced patterns of overyielding. However, this design limited our ability to infer the importance of overyielding in naturally assembled communities. Moreover, just as we observed that the mechanisms of within-species overyielding do not appear to be the same as the mechanisms of among-species overyielding, it is possible that the mechanisms of within-species overyielding change depending upon the relatedness of the population and the source of functional variation in a population. For example, kin recognition might play a role in yield responses that depend on familial kinship, and disease might play a role in yield responses that depend on a courser source of within-species variation. While evidence of the importance of among-species overyielding is mounting (Hooper et al. 2005, Balvanera et al. 2006, Cianciaruso et al. 2009), comparatively little is known about the ecological role of within-species overyielding, although recent evidence suggests it may be significant (Crutsinger et al. 2006, Crawford and Rudgers 2012). There is a continued need for more research into the exact causes and consequences of yield responses to within-species variation in natural communities.

Understanding the mechanisms behind of intraspecific overyielding will require further study, but our results indicate that overyielding by *Pseudoroegneria* was caused by a mix of size-dependent, individual-accession

dominance effects, size-dependent complementarity effects, and true complementarity effects. Thus, the ecological causes of within-species overyielding are likely to be complex. Furthermore, our results highlight that prominent causes of overyielding in species-diverse communities are not necessarily the same as the causes of overyielding in intraspecifically diverse populations, even though the ecological effects of both forms of diversity are similar. Our results also emphasize the continued need for controlled, mechanistic studies of diversity-dependent overyielding. Expanding our knowledge of how intraspecific variation affects ecosystem functioning may be crucial for predicting the effects of anthropogenic species losses and gains on community productivity (Wardle et al. 2011) and for improving our understanding of how assemblages of organisms function in nature (Hughes et al. 2008, Brooker et al. 2009, Cianciaruso et al. 2009).

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LITERATURE CITED

- Albert, C. H., W. Thuiller, N. G. Yoccoz, R. Douzet, S. Aubert, and S. Lavorel. 2010a. A multi-trait approach reveals the structure and the relative importance of intra- vs. interspecific variability in plant traits. *Functional Ecology* 24:1192–1201.
- Albert, C. H., W. Thuiller, N. G. Yoccoz, A. Soudant, F. Boucher, P. Saccone, and S. Lavorel. 2010b. Intraspecific functional variability: extent, structure and sources of variation. *Journal of Ecology* 98:604–613.
- Balvanera, P., A. B. Pfisterer, N. Buchmann, J.-S. He, T. Nakashizuka, D. Raffaelli, and B. Schmid. 2006. Quantifying the evidence for biodiversity effects on ecosystem functioning and services. *Ecology Letters* 9:1146–1156.
- Barton, K. 2013. MuMIn: Multi-Model Inference. R package, version 1.13.4. <http://CRAN.R-project.org/package=MuMIn>
- Bates, D. M., M. Maechler, B. Bolker, and S. Walker. 2014. lme4: linear mixed-effects models using Eigen and S4. R package, version 1.1-7. <http://CRAN.R-project.org/package=lme4>
- Bracken, M. E., and J. J. Stachowicz. 2006. Seaweed diversity enhances nitrogen uptake via complementarity use of nitrate and ammonium. *Ecology* 87:2397–2403.
- Brooker, R. W., R. M. Callaway, L. A. Cavieres, Z. Kikvidze, C. J. Lortie, R. Michalet, F. I. Pugnaire, A. Valiente-Banuet, and T. G. Whitham. 2009. Don't diss integration: a comment on Ricklefs's disintegrating communities. *American Naturalist* 174:919–927; discussion 928–931.
- Bukowski, A. R., and J. S. Petermann. 2014. Intraspecific plant-soil feedback and intraspecific overyielding in *Arabidopsis thaliana*. *Ecology and Evolution* 4:2533–2545.
- Burnham, K. P., and D. R. Anderson. 2002. Model selection and multimodel inference. Springer, New York, New York, USA.
- Cardinale, B. J., J. P. Wright, M. W. Cadotte, I. T. Carroll, A. Hector, D. S. Srivastava, M. Loreau, and J. J. Weis. 2007. Impacts of plant diversity on biomass production increase through time because of species complementarity. *Proceedings of the National Academy of Sciences USA* 104:18123–18128.
- Carson, W. P., and R. B. Root. 2000. Herbivory and plant species coexistence: community regulation by an outbreak phytophagous. *Ecological Monographs* 70:73–99.
- Cianciaruso, M., M. Bathala, K. Gaston, and O. L. Petchey. 2009. Including intraspecific variability in functional diversity. *Ecology* 90:81–89.
- Clausen, J., D. D. Keck, and W. M. Hiesey. 1941. Regional differentiation in plant species. *American Naturalist* 75:231–250.
- Cook-Patton, S. C., S. H. McArt, A. L. Parachnowitsch, J. S. Thaler, and A. A. Agrawal. 2011. A direct comparison of the consequences of plant genotypic and species diversity on communities and ecosystem function. *Ecology* 92:915–923.
- Crawford, K. M., and J. A. Rudgers. 2012. Plant species diversity and genetic diversity within a dominant species interactively affect plant community biomass. *Journal of Ecology* 100:1512–1521.
- Crutsinger, G. M., M. D. Collins, J. A. Fordyce, Z. Gompert, C. C. Nice, and N. J. Sanders. 2006. Genotypic diversity and ecosystem process predicts community structure and governs an ecosystem process. *Science* 313:966–968.
- Diaz, S., and M. Cabido. 2001. Vive la différence: plant functional diversity matters to ecosystem processes. *Trends in Ecology and Evolution* 16:646–655.
- Fox, J., and S. Weisberg. 2002. An {R} companion to applied regression. Sage Publications, Thousand Oaks, California, USA.
- Fox, J. W. 2005. Interpreting the “selection effect” of biodiversity on ecosystem function. *Ecology Letters* 8:846–856.
- Fridley, J. D., and J. P. Grime. 2010. Community and ecosystem effects of intraspecific genetic diversity in grassland microcosms of varying species diversity. *Ecology* 91:2272–2283.
- Garnier, E., et al. 2004. Plant functional markers capture ecosystem properties during secondary succession. *Ecology* 85:2630–2637.
- Graeber, C. E., S. Nakagawa, R. J. Laws, and I. G. Jamieson. 2011. Multimodel inference in ecology and evolution: challenges and solutions. *Journal of Evolutionary Biology* 24:699–711.
- Hooper, D. U., et al. 2005. Effects of biodiversity on ecosystem functioning: a consensus of current knowledge. *Ecological Monographs* 75:3–35.
- Hooper, D. U., and P. M. Vitousek. 1997. The effects of plant composition and diversity on ecosystem processes. *Science* 277:1302–1305.
- Hooper, D., and P. Vitousek. 1998. Effects of plant composition and diversity on nutrient cycling. *Ecological Monographs* 68:121–149.
- Hughes, A. R., B. D. Inouye, M. T. J. Johnson, N. Underwood, and M. Vellend. 2008. Ecological consequences of genetic diversity. *Ecology Letters* 11:609–623.
- Huston, M. A. 1997. Hidden treatments in ecological experiments: re-evaluating the ecosystem function of biodiversity. *Oecologia* 110:449–460.
- Johnson, J. B., and K. S. Omland. 2004. Model selection in ecology and evolution. *Trends in Ecology and Evolution* 19:101–108.
- Knops, M. J. H., et al. 1999. Effects of plant species richness on invasion dynamics, disease outbreaks, insect abundances and diversity. *Ecology Letters* 2:286–293.
- Kotowska, A. M., J. F. Cahill, Jr., and B. A. Keddie. 2010. Plant genetic diversity yields increased plant productivity and herbivore performance. *Journal of Ecology* 98:237–245.

- Kulmatiski, A., K. H. Beard, and J. Heavilin. 2012. Plant–soil feedbacks provide an additional explanation for diversity–productivity relationships. *Proceedings of the Royal Society B: Biological Sciences* 279:3020–3026.
- Latzel, V., E. Allan, A. Bortolini Silveira, V. Colot, M. Fischer, and O. Bossdorf. 2013. Epigenetic diversity increases the productivity and stability of plant populations. *Nature Communications* 4:2875.
- Loreau, M., and A. Hector. 2001. Partitioning selection and complementarity in biodiversity experiments. *Nature* 412:72–76.
- Maron, J. L., M. Marler, J. N. Klironomos, and C. C. Cleveland. 2011. Soil fungal pathogens and the relationship between plant diversity and productivity. *Ecology Letters* 14: 36–41.
- McGill, B. J., B. J. Enquist, E. Weiher, and M. Westoby. 2006. Rebuilding community ecology from functional traits. *Trends in Ecology and Evolution* 21:178–185.
- Milla, R., D. M. Forero, A. Escudero, and J. M. Iriondo. 2009. Growing with siblings: a common ground for cooperation or for fiercer competition among plants? *Proceedings of the Royal Society B: Biological Sciences* 276:2531–2540.
- Petchey, O. L. 2003. Integrating methods that investigate how complementarity influences ecosystem functioning. *Oikos* 101:323–330.
- Reusch, T. B. H., A. Ehlers, and A. Ha. 2005. Ecosystem recovery after climatic extremes enhanced by genotypic diversity. *Proceedings of the National Academy of Sciences USA* 102:2826–2831.
- Revelle, W. 2013. psych: procedures for personality and psychological research. Northwestern University, Evanston, Illinois, USA. <http://CRAN.R-project.org/package=psych>
- Roscher, C., J. Schumacher, W. W. Weisser, B. Schmid, and E.-D. Schulze. 2007. Detecting the role of individual species for overyielding in experimental grassland communities composed of potentially dominant species. *Oecologia* 154: 535–549.
- Schnitzer, S. A., et al. 2011. Soil microbes drive the classic plant diversity–productivity pattern. *Ecology* 92:296–303.
- Symonds, M. R. E., and A. Moussalli. 2010. A brief guide to model selection, multimodel inference and model averaging in behavioural ecology using Akaike’s information criterion. *Behavioral Ecology and Sociobiology* 65:13–21.
- Tilman, D., D. Wedin, and J. Knops. 1996. Productivity and sustainability influenced by biodiversity in grassland ecosystems. *Nature* 379:718–720.
- van Ruijven, J., and F. Berendse. 2005. Diversity–productivity relationships: initial effects, long-term patterns, and underlying mechanisms. *Proceedings of the National Academy of Sciences USA* 102:695–700.
- Wardle, D. A., R. D. Bardgett, R. M. Callaway, and W. H. Van der Putten. 2011. Terrestrial ecosystem responses to species gains and losses. *Science* 332:1273–1277.
- Yang, L., D. Z. Atwater, and R. M. Callaway. 2015. Root contact responses and the positive relationship between intraspecific diversity and ecosystem productivity. *Annals of Botany Plants* 7:plv053.

SUPPLEMENTAL MATERIAL

Ecological Archives

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