

# Ecotypic diversity of a dominant grassland species resists exotic invasion

Lixue Yang · Ragan M. Callaway · Daniel Z. Atwater

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**Abstract** Many species are characterized by high levels of intraspecific or ecotypic diversity, yet we know little about how diversity within species influences ecosystem processes. Using a common garden experiment, we studied how intraspecific diversity within the widespread and often dominant North American native *Pseudoroegneria spicata* (Pursh) Á. Löve. affected invasion by *Centaurea stoebe* L. We experimentally manipulated *Pseudoroegneria* intraspecific diversity by changing the number of *Pseudoroegneria* ecotypes in common garden plots, using ecotypes collected throughout western North America. Invader biomass was 46% lower in mono-ecotype *Pseudoroegneria* plots than in control plots

without any plants prior to invasion, and plots with 3–12 *Pseudoroegneria* ecotypes were 44% less invaded by *Centaurea* than the mono-ecotype plots. Across all plots, the total biomass of invading *Centaurea* plants was negatively correlated with total *Pseudoroegneria* biomass, but biotic resistance provided by high ecotypic diversity of *Pseudoroegneria* was not explained only by the increase in productivity that occurred with ecotypic diversity. Relative to *Pseudoroegneria* yield, *Centaurea* yield was lowest when *Pseudoroegneria* overyielded due to size-independent “complementarity” effects. This was not observed when overyielding was due to size-dependent effects. Our results suggest that the intraspecific diversity of a widespread and dominant species has the potential to impact invasion outcomes beyond its effects on native plant productivity and that mechanisms of biotic resistance to invaders may be to some degree independent of plant size.

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L. Yang  
School of Forestry, Northeast Forestry University,  
Harbin 150040, Heilongjiang Province, China

R. M. Callaway · D. Z. Atwater  
Division of Biological Sciences and the Institute on  
Ecosystems, The University of Montana, Missoula,  
MT 59812, USA

*Present Address:*  
D. Z. Atwater (✉)  
Department of Plant Pathology, Physiology, and Weed  
Science, Virginia Tech, 435 Old Glade Rd. 0330, Blacksburg,  
VA 24061, USA  
e-mail: danatwater@gmail.com

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## Introduction

The relationship between species diversity and ecosystem processes has been widely studied and is well understood. At the scale of local communities, species

diversity correlates positively with resistance to exotic invasion (Maron and Marler 2007, 2008), consumer diversity (Knops et al. 1999), nutrient cycling and resource consumption (Tilman et al. 1996; Hooper and Vitousek 1997; Balvanera et al. 2006; Bracken and Stachowicz 2006), and productivity (reviewed in Cardinale et al. 2007). Increases in all of these processes are thought to be due in part to increased functional diversity in species-diverse communities (Díaz et al. 2001) through more efficient resource use (Tilman et al. 1996; Hooper and Vitousek 1997; Bracken and Stachowicz 2006) and through reducing the strong effects of pathogens and consumers that occur when diversity is low (Carson and Root 2000; Maron et al. 2011; Schnitzer et al. 2011). Most studies of diversity-dependent effects on ecosystem processes have focused on the diversity of species (Hooper et al. 2005; Balvanera et al. 2006) or functional groups (Díaz et al. 2001), as these are generally define the key functional units in ecosystems (McGill et al. 2006). However, a great deal of Earth's functional diversity also resides *within* species, and intraspecific diversity can also strongly influence ecological processes (Hughes et al. 2008; Baron et al. 2015; Schöb et al. 2015).

Traits vary substantially among individuals within a species, and this can lead to high functional diversity within a single plant population (Garnier et al. 2004; Albert et al. 2010). Species also vary across their local and regional distributions with many natural populations characterized by a great deal of intraspecific variation and locally adapted genotypes (Clausen et al. 1941; Cordell et al. 1998). An extensive body of research has documented the effects of intraspecific plant diversity on ecosystem processes such as trophic interactions and disease dynamics (Hughes et al. 2008) and a number of studies have examined variation in competitive interactions associated with intraspecific variation (Turkington and Harper 1979; Turkington and Mehrhoff 1991; Jensen and Ehlers 2010; Thorpe et al. 2011; Gibson et al. 2014; Schamp and Aarssen 2014; Wang et al. 2014; Baron et al. 2015). Through processes that remain poorly understood (Atwater and Callaway 2015), intraspecific diversity also commonly increases productivity. For example, genotypic diversity of *Solidago altissima* L. increased ecosystem productivity at a scale similar to that produced by interspecific diversity (Crutsinger et al. 2006). Crawford and Whitney (2010) found that higher levels of

genetic diversity increased the overall biomass of *Arabidopsis thaliana* (L.) Heynh. in a greenhouse experiment (also see Kotowska et al. 2010). Crawford and Rudgers (2012) found no independent effects of either species diversity or genetic diversity, but instead that interactions between species diversity and genetic diversity affected ecosystem productivity.

Understanding the consequences of diversity within species may be particularly important in the context of exotic invasions (Kettenring et al. 2014). For example, there is a large body of literature on the importance of genetic diversity within populations of invasive species themselves (reviewed in Lee 2002; Roman and Darling 2007). There also are reasons to explore the role of genetic diversity within native species during invasion by exotic plants. First, invasive species have strong impacts on native plant communities and by doing so may reduce both genetic and species diversity of native plants (Mealor et al. 2004; Strauss et al. 2006), potentially weakening any positive effects of genetic diversity on ecosystem functioning. Second, through the same processes by which high species diversity improves resistance to exotic invasion (Fargione et al. 2003; Zavaleta and Hulvey 2004; Maron and Marler 2008), high genetic diversity may also increase resistance of native plant populations to invasion. For example, Weltzin et al. (2003) found that intraspecific diversity of *A. thaliana* decreased the size of a competing congener. Crutsinger et al. (2008) experimentally demonstrated that higher genotypic diversity of *S. altissima* resisted colonization in general by other species. However, a study of the effects of varietal diversity of *Poa pratensis* L. on *Taraxacum officinale* F.H. Wigg., revealed no relationship between diversity and productivity of either species (Vellend et al. 2010). Similarly, Chang and Smith (2012) saw no direct effect of naturally variable genetic diversity of *Andropogon gerardii* Vitman on *A. bladhii* (Retz.) S.T. Blake invasion success, although path analysis revealed indirect effects of diversity mediated by changes in community diversity and specific leaf area. More recently, Schöb et al. (2015) showed that genetic diversity in *Hordeum vulgare* L. led to increases in yield and weed resistance. Despite these recent advances, there is a continued need to understand how consistently genetic diversity influences ecosystem productivity and invasion resistance, and what mechanisms may be responsible.

Here we ask (1) whether high intraspecific genetic diversity in a widespread dominant intermountain grassland species provides more resistance to invasion than low intraspecific diversity, (2) whether intraspecific diversity leads to increased productivity in invaded communities, and (3) whether increased biotic resistance to invaders might be independent of effects of diversity on native plant size. In other words, does intraspecific diversity cause native plant communities to become less invasible even when their increased yield is accounted for? We explored this relationship in a common garden experiment in which we varied the numbers of native *Pseudoroegneria spicata* ecotypes in plots and then experimentally invaded these plots with seeds of *Centaurea stoebe*, a strong exotic invader that interacts with *Pseudoroegneria* throughout much of its range.

## Methods

Experimental design, planting, maintenance and harvesting

*Pseudoroegneria spicata* ecotypes were collected from 12 locations spanning a large part of the North American distribution of the species (Supplementary Fig. S1), and were grown at four levels of ecotypic richness (1-, 3-, 8-, and 12-ecotypes) in plots in a common garden. With one exception, seeds were field collected or acquired from true-bred lines collected and managed by the USDA Plant Germplasm Introduction and Testing Research Station in Pullman, WA, USA. The one exception was the purchase from a commercial vendor of seeds of a wild-selected genotype from southeastern Washington. The genetic diversity of these populations is unknown. We manipulated ecotypic diversity, rather than within-population variation, to maximize genetic variation and increase our chances of detecting effects of within-species diversity.

Seeds from each ecotype were planted into 500 cm<sup>3</sup> conical starter pots and grown for two months in a greenhouse at the University of Montana. Then transplants were placed into 96 cm × 64 cm common garden plots located at Fort Missoula, Missoula, MT, USA, early in the spring of 2010. Each plot contained 24 plants set in rows 16 cm apart. Adjacent plots were separated by 50 cm of bare ground that was

maintained by manual weeding. We established plots at four levels of ecotypic richness (1, 3, 8, and 12 ecotypes per plot). All co-planted ecotypes were equally represented. Ecotypes were randomly selected for each 3- and 8-ecotype plot and planting position was randomized. In this study the 12-ecotype plots all contained an identical assortment of ecotypes. Because the most rich treatment is fixed with respect to genotypes, one cannot separate effects of diversity at the highest level (12 ecotypes) from the particular ecotypes included.

For the 3-, 8-, and 12-ecotype plots, there were six replicates. Because of poor germination rates and problems with seed availability, we did not have enough seedlings to plant monoculture plots for each ecotype, and we could not establish six replicates for any single ecotype. We planted monoculture plots for six of the twelve ecotypes, and had 1–2 replicates for each monoculture. Monoculture yields for the remaining six ecotypes were estimated by fitting linear models of yield against richness for species with known monoculture yields, and then extrapolating monoculture yield estimates for ecotypes without known monoculture yields (Atwater and Callaway 2015). Estimated monoculture yield and relative overyielding closely matched actual values (Supplementary Fig. S2). For all analyses requiring monoculture yields, we present models based only on known monoculture yields for those six ecotypes, and also analyses based on estimated monoculture yields for all ecotypes.

*Pseudoroegneria* plants were grown for two full seasons (2010, 2011) to establish mature *Pseudoroegneria* stands for invasion, and during this time the plots were kept free of other species, mostly exotics, by hand pulling. In the first season we watered as necessary to reduce transplant stress, in the second season plots received only natural precipitation. In the fall of 2011 all aboveground biomass of *Pseudoroegneria* was removed, mimicking heavy grazing, a form of disturbance often associated with invasion, and on 10 May, 2012 we sowed 1.5 g of *Centaurea stoebe* seeds in each plot containing *Pseudoroegneria*, as well as into eight empty control plots. This timing enabled us to approximate the phenology of *Centaurea* while also allowing us to remove the first flush of non-target weeds. At the time of planting we stopped weeding species from the plots. To ensure establishment of *Centaurea* seedlings, we hand-watered the plots every

day until June 1st. Afterwards we watered each plot every third day unless interrupted by natural rainfall. On 28 August, 2012, we harvested, dried, and weighed the aboveground biomass of *Pseudoroegneria*, *Centaurea*, and all other exotic species in each plot.

### Diversity analyses

We used ANCOVA analysis to determine the relationship between plot richness and weed performance, using natural log transformed total *Centaurea* biomass as a response variable, richness as a fixed factor with a polynomial contrast, and *Pseudoroegneria* yield as a covariate. Interactions were not significant in any model, so they were excluded from final analysis (after Engqvist 2005). Type I sums of squares were calculated with the effect of *Pseudoroegneria* yield being tested first.

To determine whether invaded plots experienced diversity-dependent overyielding, we calculated the net diversity effect and partitioned it according to Fox (2005). Overyielding is defined as the difference in per-plant yield of species grown in polyculture versus monoculture (Loreau and Hector 2001). A value of zero means that an ecotype had the same per-plant yield in monoculture that it did in polyculture. A value of 100 g means that an ecotype averaged 100 g more per-plant yield in polyculture than in monoculture. Overyielding is evaluated at the plot scale and is calculated separately at each level of diversity. The relative overyielding of individual ecotypes ( $\Delta RY$ ) can also be calculated, as the proportional difference in yield of each ecotype grown in polyculture versus monoculture. By correlating  $\Delta RY$  and monoculture yield, it is possible to determine how much plot overyielding is due to competitive dominance of large ecotypes (i.e., “selection effects” *per* Loreau and Hector 2001) and how much is due to size-independent interactions among ecotypes [called “complementarity effects” by Loreau and Hector (2001)].

We used ANOVA analyses with richness as a fixed factor with a polynomial contrast to evaluate the effects of ecotypic richness on net overyielding (DE—net diversity effect), overyielding that was not due to size-specific effects (SIE—size-independent effects), overyielding that was due to size-dependent dominance effects (SDDE), and overyielding that was due to

size-dependent “complementarity” effects (SDCE). Positive diversity effects (DE and SIE) indicate that ecotypes performed better in mixtures than in ecotypic monoculture. Positive SDDE indicates large ecotypes overyielding at the expense of smaller ecotypes (i.e. a “zero sum game”), and negative SDDEs indicate smaller ecotypes overyielding at the expense of larger ecotypes. Positive SDCE, on the other hand, indicates overyielding of large ecotypes that does not occur at the expense of small ecotypes. Likewise, negative SDCE would result if small ecotypes overyielded without affecting the performance of large ecotypes. Selected relationships between DE, SIE, SDDE, SDCE and *Centaurea* biomass were evaluated using Pearson correlations.

This partitioning is performed as follows (Loreau and Hector 2001; Fox 2005):

$$DE = SIE + SDDE + SDCE$$

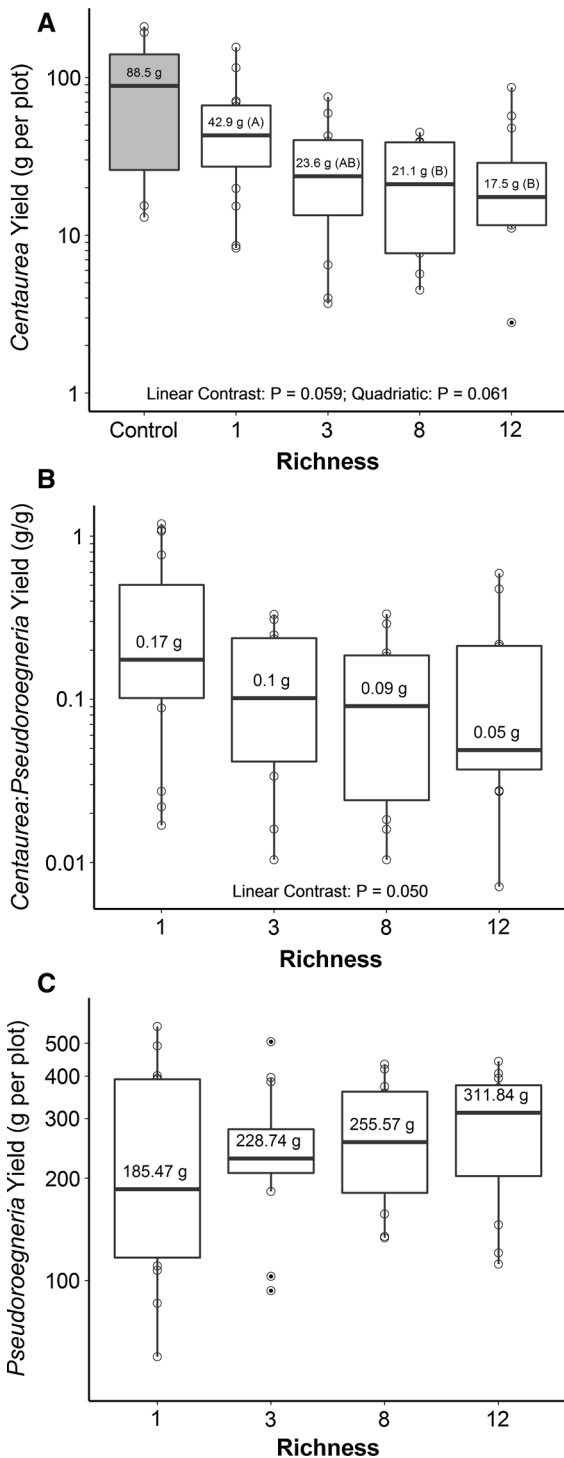
$$SIE = N\overline{M\Delta RY}$$

$$SDDE = NCov\left(M, \frac{RY_O}{RYT_O} - RY_E\right)$$

$$SDCE = NCov\left(M, RY_O - \frac{RY_O}{RYT_O}\right)$$

where  $N$  is the plot richness,  $M$  is the monoculture yield,  $RY_O$  is the relative observed yield (the species observed yield relative to its monoculture yield),  $RY_E$  is the relative expected yield (the relative expected yield given the density of the ecotype in the plot), and  $RYT_O$  is the total relative observed yield for the plot (see Loreau and Hector 2001; Fox 2005 for details).

We investigated the performance of individual *Pseudoroegneria* ecotypes and their effects on *Centaurea* using linear mixed models with package “lmer4” (Bates et al. 2013) in R version 3.0.1 (R Core Team 2012), with per-plant average biomass (log-transformed), and relative overyielding (log transformed  $\Delta RY$ ). Significance of parameter estimates was calculated using Satterthwaite approximation (package “lmerTest”; Kunetsova et al. 2014). Plot was modelled as a random factor. In these models we used richness as a fixed factor with a planned polynomial contrast, and used monoculture yield as a covariate. These analyses were performed separately



◀ **Fig. 1** **a** *Centaurea* yield, **b** ratio of *Centaurea* yield to *Pseudoroegneria* yield (*C:P*), and **c** *Pseudoroegneria* yield at each level of richness. For each box plot, the heavy horizontal line shows the median (indicated also by the quantity above each line), and the light horizontal lines show the interquartile range. Vertical lines indicate 95% intervals. Results of Tukey HSD tests are shown in parentheses for *Centaurea* yield against richness. Quadratic and cubic effects of richness on *C:P* were not significant, and effects of richness on *Pseudoroegneria* yield were not significant

relative overyielding [as reported in Atwater and Callaway (2015)] persisted after invasion.

**Results**

*Pseudoroegneria* productivity

Increases in *Pseudoroegneria* yield with richness were not statistically significant (Fig. 1c). Likewise, the strength of the overall diversity effect (DE) did not vary with richness, however size-independent “complementarity” effects (SIE) and size-dependent “dominance” effects (SDDE) both varied with plot richness (Table 1). In general, SIE was positive and increased with richness (Fig. 2), whereas SDDE was strongly positive in the 3-ecotype plots and negative in the 8- and 12-ecotype plots (Fig. 2). For the six ecotypes with known monoculture yields, the quadratic contrast was dominant, driven by underyielding of the 8-ecotype plots (Fig. 2a). For all ecotypes, using projected monoculture yields, effects of richness were roughly linear, with DE and SIE being much greater in the 8- and 12-ecotype plots compared to the 3-ecotype plots (Fig. 2b). Taken together, these results indicate that small plants tended to overyield at the expense of large plants, but that most of the diversity effect was due to size-independent effects (Fig. 2) which may have intensified with increased richness (Table 1; Fig. 2).

Mixed effects models revealed that richness interacted with monoculture yield to affect log-transformed per-plant *Pseudoroegneria* yield and  $\Delta Y$  (Table 2). These effects of richness were complex, being contingent upon monoculture yield (Supplementary Fig. S3). In general, ecotypes with large monoculture yields had higher log-transformed biomass in polyculture, although these effects were

on the six ecotypes with known monoculture yield, as well as on all ecotypes, using predicted monoculture yields. This was done to determine whether the positive effects of ecotypic richness on biomass and

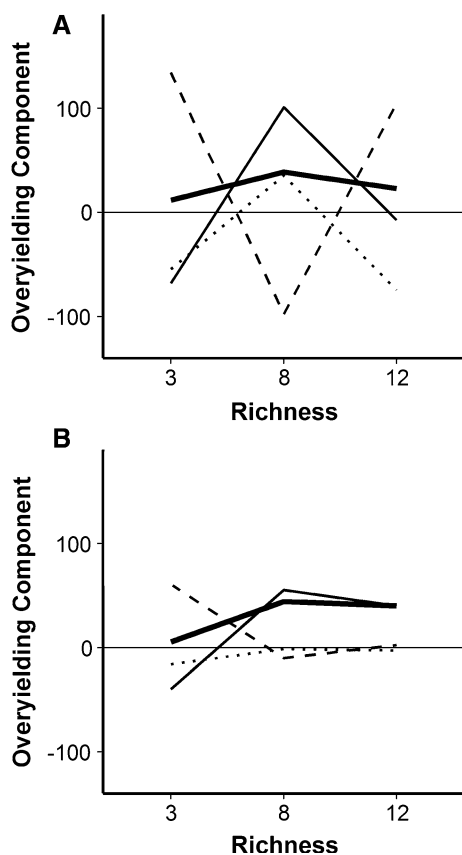
**Table 1** Results of ANOVA analyses showing the effects of richness on net diversity effect (DE), size-independent effect (SIE), size-dependent dominance effect (SDDE), and size-dependent complementarity effect (SDCE) on *Pseudoroegneria* yield

	df	Actual				Projected			
		DE	SIE	SDDE	SDCE	DE	SIE	SDDE	SDCE
Richness	2	0.130	5.905**	5.054*	2.299	0.595	3.474*	4.847*	1.168
L-contrast	31/33	n/a	1.202	-0.362	n/a	n/a	2.062*	-2.400*	n/a
Q-contrast	31/33	n/a	-3.280**	3.174**	n/a	n/a	-1.642	1.984 <sup>a</sup>	n/a

Results of significant linear (L) and quadratic (Q) contrasts are shown. Test statistics are  $F$ -statistics for richness and  $t$ -statistics for contrasts. Models using the six ecotypes with known monoculture yields are shown (actual) alongside models using projected monoculture yields for all twelve ecotypes (projected). Error  $df = 31$  for actual models and 33 for projected

<sup>a</sup>  $P = 0.056$

\*  $P \leq 0.05$ ; \*\*  $P \leq 0.01$



**Fig. 2** Relationship between richness and *Pseudoroegneria* overyielding for **a** actual and **b** projected yields. The **thick solid line** shows median net diversity effect (DE), and the overyielding components are shown by the **thin solid** (SIE), **dashed** (SDDE), and **dotted** (SDCE) lines. Results are calculated from actual monoculture yields for six ecotypes, and from projected monoculture yields

strongest in the 3-ecotype plots. Likewise, monoculture yield had positive effects on  $\Delta RY$  in the 3-ecotype plots and negative effects in the 8- and 12-ecotype plots (Table 2; Supplementary Fig. S3 G–H).

#### Invasion by *Centaurea*

Plots with greater *Pseudoroegneria* biomass were less invaded by *Centaurea* than the control plots (Table 3), but ANCOVA analysis showed that intraspecific ecotypic richness also had effects on *Centaurea* biomass even when effects of richness on *Pseudoroegneria* yield were accounted for ( $F_{3,47} = 2.835$ ,  $P = 0.048$ ; Table 3). Polynomial contrasts indicated that the relationship between richness and *Centaurea* mass was close to linear ( $P = 0.059$ ) with a marginally non-significant quadratic component as well ( $P = 0.061$ ). Because this appeared to be driven primarily by strong *Centaurea* performance in the monoculture plots (Fig. 1a), we performed a post-hoc Tukey HSD test which indicated similar levels of *Centaurea* biomass in plots containing three to twelve ecotypes, and increased biomass in the monocultures (Fig. 1a). In these analyses *Pseudoroegneria* yield was a highly significant covariate (Table 3), thus richness had effects on *Centaurea* yield even when the effect of *Pseudoroegneria* mass was accounted for. Plots with strong net diversity effects (DE) had similar absolute *Centaurea* yield to plots with weak effects, but much lower relative *Centaurea* yields ( $DE_{\text{actual}}: r = -0.070$ ,  $P < 0.001$ ;  $DE_{\text{projected}}: r = 0.072$ ,  $P < 0.001$ ; Table 4). Relative *Centaurea* yield ( $\ln\text{-C:P}$ ) was also negatively correlated with SIE

**Table 2** Results of linear mixed models of log-transformed *Pseudoroegneria* per-plant biomass and relative overyielding ( $\Delta Y$ ), with richness as a fixed factor with a linear contrast and monoculture yield as a covariate

	Actual			Projected		
	<i>B</i>	<i>t</i>	<i>P</i>	<i>B</i>	<i>t</i>	<i>P</i>
Log-biomass (g)						
Richness (L)	0.086	0.370	0.712	0.255	1.263	0.208
Richness (Q)	-0.639	-3.064	0.003	-0.401	-2.222	0.028
Monoculture yield	0.0035	11.626	<0.001	0.0035	10.705	<0.001
Richness (L) × Mono.	-0.005	-0.859	0.392	-0.0008	-1.269	0.206
Richness (Q) × Mono.	0.0012	2.510	0.013	0.0009	1.677	0.095
$\sigma$ (Plot)	0.385			0.313		
$\sigma$ (Residual)	0.662			0.702		
Overyielding ( $\Delta Y$ )						
Richness (L)	0.135	4.594	<0.001	0.118	4.266	<0.001
Richness (Q)	-1.440	-5.617	<0.001	-0.087	-3.546	<0.001
Monoculture Yield	0.0001	2.810	0.006	0.0001	2.510	0.013
Richness (L) × Mono.	-0.0003	-3.249	0.001	-0.0003	-3.254	0.001
Richness (Q) × Mono.	0.0003	3.753	<0.001	0.0002	2.498	0.013
$\sigma$ (Plot)	0.018			0.035		
$\sigma$ (Residual)	0.097			0.101		

For richness, results of significant linear (L) and quadratic (Q) contrasts are shown. Estimated random effect distributions of plot were also estimated ( $\sigma$ ). Parameter estimates (*B*), *t*-statistics estimated using Satterthwaite approximation, and *P* values are shown for models fitted only on ecotypes with known monoculture yields (actual) and all ecotypes, with projected monoculture yields (projected)

**Table 3** Results of ANCOVA showing the effects of richness and *Pseudoroegneria* biomass on natural log transformed *Centaurea* biomass

Response: ln- <i>Centaurea</i> yield	SS	<i>df</i>	<i>F/t</i>	<i>P</i>	Effect
Richness	4.683	3	2.835	0.048	
Linear contrast			-1.936	0.059	-
Quadratic contrast			1.921	0.061	+
Cubic contrast			-0.384	0.703	
<i>Pseudoroegneria</i> yield	13.605	1	24.711	<0.001	-
Error	25.876	47			

“Effect” indicates whether the independent variable had a positive (+) or negative (-) effect on the *Centaurea* biomass. Results of polynomial contrasts are shown. The column labelled “*F/t*” shows the *F*-statistic for ANCOVA effects and *t*-statistic for contrasts (*df* = 47). Error *df* for the model = 47

( $SIE_{actual}$ :  $r = -0.52$ ,  $P = 0.058$ ;  $SIE_{projected}$ :  $r = -0.52$ ,  $P = 0.027$ ).

**Discussion**

Our finding that intraspecific diversity contributes to biotic resistance to exotic invasion adds to a growing

body of research showing that within-species richness and ecosystem functioning are linked (e.g. Crutsinger et al. 2006; Hughes et al. 2008; Thorpe et al. 2011; Gibson et al. 2014; Schamp and Aarssen 2014; Wang et al. 2014; Baron et al. 2015; Atwater and Callaway 2015). This is important because a variety of anthropogenic causes that threaten local diversity are also likely to decrease genetic diversity within populations

**Table 4** Select correlations between net diversity effect (DE), size-independent effect (SIE), size-dependent dominance effect (SDDE), and size-dependent complementarity effect (SDCE),ln-*Centaurea* yield and ln-C:P (*Centaurea* yield relative to *Pseudoroegneria* yield)

	Actual				Projected			
	DE	SIE	SDDE	SDCE	DE	SIE	SDDE	SDCE
Ln- <i>Centaurea</i> yield	-0.51 <sup>a</sup>	-0.37	0.02	-0.12	-0.46	-0.37	-0.21	0.08
Ln-C:P	-0.70**	-0.52 <sup>b</sup>	-0.01	-0.11	-0.71**	-0.52*	-0.26	0.09

Models using the six ecotypes with known monoculture yields are shown (actual) alongside models using projected monoculture yields for all twelve ecotypes (projected)

<sup>a</sup>  $P = 0.063$

<sup>b</sup>  $P = 0.058$

\*  $P \leq 0.05$ ; \*\*  $P \leq 0.001$

(Ellstrand and Elam 1993). If the positive effects of genetic diversity on ecosystem function, such as we found for resistance to exotic invasion, are common or can be extrapolated to other functions, then changes in ecosystem functioning may begin long before species are locally extirpated.

The ecotypic diversity of *Pseudoroegneria* had strong effects on the per-plant biomass of *Pseudoroegneria* and yields of *Centaurea* in this study, with individual *Pseudoroegneria* plants being larger in the polyculture plots than in the monoculture plots (Table 2), and with *Centaurea* yielding about half as much in the 12-ecotype plots than in the 1-ecotype plots (Fig. 1a; Table 3). A large portion of this strong effect of richness on invasion appeared to be associated with the increased *Pseudoroegneria* performance in more ecotype-rich plots, as ANCOVA analysis indicated that *Pseudoroegneria* biomass had major effects on *Centaurea* yield. It is possible that some of this was driven by the greater likelihood of *Centaurea* encountering large, dominant *Pseudoroegneria* ecotypes in the diverse plots, in a process akin to the “selection effect” (Loreau and Hector 2001). However, richness also had important positive effects that were independent of *Pseudoroegneria* yield. This observation is important because it means that the intraspecific diversity of native plant populations could have a profound dual effect on biotic richness to invasion—via the superior performance of native plants, but also through strong yet cryptic ecological processes in diverse populations that are unrelated to native plant productivity. The reason for this is unclear, although overyielding of *Pseudoroegneria*

has been linked to reduced soil feedback (Luo et al. 2016) and possibly neighbor recognition (Yang et al. 2015). It is possible that this or other processes free *Pseudoroegneria* from inhibition in polyculture, allowing them to allocate more resources to competing with *Centaurea*.

Planned linear contrasts indicated that *Centaurea* yield tended to decrease linearly with diversity ( $P = 0.059$ ), but post-hoc contrasts and graphical analysis indicated that the positive effect of diversity reached its limit at three ecotypes, and did not increase further in plots with eight or 12 ecotypes (Fig. 2). Similar rapidly saturating responses to diversity have been shown for productivity (Tilman et al. 2001) but in other cases the positive effects of species diversity increase linearly to very high numbers of species (Tilman et al. 2001; Maron and Marler 2008). Why some diversity-ecosystem function relationships peak at relatively low numbers of species is not clear, but where the asymptote lies may depend on the mechanism by which richness increases function. For example, if richness attenuates density-dependent consumer effects then a relatively small increase in diversity might yield as great an increase in function as a large increase in diversity, particularly if resource niche overlap is low (Schnitzer et al. 2011). Ecosystem effects may have also peaked at three species because of our specific experimental design. We randomly sorted the ecotypes within the plots, and thus even at the lowest ecotype richness each individual had a relatively high probability of having a different ecotype as a neighbor. Thus if having neighbors that were different from oneself but not necessarily



different from each other was as important as having many different neighbors, then ecosystem function would have peaked at a low diversity (Schnitzer et al. 2011). Another possibility is that genetic diversity was high within ecotypes and saturated quickly. Although we do not know what process caused such a dramatic decline in *Centaurea* yield with increased *Pseudoroegneria* diversity, our results suggest that these effects saturated at relatively low ecotypic diversity. Thus while intraspecific diversity has strong effects on biotic resistance, only a small amount of diversity might be needed to maximize biotic resistance.

The causes of overyielding in genetically diverse systems are not as well understood as in species diverse systems (Atwater and Callaway 2015). There are several mechanisms that affect the relationship between species diversity and ecosystem function, and these can be broadly grouped into two categories. First, there are complementarity effects, in which complementary interactions among multiple species cause differences in ecosystem function. Second, there are selection effects in which particular species have dominant effects on ecosystem processes whenever they are present (Loreau et al. 2001). Complementarity effects are believed to be more important than selection effects, at least in the overyielding of diverse communities (Cardinale et al. 2007), and are usually associated with greater niche utilization in diverse communities compared to monocultures. This could lead both to increases in productivity (because resources are used more efficiently) and decreases in invasibility (because more niche space is occupied and/or because the system is more productive). As was observed prior to disturbance (Atwater and Callaway 2015), overyielding of *Pseudoroegneria* in diverse plots was due to positive size-dependent effects in the 3-ecotype plots (i.e. “selection” effects), and size-independent effects (i.e. “complementarity” effects) in the 8- and 12-ecotype plots. This suggests that competitive advantages of large ecotypes played a role when diversity was low, but that size-independent “complementarity” effects may have been more important when diversity was high. This result may be relevant for *Centaurea*, which grew particularly poorly in plots in which strong overyielding of *Pseudoroegneria* occurred. In other words, whatever the process that caused size-independent overyielding of *Pseudoroegneria*, it appears to have also been disadvantageous for *Centaurea*. This did not occur

when overyielding was due to size-dependent interactions between *Pseudoroegneria*, suggesting that yield increases due to “complementarity” effects such as resource partitioning, reduced pathogen load, or neighbor avoidance promote biotic resistance more strongly than yield increases due to competitive dominance.

In this study we manipulated within-species diversity by collecting multiple *Pseudoroegneria* ecotypes throughout the United States, rather than by using accessions collected from a single-population. We did this in order to maximize functional diversity, thereby increasing our chances of detecting effects of intraspecific variation and characterizing the relationship between *Pseudoroegneria* overyielding and *Centaurea* yield. This can be useful in studies of overyielding mechanisms (Atwater and Callaway 2015; Bukowski and Petermann 2015). While these ecotypes would not interact naturally, evidence from other studies suggests that within-population functional diversity is high enough to influence productivity and other ecosystem processes on a level similar to species diversity (e.g. Crutsinger et al. 2006; Crutsinger et al. 2008; Schöb et al. 2015). Thus the results of this study may be applicable to diverse systems at smaller scales, although we caution that effects of within-population diversity may nonetheless be somewhat weaker than those of among population diversity (Milla et al. 2009).

Our results suggest that manipulating native ecotypic diversity has the potential to contribute to the management of invasive species. For example, restoration efforts commonly use seed collected from multiple populations, particularly if locally accessed seeds are unavailable. However, it is important to note that we sampled ecotypic diversity over a very wide range of the regional distribution of *Pseudoroegneria*, and such a broad range of genetic diversity is unlikely to occur within local populations. It is at the scale of local populations that invasion is resisted, not at regional scales. We have observed substantial within- and between-population ecotypic variation in *Pseudoroegneria* in other experiments, but it will be crucial to determine in the future whether or not such population-scale diversity yields either resistance to exotic variation or any other ecosystem function.

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