

Community and ecosystem effects of intraspecific genetic diversity in grassland microcosms of varying species diversity

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Abstract. Studies of whether plant community structure and ecosystem properties depend on the genetic diversity of component populations have been largely restricted to species monocultures and have involved levels of genetic differentiation that do not necessarily correspond to that exhibited by neighboring mature individuals in the field. We established experimental communities of varying intraspecific genetic diversity, using genotypes of eight species propagated from clonal material of individuals derived from a small (100-m²) limestone grassland community, and tested whether genetic diversity (one, four, and eight genotypes per species) influenced community composition and annual aboveground productivity across communities of one, four, and eight species. Eight-species communities were represented by common grass, sedge, and forb species, and four- and one-species communities were represented by four graminoids and the dominant grass *Festuca ovina*, respectively. After three years of community development, there was a marginal increase of species diversity with increased genetic diversity in four- and eight-species communities, and genetic diversity altered the performance of genotypes in monospecific communities of *F. ovina*. However, shifts in composition from genetic diversity were not sufficient to alter patterns of community productivity. Neighborhood models describing pairwise interactions between species indicated that genetic diversity decreased the intensity of competition between species in four-species mixtures, thereby promoting competitive equivalency and enhancing species equitability. In *F. ovina* monocultures, neighborhood models revealed both synergistic and antagonistic interactions between genotypes that were reduced in intensity on more stressful shallow soils. Although the dependence of *F. ovina* genotype performance on neighborhood genetic composition did not influence total productivity, such dependence was sufficient to uncouple genotype performance in genetic mixtures and monocultures. Our results point to an important connection between local genetic diversity and species diversity in this species-rich ecosystem but suggest that such community-level dependence on genetic diversity may not feedback to ecosystem productivity.

Key words: ecosystem productivity; *Festuca ovina*; genotypic diversity; limestone grassland; maximum likelihood; neighborhood models; Sheffield, UK; species richness.

INTRODUCTION

Populations of outbreeding perennial plants can exhibit high local genetic differentiation (Linhart and Grant 1996, Whitlock et al. 2007), but the ecological significance of such differentiation remains unclear. Although Harper (1977), Antonovics (1976, 1978), and others (Turkington 1979, Aarssen and Turkington 1985) long ago challenged the assumption that intraspecific variation could be safely ignored in short-term studies of plant community dynamics, empirical studies demonstrating clear links between local genetic diversity, plant species interactions, and ecosystem properties have been slow to develop (Antonovics 2003, Vellend and Geber 2005, Hughes et al. 2008). Most of what is known about

the ecological consequences of genetic diversity comes from monospecific or mono-dominant communities. For example, eelgrass (*Zostera marina*) beds of multiple genotypes exhibit greater short-term productivity than single-clone beds (Hughes and Stachowicz 2004, Reusch et al. 2005). Similarly, mean aboveground production in monocultures of the tall goldenrod *Solidago altissima* increases with the number of planted genotypes (Crutsinger et al. 2006). There is an extensive body of research that documents effects of intraspecific plant genetic diversity on trophic interactions and disease dynamics (reviewed by Hughes et al. 2008), but such studies are largely limited to communities dominated by one species.

The influence of intraspecific variation in communities of multiple interacting plant species on community or ecosystem dynamics remains largely unexplored. If phenotypic variance in functional traits is small within populations compared to that among species, then direct

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effects of genetic diversity on species composition and ecosystem functioning should be minor. However, several lines of evidence suggest genetic diversity could indirectly influence composition and functioning by altering the nature of interactions between species. For example, there is now considerable evidence that the nature and intensity of competition between species is genotype-dependent (Turkington and Harper 1979, Kelley and Clay 1987, Taylor and Aarssen 1990, Ehlers and Thompson 2004, Gustafson et al. 2004, Lankau and Strauss 2008). Fridley et al. (2007) have shown that such genotype dependency can be sufficient to reverse dominance relations. If strong genotype dependence of species interactions is common in plant communities, then intraspecific genetic diversity may act to dilute interaction strengths between species, thus promoting species diversity and compositional stability (Vellend 2006). In what remains the only experimental test of this hypothesis, Booth and Grime (2003) found that experimental grassland assemblages of 11 species, each planted with multiple genotypes, maintained a more predictable species composition than those containing populations of a single clone. Although Booth and Grime (2003) did not detect an associated effect of genetic diversity on ecosystem productivity, changes in species composition in other systems may be sufficient to drive ecosystem effects, depending on the extent of functional differences between species (Fridley 2001).

Attempts to document the nature of interactions between intraspecific genotypes have been few, even within monospecific communities (Boyden et al. 2008). Indirect evidence from comparisons of monoculture and mixture yields in agronomic studies (Smithson and Lenne 1996) and genotype richness experiments (Reusch et al. 2005, Crutsinger et al. 2006) suggests genotypes can exhibit complementary resource use, but mechanisms for such behavior at the intraspecific level remain unclear. Genetically based variance in root and shoot morphology and foraging behavior could lead to significant differences in competitive abilities between neighboring genotypes (Aarssen 1989) relating to both the rate of resource acquisition (competitive effect) and tolerance of resource shortage (competitive response; Cahill et al. 2005). However, to our knowledge, no studies have addressed the extent to which competitive effects and responses vary among intraspecific genotypes that naturally co-occur.

In this paper, we report an experimental study of how intraspecific genetic diversity influences community composition and productivity across species monocultures and multi-species mixtures. We constructed synthesized communities of one, four, and eight species, each planted with one, four, or eight genotypes, all propagated from mature individuals located within a 100-m² quadrat in species-rich limestone grassland. Our primary objective was to test whether genotypic diversity drives annual aboveground productivity in either species monocultures (here represented by the

common limestone grass *Festuca ovina*) or more complex communities represented by either a mixture of four common graminoids or eight herbaceous species of varying growth form. Our secondary objective was to determine whether ecosystem effects are driven by changes in genotype or species composition resulting from genotype-dependent neighborhood interactions.

METHODS

Experimental design and mesocosm construction

Experimental units consisted of fifty-four 55 × 55 cm wooden boxes, 12 cm deep, placed in six replicate blocks on scaffolding at Tapton Experimental Garden at the University of Sheffield, Sheffield, UK. To mimic natural variation in substrate conditions, we deliberately varied soil depth and composition within each experimental unit. Within each box, soil depth was manipulated by insertion of two wooden blocks measuring 55 × 10 × 5 cm on one box half, separated by a “crevice” soil space 4 cm wide. Soil crevices were further lengthened to simulate natural bedrock crevices by attaching four PVC pipes (46 cm long × 3.2 cm internal diameter) to the box bottom, equally spaced along the crevice length (55 cm), each filled with native rendzina soil (described in this section). Boxes were filled to 10 cm height with native soil removed from the field. Each box was filled with two soil types in a 2 × 2 checkerboard pattern to simulate the natural edaphic variation present in limestone dale communities: a higher pH rendzina soil derived from a mid-slope position that typically harbors a more calcicolous vegetation, and a more acidic podzol soil excavated from near the top of a slope typical of more calcifuge vegetation (Balme 1953). Excavated soil (top 20–30 cm) was slightly dried, mixed in a cement mixer for homogenization, and added to boxes in aliquots. All boxes contained identical depth and soil type configurations, but crevice and soil type orientation was randomly determined among boxes. Within each block, nine composition treatments were assigned randomly to boxes, including a factorial combination of three levels of intraspecific genetic diversity (GD; one, four, and eight genotypes) and three different species compositions of varying species diversity (SD; one, four, and eight species).

Planting, maintenance, and harvesting

Eight unique genotypes of eight species (Table 1) were selected from a library of clonal lines maintained in a common garden at Tapton since 1995. Genotypes were originally collected from a 10 × 10 m quadrat at Cressbrookdale, North Derbyshire, UK (Booth and Grime 2003) and subjected to molecular analysis by Whitlock (2004) to confirm genetic uniqueness. Individual transplants were prepared by splitting apart separate tussocks (grasses), potted genets (*Carex*), or spreading clumps (*Succisa* and *Campanula*) and clipping above- and belowground tissues to a standard size constant within species. All individuals were transplant-

TABLE 1. Limestone grassland species used in the experiment at Tapton Experimental Garden at University of Sheffield, Sheffield, United Kingdom, in 2006 and 2007 listed with growth attributes and treatment effects.

Species	N	Growth form	Growth attributes		
			Reproduces clonally?	Individual size (mg)†	Genotype size range
<i>Festuca ovina</i> L.	1584	tufted or mat-forming grass	N	362 ± 5.7	1.9
<i>Koeleria macrantha</i> (Ledeb.) Schultes	432	loosely tufted grass	N	597 ± 16.1	1.8
<i>Helictotrichon pratense</i> (L.) Besser.	432	densely tufted grass	N	175 ± 6.4	3.2
<i>Carex caryophyllea</i> Latour.‡	432	rhizomatous sedge	Y	422 ± 22.3	4.0
<i>Carex flacca</i> Schreber‡	144	rhizomatous sedge	Y	517 ± 32.8	2.5
<i>Carex panicea</i> L.‡	144	rhizomatous sedge	Y	318 ± 27.8	3.0
<i>Succisa pratense</i> Moench	144	rosette forb	N	3884 ± 142.8	1.8
<i>Campanula rotundifolia</i> L.‡	144	rhizomatous forb	Y	107 ± 15.9	8.0

Notes: N is the total number of individuals used across the experiment, and genotype size range is the ratio of the mean biomass of largest and smallest genotypes. The last eight columns show levels of statistical significance of experimental treatments in linear models (LME) of species performance, including genetic identity (genotype), genetic and species diversity treatments (GD and SD), soil depth and substrate type, and selected interactions. Four pairwise interactions (soil depth × GD, soil depth × soil type, soil type × GD, and SD × GD) were not significant for any species and are not shown. Genotype effects could not be measured for clonal species, and SD effects could not be measured for four species present only in eight-species communities; this is indicated by empty cells. For clonal species, size is estimated as quadrat mass divided by the number of planted individuals, and genotype size range was derived from GD1 (single genotype) boxes only.

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

† Values are mean ± SE.

‡ Species harvested by quadrants within experimental units rather than by individual.

ed during a 3-wk period in November/December 2004. Each box was planted with 64 individuals in an equally spaced 8 × 8 grid. For the eight species (SD)–8 genotype (GD) treatment, planting positions of each genotype and species were determined randomly, and each genotype was represented once in each box. For the SD8–GD4 and SD4–GD8 treatments, each genotype was planted twice, and replicate compositions were planted on crevice and non-crevice box sides, with planting positions otherwise determined randomly. For the other six treatments, genotypes were represented by 4, 8, 16, 32, or 64 individuals and replicate compositions were planted in each box quadrant, but randomly within quadrats.

For all but two of the nine composition treatments, genotypes were represented equally across treatments and replicates. *Festuca ovina* genetic monocultures included a box for each genotype ($N = 8$), accompanied by a reduction of two replicates for the compositionally identical SD8–GD8 boxes ($N = 4$). For the SD4 and SD8 boxes where each species was represented by a single genotype, it was not possible to include all eight genotypes of each species in six replicates, so two of each species were randomly excluded from these treatments.

Plants in all boxes were clipped annually in the fall at a height of 2.5 cm to simulate sheep and cattle grazing. Boxes were regularly weeded and flowering stalks of transplants were removed to prevent sexual diversification. Fall clippings were sorted by species and weighed in 2005 and 2006, but biomass estimates did not include flowering stalks. In August 2007, all plants were harvested at 2.5 cm height, separately by individual where possible (grasses and *Succisa*) and by box quadrant for rhizomatous *Carex* and *Campanula*, and

dried to constant dry mass. Inflorescences were harvested in the same manner for each species in June and August 2007.

Biomass and diversity analyses

Because species and genotype performance was qualitatively similar between years but complete biomass data including vegetative and reproductive structures were only available for 2007, our analysis focuses on productivity patterns present in this third year of the experiment. Treatment effects on annual aboveground biomass at final harvest in 2007 were analyzed using three different sizes of experimental unit. First, the biomass of each box ($N = 54$) was subject to ANOVA with independent predictor variables of block, SD, and GD, including their interaction. Second, the biomass of each box quadrant ($N = 216$) was modeled in a mixed effects linear model (LME) to account for the nesting of soil type and depth variables within blocks and boxes (both random effects), including these previously noted predictor variables with soil type, depth, and associated interaction terms (fixed effects). Because soil type effects were minor, soil types were subsequently pooled within boxes and separate ANOVAs were performed within the three community types relating biomass to genotypic diversity and soil depth, with multiple mean comparisons performed via Tukey hsd tests. Third, individual total aboveground biomass (sum of vegetative and reproductive) was modeled separately by species following the LME approach nested by box and block, with predictor variables varying by species, depending on whether they were used across species diversity treatments and could be harvested by individual or by quadrat (Table 1). Full models (grass species) included

TABLE 1. Extended.

Geno- type	Treatment effects												
	Genetic diversity (GD)	Species diversity (SD)	Depth	Soil type	Depth × geno- type	GD × geno- type	SD × geno- type	Depth × SD	Soil type × SD	Soil type × geno- type	Depth × SD	Soil type × SD	Soil type × geno- type
***	NS	***	***	NS	***	***	*	***	***	***	***	***	***
***	NS	NS	***	***	NS	NS	NS	NS	NS	NS	NS	NS	NS
***	*	**	***	NS	**	***	NS	NS	NS	NS	NS	NS	NS
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genotype identity, GD, SD, soil type, depth, and two-way interactions. Clonal species could not be modeled with respect to genotype and four species were only in one-species diversity treatment (Table 1). Biomass values for each test were log-transformed to normalize residuals. All statistical analyses were performed in R 2.6.2 (R Development Core Team 2006).

We measured effects of GD treatments on community structure in SD4 and SD8 treatments using the exponent-transformed Shannon diversity (H') index that describes the number of equally abundant species required for the observed H' . Diversity contrasts were performed separately within SD4 and SD8 treatments using Student's t tests.

Neighborhood interaction modeling of Festuca ovina genotypes

To quantify the nature and strength of interactions between genotypes of *Festuca ovina* in *Festuca*-only (SD1) communities, we modeled the annual growth of individuals, harvested at 2.5 cm height in 2006 (leaves only) and 2007 (leaves and culms), in relation to planted neighbor identity. We used the well known plant hyperbolic competition model (Pacala and Silander 1990, Freckleton and Watkinson 1997, 2001):

$$\text{Growth}_{i,j} = \text{PG} \left(1 + a_j \left[\sum_k \alpha_{jk} N_k \right] \right)^{-1} \quad (1)$$

where the potential growth (PG) of individual i of genotype j is reduced in the presence of neighbor genotype k , with competitive effect α_{jk} per individual of k multiplied by the number of individuals of k in the neighborhood (N_k), including the effect of each genotype on itself (α_{jj}). The parameter a_j describes the sensitivity of a target genotype to crowding (Freckleton and Watkinson 1997). We considered eight individuals in the neighborhood of each individual planted (four closest neighbors plus diagonal neighbors). For the 28 individuals that neighbored the edges of each box, rather than significantly reduce focal individual sample size, we created an additional neighbor entity that described the

effect of the box wall, thereby retaining pairwise interactions of these plants with neighboring interior individuals. Potential growth was estimated separately for each genotype as

$$\text{PG} = \beta_0 \text{SIZE} + \beta_1 \text{DEPTH} + \beta_2 \text{SOIL} + \beta_3 \text{YEAR} \quad (2)$$

where SIZE accounted for genetic differences in growth rate in the absence of neighbors, estimated from pot data, DEPTH was a dummy variable for soil depth (shallow vs. deep), SOIL described soil type (rendzina vs. podzol), and YEAR described when biomass samples were harvested (2006 or 2007, accounting for both year effects and the addition of culms in 2007).

The composite model described by Eqs. 1 and 2 was fit via maximum likelihood estimation following the approach of Uriarte et al. (2004) and Canham and Uriarte (2006), via optimization of a normal likelihood function describing the difference between observed and predicted values of annual growth of individuals of each genotype. Optimization was achieved with the method of Byrd et al. (1995) through the “optim” function in R 2.6.2. Note that although the likelihood model for normal errors assumes independence among observations (individuals), the parameter estimates are generally insensitive to spatial autocorrelation (Uriarte et al. 2004).

For modeling the influence of neighbor genotypes on the growth of eight genotypes in *Festuca* monocultures, we tested whether the addition of neighbor identity significantly improved model fit via AIC_c tests (Burnham and Anderson 2001), comparing the above composite model to a simple linear least-squares regression version of Eq. 2. We also tested whether DEPTH, SOIL, or YEAR coefficients were needed in neighborhood models via AIC_c; in models for several genotypes, SOIL coefficients were insignificant (Appendix: Table A2). For comparing differences in competition coefficients between *Festuca* genotypes on deep and shallow soils, models were run on separate deep and shallow data sets. Coefficients of determination (R^2) for the neighborhood model were estimated by parameter-

izing the model with final maximum likelihood estimates and regressing predicted values against observed.

Neighborhood modeling in four-species mixtures

To assess whether differences in community structure in four-species mixtures (SD4) in response to GD were the result of changes in interaction intensity between species, we applied this neighborhood model to estimate interaction coefficients between species in the three GD treatments. As for *Festuca*, individuals of *Koeleria macrantha* and *Helictotrichon pratense* were harvested by individual at 2.5 cm height in 2006 and 2007, and we modeled the annual growth of individuals in relation to the planted position of eight neighbors, including the coefficient for the box wall. Because the fourth species in SD4 communities, the rhizomatous *Carex caryophyllea*, could not be harvested by individual, we were only able to estimate its competitive effect (based on planted position) and could not use it as a model target species. Models estimating parameters in Eqs. 1 and 2 were fit via maximum likelihood following the method for *Festuca* genotypes, including AIC_c tests for whether the addition of neighbor (species) identity significantly improved model fit. We also attempted to fit SD4 neighborhood models separately by soil depth treatment, but constraints due to sample size (in some cases <100 target individuals) produced non-converging optimization results and in only two out of 18 cases (three species \times three GD treatments \times two depths) were models including neighbor identity supported. For the same reason, pairwise species interactions in eight-species mixtures could not be quantified.

RESULTS

Variation in performance among intraspecific genotypes

For those non-clonal species with known genetic identities across all harvested individuals, species performance was strongly dependent on genetic identity (Table 1), although all planted individuals of all species survived through the duration of the experiment. Different genotypes varied in size by about a factor of two (or three in the case of *Helictotrichon pratense*), and the effect size of genetic identity rivaled that of the most important environmental variable, soil depth (Table 1). For clonal species, genetic identity effects on performance were estimated using GD1 (single genotype) boxes, and genotypes varied in mean size from over twofold (*Carex flacca*) to eightfold (*Campanula rotundifolia*, although note the small sample size). For *Festuca ovina* and *Helictotrichon*, the relative performance of different genotypes varied according to soil depth and GD treatment. *Festuca* genotypes also responded differently to SD (species diversity) treatments and soil substrate, and both soil type and depth effects varied according to SD treatment (Table 1). Overall, soil substrate type effects were generally less than those of depth; growth of *Koeleria macrantha* was greater in the more acidic podzol, while that of *C. caryophyllea* and *Succisa*

pratense was greater in the rendzina. For *C. caryophyllea* and *Helictotrichon*, present in both four- and eight-species mixtures, performance was reduced in eight-species mixtures; however, *Festuca* performance was significantly greater in four- and eight-species mixtures compared to its monoculture yield ($P < 0.001$).

Productivity effects of genetic diversity across community types

Intraspecific genotypic diversity was not a significant driver of annual aboveground productivity, regardless of community type or soil depth (Fig. 1; Appendix: Table A1). Across community types, production in 2007 was about twice as high in deeper substrate ($P < 0.0001$) and rendzina soil types were slightly ($\sim 15\%$) more productive than more acidic podzol soils ($P < 0.001$). The presence of the large rosette forb *Succisa pratensis* in eight-species mixtures nearly doubled the mean biomass of those communities compared to one- and four-species communities ($P < 0.001$), but mean biomass did not otherwise depend on species composition. Although biomass patterns in relation to genetic diversity were not significant at the $\alpha = 0.05$ level, there were marginal GD effects in the eight-species mixtures on shallow substrate, where GD8 mixtures were $\sim 15\%$ more productive on average than GD1 mixtures ($P = 0.16$, Tukey hsd test; Fig. 1). Similarly, GD4 mixtures were 13% more productive than GD1 or GD8 mixtures for eight-species communities on deep soils, although differences were not significant ($P > 0.2$, Tukey hsd test; Fig. 1). There were also no effects of genotypic diversity at the whole-box level, where soil depth and type effects were pooled within communities (ANOVA, $N = 54$, GD main effect $P > 0.3$; GD–SD interaction $P > 0.7$).

Genotype interactions in Festuca ovina monocultures

Although the mean productivity of monospecific communities of *Festuca ovina* did not depend on GD, the individual performance of genotypes in genetic monocultures did not predict their performance in genotypic mixtures, in either deep or shallow soils (Fig. 2; $P > 0.3$ in simple pairwise least-squares regressions of mean biomass per genotype in different GD treatments). However, mixture performance was consistent across four- or eight-genotype mixtures (Fig. 2; $P < 0.01$ for both deep and shallow soils), suggesting that consistent competitive outcomes were established in mixtures of four genotypes. Among the most productive genotypes in monoculture was *F. ovina* genotype 11 (Fo11), which was consistently the least productive genotype in mixture (Fig. 2). Conversely, genotype Fo3 was the least productive in monoculture but always among the greatest contributors to mixture biomass (Fig. 2).

In neighborhood models fit separately for each *F. ovina* genotype, models that included neighbor identity outperformed non-neighborhood models (Eq. 2) for every genotype, with improvements in model R^2

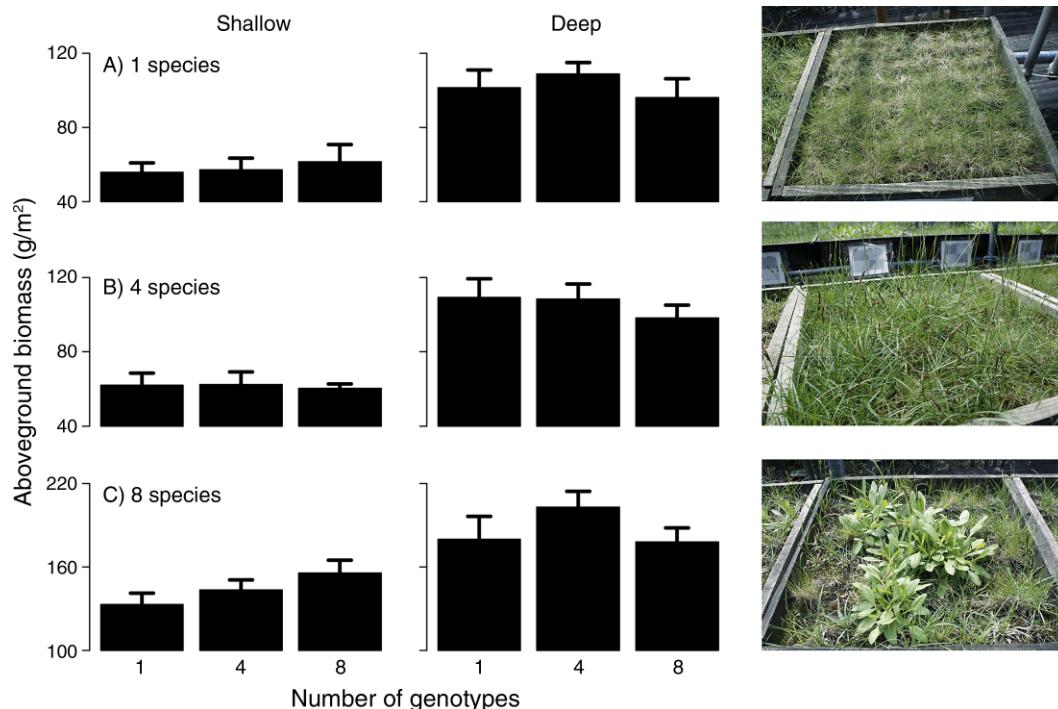


FIG. 1. Annual aboveground production in 2007 (mean + SE, $N = 6$ replicates) at Tpton Experimental Garden at the University of Sheffield, Sheffield, United Kingdom, in relation to intraspecific genotypic richness in communities of (A) one grass species, (B) four graminoid species, and (C) eight-species mixtures that included graminoids and forbs. “Shallow” panels describe communities grown on shallow (5 cm) substrate, and “deep” panels describe those on deep (10–12 cm) substrate. Note that in panel (C) the y -axis is scaled differently. There was no significant variation in biomass attributable to genotypic richness within each community and soil depth type (Tukey hsd tests, $\alpha = 0.05$).

averaging $\sim 10\%$ (Appendix: Table A2). Table A3 in the Appendix lists the standardized community interaction matrix for all eight genotypes, where intergenotypic competitive effects and responses given in Appendix: Table A2 are relativized by intragenotype values. Estimated across soil types, mean competitive effects varied from 0.66 (Fo11) to 2.53 (Fo15), with values above unity indicating strong competitive suppression of neighbors (particularly genotypes Fo15, Fo8, and Fo13), and those below unity indicating weak neighbor suppression (Fo11, Fo3). Mean competitive responses varied from 0.76 (Fo7) to 2.18 (Fo5), with values above unity indicating strong sensitivity of a genotype to neighbor interference (particularly Fo11, Fo5), and those near or below unity (Fo3, Fo7, Fo10, Fo8) indicating weaker neighbor response when compared to neighbors of the same genotype. Fig. 3A illustrates mean competitive effect and response relationships of each genotype. Fitted competition coefficients were generally consistent within genotypes, regardless of neighbor identity (note standard errors in Fig. 3A). Exceptions were the competitive effect of Fo15 and the competitive response of Fo11, which were nonetheless consistently larger than other genotypes (Fig. 3A).

When interaction models were fitted to each genotype separately by soil depth, genotypic differences in

standardized competitive effects and responses were significantly reduced in shallow soils (Fig. 3B; Appendix: Table A4). In deep soils, standardized competitive effects ranged from 4.02 (Fo15) to slightly < 0 (Fo3), and competitive responses ranged from 0.76 (Fo3) to 5.96 (Fo11). In contrast, shallow-soil coefficients were significantly closer to unity for both competitive effects (range 0.60–1.77) and responses (0.65–2.66), although effects and responses among genotypes were positively correlated among substrate types (effects correlation marginal, Pearson $r = 0.67$, $P = 0.06$; responses, Pearson $r = 0.95$, $P < 0.001$). Thus, although the nature of intergenotypic interactions was largely insensitive to substrate type, genotypes were more competitively equivalent on shallow soils.

Community-level effects of genetic diversity in species mixtures

There were small but significant effects of genotype richness on species evenness in both four- and eight-species mixtures, but effects were restricted to communities on deep soils (Fig. 4). Effective species richness (e^H) was marginally higher for four- and eight-genotype communities in four-species mixtures on deep soils compared to single-genotype mixtures (t tests, $P < 0.1$ for both contrasts), but not on shallow soils. The same

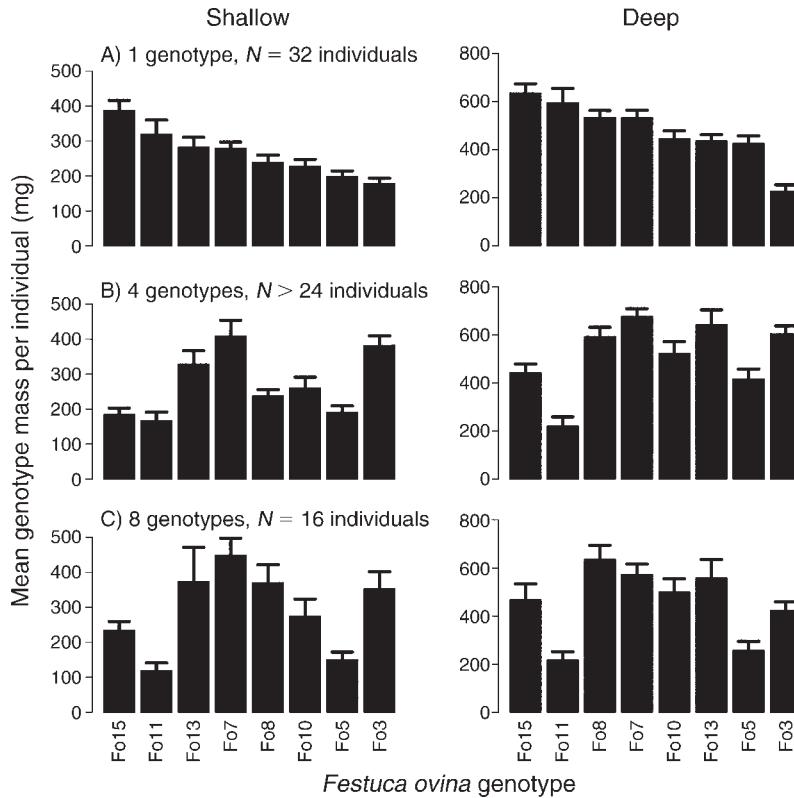


FIG. 2. Mean (\pm SE) aboveground biomass in 2007 of individuals of eight genotypes of *Festuca ovina*, grown in *Festuca*-only monocultures containing one, four, or eight genotypes. Shallow panels indicate individuals on shallow substrate; deep panels, on deep substrate. Genotypes are listed in order of monoculture biomass separately by soil depth. Note that y-axes are scaled differently for deep and shallow treatments.

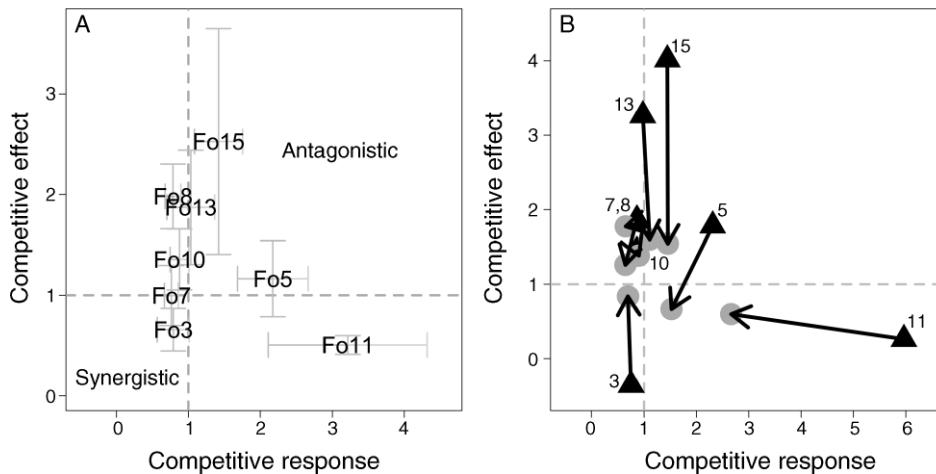


FIG. 3. (A) Mean (\pm SE, $N = 8$) competitive effect and response of eight genotypes of *Festuca ovina* in genotypic mixtures across substrate treatments, as determined by neighborhood interaction models of individual performance in 2006 and 2007. Competitive effects and responses are relative to intragenotypic competition; values < 1 for both effects and responses indicate synergistic genotypes that had net positive effects on yield, while values > 1 indicate antagonistic genotypes that reduced mixture yield. (B) Competitive effects and responses of the same eight genotypes estimated separately by soil depth using individuals and neighborhoods on either deep (black triangles) or shallow (gray circles) soils. Arrows connect values of each genotype across soil depths. The arrows emphasize how all competition values on deep soils are brought in toward the 1:1 point (competitive equivalency) on shallow soils.

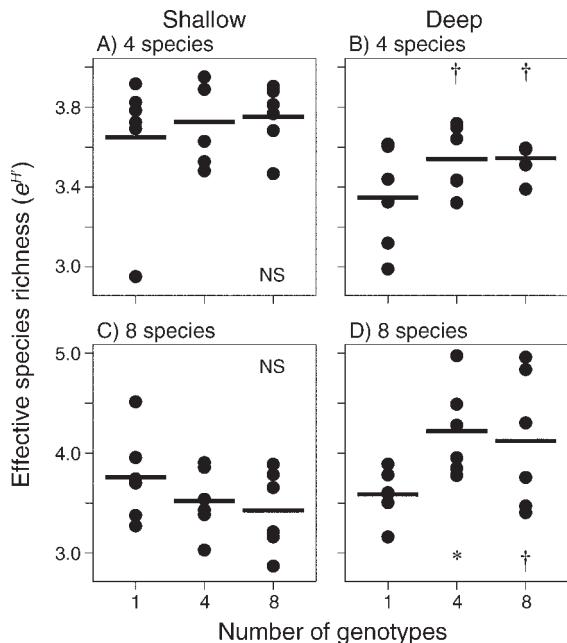


FIG. 4. Effective species richness ($e^{H'}$, where H' is the Shannon diversity index) in (A, B) four-species and (C, D) eight-species mixtures planted with one, four, or eight genotypes per species, across (A, C) shallow and (B, D) deep soils. Bars indicate treatment means. Asterisks (*) and daggers (†) indicate that four- or eight-genotype treatments were more diverse than one-genotype treatments in two-tailed t tests; four- and eight-genotype treatments were not significantly different.

* $P < 0.05$; † $P < 0.1$.

was true for eight-species mixtures, where the four-genotype treatment ($P < 0.05$) and the eight-genotype treatment ($P < 0.1$) were more diverse than the one-genotype treatment, but only on deep soils.

Neighbor species identity was a significant driver of individual performance for *Koeleria macrantha* and *Helictotrichon pratense* in four-species mixtures, but only in GD1 and GD4 treatments, and *Festuca ovina* neighborhood models did not outperform non-neighborhood models for any GD treatment (Appendix: Table A5). Standardized competitive effects and responses estimated for *Festuca*, *Koeleria*, and *Helictotrichon* across GD treatments suggest that the intensity of species interactions decreased as genetic diversity increased (Fig. 5; Appendix: Table A6). In the GD1 treatment, *Koeleria* was the competitive dominant (competitive effect mean 3.2) and *Helictotrichon* was most often suppressed (competitive response mean 3.5), with *Festuca* competitively neutral. In GD4 and GD8 communities, however, competitive effects and responses of all three species did not exceed 1.04 and were typically below unity (Fig. 5), supporting the shift toward greater equitability in these treatments. The overall result from interaction models in the four-species mixtures is that the competitive hierarchy established in

GD1 communities gave way to competitive equivalence as GD increased.

DISCUSSION

Patterns of composition and productivity from our experimental microcosms of varying intraspecific genetic diversity in communities of one, four, and eight species suggest that local genetic diversity can play a significant role in community structure, but with only minor consequences for ecosystem processes. This was true in both species-poor and species-rich communities. In monocultures of the dominant calcareous grass *Festuca ovina*, individual performance was strongly dependent on neighborhood genetic composition, but neighbor effects could be either synergistic or antagonistic, resulting in similar mixture and monoculture yields. In four- and eight-species communities, intraspecific genetic diversity marginally increased species equitability in deep soils, but not to the extent that overall productivity was affected. The decoupling of composition and productivity in our system may result in part from chronic nutrient stress that puts strong constraints on the ability of different assemblages to alter aboveground productivity levels. In a prior experiment with many of the same species and using the same unproductive native soils, Booth and Grime (2003) also failed to detect a response of community productivity to intraspecific genetic diversity after five years. In addition, like the Booth and Grime study, our experiment involved genotypes of mature individuals sampled from a small

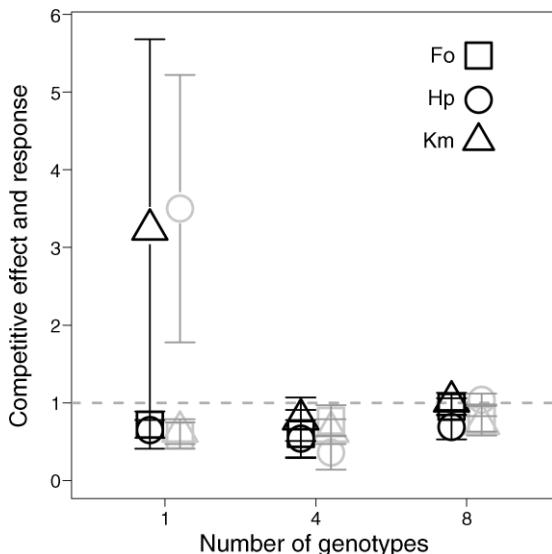


FIG. 5. Consequences of genetic diversity for species-level interactions in four-species mixtures. Mean (\pm SE) competitive effect (black symbols) and response (gray symbols) for three of the four species (*Carex caryophylla* could not be included as a target in neighborhood models) in communities planted with one, four, and eight intraspecific genotypes. Fo is *Festuca ovina*; Hp, *Helictotrichon pratense*; and Km, *Koeleria macrantha*. Competitive effects and responses are relative to intraspecific competition.

area (100 m²). Consequently, the range of variation in intraspecific genotype performance was relatively small (twofold for *Festuca*) compared to that reported from other genetic diversity studies. For example, Crutsinger et al. (2006) reported a fourfold range of monoculture yields across genotypes of *Solidago altissima*, and Reusch et al. (2005) reported a corresponding sixfold range for genotypes of *Zostera marina*. Both of these studies reported mean yield advantages in genetic mixtures, although mixtures did not outperform those of the best genetic monocultures. In contrast, Münzbergová et al. (2009) reported no effects of genetic diversity on yield in genotypic mixtures of *Festuca rubra*, and the range of monoculture performance was similar to what we measured for *Festuca ovina*.

Although we do not know the details of genotype sampling of these other studies, we suggest that the spatial extent of genotype sampling and degree of establishment of selected clones are important considerations in the design of genetic diversity experiments. The inclusion of genotypes that are ill-adapted for a chosen environmental regime will produce a large range of monoculture yields; if those same genotypes are outcompeted in mixtures, then mean mixture yields will be consistently higher than mean monoculture yields, although maximum yields will not (Fridley 2001, Loreau and Hector 2001). Although both Reusch et al. (2005) and Crutsinger et al. (2006) reported evidence of complementary interactions between genotypes, the bulk of evidence for several single-species experiments and now two multi-species experiments (including the present study and Booth and Grime [2003]) suggests short-term productivity levels of genetically diverse communities are similar to those of genetically depauperate communities that include high-performing genotypes. The same general conclusion has been reached by some for species-level diversity studies, although this subject is not without controversy (Hooper et al. 2005, Cardinale et al. 2006). This is not to deny the existence of complementary interactions between intraspecific genotypes (of which we also find evidence), but to acknowledge that their influence on productivity is small, and we hypothesize this is particularly true in unproductive systems (Fridley 2003).

Although we did not detect a large influence of intraspecific genetic diversity on aboveground productivity, community composition was mediated in part by the composition and diversity of genotypes within species. In multi-species communities on deep soils, the effect of increasing genetic diversity was to increase species equitability. This was detected as both a marginal increase in the modified Shannon diversity metric and a reduction in competitive interaction coefficients to near unity in communities where species were represented by more than one genotype. These results mirror those of Booth and Grime (2003) and are consistent with a related experiment by Fridley et al. (2007) which demonstrated how pairwise interactions

between *Koeleria macrantha* and *Carex caryophyllea*, both present here in four-species mixtures, could be reversed by altering genotype identity. In the present study, the neighborhood model indicated *Koeleria* as the more frequent competitive dominant and *Helictotrichon pratense* as the species most often competitively suppressed, but only in genetic monocultures. In genetic mixtures, *Koeleria* and *Helictotrichon* became competitively equivalent, which was the predicted outcome of genetic mixtures in the Fridley et al. (2007) study. Taken together, these three studies suggest the high fine-scale species diversity in this ecosystem is maintained in part by the dilution of interaction intensities that accompanies high levels of local genetic diversity in these outbreeding species. Whether this is a general phenomenon in species-rich ecosystems remains to be seen (Herben et al. 2001), but our study should be added incentive for the exploration of whether species diversity and intraspecific genetic diversity covary among ecosystems (Antonovics 1978, Vellend 2003, Vellend and Geber 2005). We also note that our experiment included a maximum of eight species per 0.3 m², whereas the mean species richness for this ecosystem at this scale is several times greater; we expect that pairwise interaction intensities between species would be further diluted as more species are added to plant neighborhoods (Aarssen 1989).

Species composition and species richness covaried in our experimental design, so we have limited scope for quantifying the extent to which the community-level effects of genotypic diversity described here were merely the result of including these particular species combinations. Of particular significance is the representation of clonal vs. non-clonal species at different levels of species richness: differential neighborhood effects that we detected in *F. ovina* monocultures and four-species mixtures may have been in part the product of including mostly immobile species in these communities. The clonal *Carex* species, for example, may respond less to neighborhood genotypic diversity due to their ability to escape their initial (planted) neighbors. Indeed, the ability of many species in this system to escape local competitive environments via lateral expansion may be an additional mechanism of reduced interaction intensities favoring species coexistence (Campbell et al. 1991).

Our study is among the first to demonstrate that the nature and intensity of intraspecific competition can vary considerably between intraspecific genotypes that naturally co-occur as mature individuals, and that the intensity of such interactions is strongly mediated by microsite conditions. Here, the annual growth of *Festuca ovina* individuals was influenced as much by the genetic composition of neighbors than by either substrate conditions or inherent genotype attributes. Furthermore, genotypes were well characterized by whether they exhibited large competitive effects, whether they were consistently suppressed by neighbors of different genetic identity, or whether they benefited from growing in

genetic mixtures. Consistent with the hypothesis that competitive intensity increases along resource gradients (Grime 1973, Goldberg and Novoplansky 1997), the intensity of these interactions decreased on shallow soils, suggesting that environmental stress, and possibly limits to growth from frequent moisture deficit, reduced the importance of neighbor relations to genotype performance. Our results confirm that the rich array of intraspecific interactions found in laboratory and greenhouse genotype studies may also apply to neighboring genotypes in mature vegetation under natural conditions. For example, Cahill et al. (2005) quantified competitive effects and responses of 11 mutant genotypes of *Arabidopsis thaliana*, and similarly found wide variance in competitive effect and response coefficients among genotypes that increased in intensity in more fertile soil. Similarly, using genotypes sampled from different habitats, Vermeulen et al. (2009) found that genotypes of *Potentilla reptans* exhibited contrasting strategies of carbon acquisition that maximize either light interception or tolerance of low light levels.

A clear dependence of *Festuca ovina* performance on the genetic identity of its neighbors begs the question as to the mechanism of neighbor influence. Such a mechanism must explain how the performance of one of the highest yielding genotypes in monoculture can become the worst performing genotype when grown in genetic mixtures (Fo11; Fig. 2). Although our results offer no definitive test of mechanism, we suspect that genotypes of *Festuca* interact differently with soil microbes, and thus indirectly determine either the nutrient regime or interaction strength of surrounding individuals with the microbial community. This hypothesis is based on several observations. First, although individuals of *Festuca* were planted closely enough to have minor canopy interactions after three years, our study system was grazed annually and *Festuca* has only limited ability to modify the light environment of its neighbors. Second, neither mixture nor monoculture performance of genotypes was related to overall genotype size (M. Bilton, *unpublished data*), suggesting that the mechanism of competitive dominance was not directly related to the size of above- or belowground foraging structures (cf. Cahill et al. 2005). Third, *Festuca ovina* is an obligate vesicular-arbuscular mycorrhizal species that is strongly influenced by the extent of mycelium development in nutrient-poor soils (Grime et al. 1987), and our use of ambient soils of low nutrient content and native fungal inoculum allowed ample opportunities for physiological differences of belowground morphology and root chemistry between genotypes to influence mycorrhizal relations. Although it has been shown that interactions at the species level can be mediated by the composition and abundance of mycorrhizae in herbaceous assemblages (Grime et al. 1987, van der Heijden et al. 1998), we know of no evidence that genotypic interactions can be mediated in the same way; and there now exists a novel opportunity

to pursue whether plant-microbe interactions underlie effects of genetic diversity in strongly mycorrhizal species. It is also possible that such microbial interactions are pathogenic; although we did not observe foliar pathogens on *Festuca* genotypes, the rapid spread of pathogens in a genetic monoculture could explain why the performance of a genotype like Fo3 is consistently enhanced when surrounded by intragenotypic neighbors (Schmid 1994, Mundt 2002).

Greater understanding of the extent to which communities and ecosystems depend on the genetic diversity of their constituent populations will depend on further detailed exploration of how genotypes differ at scales relevant to interactions between individuals in nature. Just as species-level diversity studies derive significant insights from manipulations of species that naturally co-occur, genotype-level studies will ideally involve levels of genetic differentiation that typify that of established neighbors. In our study involving neighboring genotypes of long-lived grassland species, ambient levels of genetic differentiation were implicated in the maintenance of species diversity but failed to reveal a significant ecosystem effect, regardless of whether the assemblage involved one or many species. This may not be true in other systems where genetic diversity is strongly linked to dominant species performance, for example through the influence of herbivores or pathogens (Hughes et al. 2008), which we expect to be more significant in nutrient-rich systems (Blumenthal et al. 2009). Additional experimental studies in multi-species communities across a wide range of ecological conditions are required before any generalizations can be made about where genetic diversity effects should be most prominent in natural ecosystems.

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APPENDIX

Statistical summary tables, including output from linear mixed models of aboveground biomass and neighborhood models of *Festuca* genotypes and four-species mixtures (*Ecological Archives* A091-162-A1).