Genetic Identity Affects Performance of Species in Grasslands of Different Plant Diversity: An Experiment with *Lolium perenne* Cultivars

CHRISTIANE ROSCHER1,2,*, JENS SCHUMACHER2,3, WOLFGANG W. WEISSER1 and ERNST-DETLF SCHULZE2

1Institute of Ecology, Friedrich Schiller University Jena, Dornburger Straße 159, D-07743 Jena, Germany, 2Max Planck Institute for Biogeochemistry, POB 100164, D-07701 Jena, Germany and 3Institute of Stochastics, Friedrich Schiller University Jena, Ernst-Abbe-Platz 2, D-07743 Jena, Germany

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- **Background and Aims** Recent biodiversity research has focused on ecosystem processes, but less is known about responses of populations of individual plant species to changing community diversity and implications of genetic variation within species. To address these issues, effects of plant community diversity on the performance of different cultivars of *Lolium perenne* were analysed.
- **Methods** Populations of 15 genetic cultivars of *Lolium perenne* were established in experimental grasslands varying in richness of species (from 1 to 60) and functional groups (from 1 to 4). Population sizes, mean size of individual plants, biomass of individual shoots and seed production were measured in the first and second growing season after establishment.
- **Key Results** Population sizes of all cultivars decreased with increasing community species richness. Plant individuals formed fewer shoots with a lower shoot mass in more species-rich plant communities. A large proportion of variation in plant size and relative population growth was attributable to effects of community species and functional group richness, but the inclusion of cultivar identity explained additional 4–7 % of variation. Cultivar identity explained most variation (28–51 %) at the shoot level (biomass of individual tillers and reproductive shoots, seed production, heading stage). Coefficients of variation of the measured variables across plant communities were larger in cultivars with a lower average performance, indicating that this variation was predominantly due to passive growth reductions and not a consequence of larger adaptive plastic responses. No single cultivar performed best in all communities.
- **Conclusions** The decreasing performance of *Lolium perenne* in plant communities of increasing species richness suggests a regulation of competitive interactions by species diversity. Genetic variation within species provides a base for larger phenotypic variation and may affect competitive ability. However, heterogeneous biotic environments (= plant communities of different species composition) are important for the maintenance of intra-specific genetic variation.

**Key words:** Biodiversity, competition, genetic variation, growth reduction, *Lolium perenne*, phenotypic plasticity, species richness.

**INTRODUCTION**

Over the last decades, the threat to biodiversity has raised concerns about the consequences of species loss for ecosystem functioning. A number of experimental studies have provided evidence that decreasing biodiversity can negatively affect ecosystem-level processes such as a decline in above-ground productivity or an increase in the number of invasive species (Hooper et al., 2005; Balvanera et al., 2006). Recent studies also explored the effects of plant community diversity on plant species themselves. In some of these studies, species were introduced into existing communities by seed addition or as transplanted individuals (Joshi et al., 2000; Symstad, 2000; Diemer and Schmid, 2001; Scherber et al., 2006; Mwangi et al., 2007). Other studies investigated the effects of community-level species richness on individual populations of plants within the community (Hector et al., 2002; van Ruijven and Berendse, 2003; Dimitrakopoulos and Schmid, 2004; Jumpponen et al., 2005; Roscher et al., 2007). Different responses of individual species, i.e. negative, neutral or positive effects of community species richness on species biomass production, indicate that species idiosyncrasy may be a key factor in these studies.

Plant performance does not only depend on environment, but also on genetic constitution. Genetic differentiation between plant populations in response to the abiotic or biotic environment is a common phenomenon (Bradshaw, 1984; Linhart and Grant, 1996). Genotype-specific responses to competition have been reported for several plant species (e.g. Turkington and Harper, 1979; Aarsen and Turkington, 1985; Kelley and Clay, 1987; Fridley et al., 2007). Natural selection that allows plant populations to evolve on ecologically relevant time scales (Thompson, 1998), and recent competition models among genotypes and species (Vellend, 2006) all emphasize the importance of genetic diversity for population performance, community composition and stability. Two main hypotheses propose causal, but opposite effects of community species richness on the range of genetic variation within a population (Vellend and Geber, 2005). The first hypothesis predicts a negative effect of community species richness on
population-level genetic diversity because a diverse community of competing species restricts the chance for individual species to use different parts of a heterogeneous environment and only a subset of genotypes of a species may be able to coexist (stabilizing selection). The second hypothesis postulates a positive effect, because different local assemblages of species may favour different genotypes in competition (diversifying selection). So far, experimental studies on the relationship between population-level genetic diversity and community-level processes have been scarce. Booth and Grime (2003) demonstrated in a microcosm experiment with calcareous grassland species that community composition was more stable over a 5-year study period if component populations were made up of a larger number of genotypes.

Plant individuals are generally capable of phenotypic adaptations to the prevailing environmental conditions. Phenotypic plasticity is jointly determined by a genetic component, an environmental component and genotype–environment interactions (Schlichting, 1986; Pigliucci, 1996; Fordyce, 2006), but ultimately depends on the capacity of a genotype to produce different phenotypes under different environmental conditions (Via et al., 1995; Booy et al., 2000). Although the potential fitness benefits of phenotypic plasticity are generally accepted, trait plasticity does not necessarily have an adaptive value (Sultan, 1987). Phenotypic plasticity may evolve by genetic correlation with other traits under selection (Pigliucci et al., 2006). In addition, phenotypic responses may be the result of passive reductions in growth due to environmental stress such as resource limitation (Sultan, 1987, 2000; Dorn et al., 2000) that cannot be easily disentangled from active plastic responses (van Kleunen and Fischer, 2005; Valladares et al., 2007).

Despite several lines of evidence that genetic identity is important for species responses to plant community diversity, this issue has so far been largely ignored in biodiversity–ecosystem functioning research.

In the present study, experimental grasslands varying in species number (from 1 to 60) and number and identity of functional groups (from 1 to 4: grasses, small herbs, tall herbs, legumes) were used. *Lolium perenne* (Poaceae) was chosen as the model species and small populations of 15 different cultivars of this species were established in each experimental community. *Lolium perenne* is one of the most important grass species in central Europe and worldwide used for the sowing and regeneration of temperate agricultural grassland. Its productivity is stimulated by nitrogen fertilization and it has a high fodder quality and grazing tolerance. Beside its importance as a fodder crop, perennial ryegrass is frequently used as a turf species in lawns (Beddows, 1967). There has been a large number of studies on physiological, morphological and phenological differences among *L. perenne* cultivars, including leaf length and tillering (Hazard et al., 1996; Gautier et al., 1999; Bahmani et al., 2000), growth rate and carbohydrate accumulation ( Humphreys, 1989; Turner et al., 2001), heading and tiller turnover (Laidlaw, 2004), induction of flowering (Aamlid et al., 2000) and seed production (Elgersma, 1990). With the expectation of variation among *L. perenne* cultivars in the present model system, the main questions were as follows. (1) Do cultivars vary in their response to the richness of species and functional groups of local communities? (2) Do cultivars differ in the extent of phenotypic plasticity induced by different numbers and assemblages of plant communities? (3) Is the amount of phenotypic plasticity across plant communities, assessed as coefficient of variation, positively or negatively related to the overall performance of individual cultivars?

**MATERIALS AND METHODS**

**Design of The Jena Experiment**

The study was implemented as part of The Jena Experiment, a large integrated biodiversity study (Roscher et al., 2004). The experimental site is located in the floodplain of the river Saale in Jena (Thuringia, Germany, 50°55′N, 11°35′E, 130 m a.s.l.). Mean annual air temperature in the region is 9.3 °C, and average annual precipitation amounts to 587 mm (Kluge and Müller-Westermeier, 2000). The site was converted from grassland to an arable field around 1960 and received high fertilizer inputs for the last decades prior to the establishment of the experiment in 2002. The soil is a Eutric Fluvisol developed from up to 2 m thick fluvial sediments almost free of stones. Due to the fluvial dynamics of the Saale river, soil texture ranges from sandy loam near the river to silty clay with increasing distance from the river. Because of this gradient in soil characteristics, the site was divided into four blocks parallel to the river, such that effects of soil heterogeneity could be separated from biodiversity effects. Central European species-rich semi-natural grasslands traditionally mown twice a year (Arrhenatherion communities according to the vegetation classification of Ellenberg, 1988) served as the ‘target’ community to create a species pool for the experiment. Sixty species common to these grasslands were chosen and categorized into four functional groups: grasses (16 species), legumes (12 species), small herbs (12 species) and tall herbs (20 species) (Roscher et al., 2004). The experiment had a near-orthogonal design based on the factors species number (1, 2, 4, 8, 16, 60 species) and number of functional groups (1, 2, 3, 4 functional groups) with the limitation that it is not possible to have more functional groups than species in a mixture. Each species richness level was replicated 16 times except for the 16-species mixtures with 14 replicates because species number in two functional groups was too small to create mixtures containing legumes or small herbs only and the 60-species mixture with four replicates (Table 1). In total, 82 plots of 20 × 20 m size were established. Mixtures were created by drawing at random (with replacement). A complete species list of all experimental communities is given in Table S1 in the Supplementary Information, available online.

Plots were sown from 11 to 16 May 2002. The numbers of seeds sown aimed at a density of 1000 seedlings m⁻² divided equally among species and were corrected for germination rates following laboratory tests (Roscher et al., 2006). In addition, phenotypic responses to different environmental conditions (Schlichting, 1986; Pigliucci, 1996; Fordyce, 2006), but ultimately depends on the capacity of a genotype to produce different phenotypes under different environmental conditions (Via et al., 1995; Booy et al., 2000). Although the potential fitness benefits of phenotypic plasticity are generally accepted, trait plasticity does not necessarily have an adaptive value (Sultan, 1987). Phenotypic plasticity may evolve by genetic correlation with other traits under selection (Pigliucci et al., 2006). In addition, phenotypic responses may be the result of passive reductions in growth due to environmental stress such as resource limitation (Sultan, 1987, 2000; Dorn et al., 2000) that cannot be easily disentangled from active plastic responses (van Kleunen and Fischer, 2005; Valladares et al., 2007).

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PO4-P storage varied between 0.5 and 2.2 g m\(^{-2}\), while PO4-P storage was at an optimum level based on a classification used for CAL extraction (Oelmann, 2006).

Although removal of mown plant material without fertilizer addition results in a continuous export of nutrients during the course of the experiment, nitrogen mineralization contributes N uptake by plants. Phosphorous supply is at an advanced level based on knowledge from agricultural cultivar standard (calcium–lactate–acetate) extraction (Oelmann, 2006).

Sub-experiment with Lolium perenne cultivars

The grass *Lolium perenne* L. (perennial ryegrass) is a hemicryptophyte with a loose to densely tufted growth. Longevity of plant individuals ranges from annual or biennial life span to prevailing persistent forms. The individual tillers are annual or winter-anual, depending on the time taken to develop an inflorescence. Under suitable conditions plantlets may be formed in the leaf-axils and allow for clonal propagation. However, reproduction by seeds is the main mode of propagation (Beddows, 1967).

Perennial ryegrass is a self-incompatible, wind-pollinated species. In common with many other temperate grasses, *L. perenne* requires low temperatures and/or short days for primary flower induction, and floral initiation depends on subsequent day length (Cooper, 1960; Heide, 1994).

*Lolium perenne* was not included in the pool of 60 species used to create the experimental communities because of the planned sub-experiment. Accessions of 15 cultivars of *L. perenne* were provided by the Institute of Plant Genetics and Crop Research Gatersleben (IPK, Germany). This selection covered a range of variability based on knowledge from agricultural cultivar standard tests on productivity, the capability to form dense swards and phenology (E. Willner, Institute for Plant Genetics and Crop Research Gatersleben, Germany, pers. comm.), but was restricted to diploid cultivars with a perennial life cycle. Accessions of seven forage cultivars (‘Barlano’, ‘Borvi’, ‘Duramo’, ‘Fennema’, ‘Gremie’, ‘Kerdion’, ‘Lilasso’) and accessions of eight turf cultivars (‘Bellatrix’, ‘Bianca’, ‘Entrar’, ‘Gator’, ‘Juwel’, ‘Langa’, ‘Lisuna’, ‘Lorina’) were included. Molecular analyses with SNP markers gave evidence that all cultivars used for this study were genetically different (S. Nestman, Max Planck Institute for Biogeochemistry, Jena, unpubl. res.). Preliminary germination tests served to control for differences in seed viability and to determine germination rates. Tests were performed on filter paper with three replicates per cultivar subjected to a standardized temperature and light regime (16 h day at 20 °C and a night temperature of 12 °C). Per replicate, 50 seeds were placed in a Petri dish and checked for germination every other day. Germination tests were terminated after 40 d.

The experiment with 15 *Lolium* cultivars was nested in the large-area plots of The Jena Experiment and was sown at the same time as the experimental communities. An area of 3 × 4 m in each 20 × 20 m plot was divided into 15 sub-plots of 0.8 × 1.0 m size. Cultivars were distributed randomly among these small sub-plots in each plot. The aimed sowing density amounted to 100 individuals m\(^{-2}\) (adjusted for average germination rates of 80 % from laboratory tests), in addition to the 1000 seedlings in the mixture of the plant community. In addition, in each experimental block a small-area monoculture of *Lolium* cultivars was established. Here, plots of 3.5 × 3.5 m were divided for the cultivars into sub-plots each 1/15th of the total plot area. The aimed sowing density was 1100 individuals m\(^{-2}\) to achieve a total plant density similar to the density in the experimental communities containing *Lolium* cultivars as additional species.

### Sub-experiment with Lolium perenne cultivars

**Table 1. Summary of the experimental design showing the number of replicates for each combination of species number and functional group number**

<table>
<thead>
<tr>
<th>Species number</th>
<th>1</th>
<th>2</th>
<th>4</th>
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<th>16</th>
<th>60</th>
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<tr>
<td>Functional group no.</td>
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<tr>
<td>No. of plots</td>
<td>16</td>
<td>16</td>
<td>16</td>
<td>16</td>
<td>14</td>
<td>4</td>
</tr>
</tbody>
</table>

2004). Plots were mown twice a year in early June and in the middle of September according to the typical management regime of extensively used hay-meadows. In the year of sowing, mowing first took place 8 weeks after sowing at the beginning of July. Plots were regularly weeded by hand to prevent establishment of unsown species. Plots remained unfertilized during the whole experiment. In the beginning of the experiment (autumn 2002) mineral NO3-N storage in the soil (30 cm depth) varied between 0.5 and 2.2 g m\(^{-2}\), while PO4-P storage was in a range 23.7–82.7 g m\(^{-2}\) (Oelmann, 2006).

Although removal of mown plant material without fertilizer addition results in a continuous export of nutrients during the course of the experiment, nitrogen mineralization compensates N uptake by plants. Phosphorous supply is at an optimum level based on a classification used for CAL (calcium–lactate–acetate) extraction (Oelmann, 2006).

### Measurements

The number of established *Lolium* individuals per sub-plot was counted after first mowing in July 2002 to determine initial population sizes 10 weeks after sowing. Counting of plant individuals was repeated 1 year later in July 2003. Relative growth rates of populations were calculated using the equation

\[
\text{RGR} = (\log_e N_{t2} - \log_e N_{t1})/a
\]

where \(N_{t1}\) is the number of individuals in the first year, \(N_{t2}\) is the number of individuals in the second year, and \(a\) is the length of the time interval (= 1 year; Harper, 1977).

The number of vegetative tillers per *Lolium* individual was determined as a measure of plant individual size in early October in both years. Five individuals per cultivar and plot were selected at random, and the number of tillers per tussock was counted. The size of individuals with >100 tillers was estimated by counting the tillers in part of the tussock. Tillers of plantlets developing in leaf-axils which occurred in a few individuals (see above) were counted as tillers of the established plant individual. Counts per plot and cultivar were averaged. Mean relative growth rates of plant individuals were calculated according
to the above equation using the numbers of tillers counted in both years.

The average size of flowering shoots and vegetative tillers was determined in the second year of the experiment in 2003. Flowering shoots were studied just before the first mowing between 4 and 10 June 2003 in mixtures with one, four and 16 species only. Eight randomly selected flowering shoots per cultivar and plot were harvested. They were classified on a five-part scale of flowering stages defined according to the relative position of the developing spike to the uppermost leaf of the flowering shoot: 0 = spike completely covered by the sheath of the uppermost leaf; 1 = spike conspicuously overtopped by the developing blade of the uppermost leaf, inflorescence not fully developed; 2 = top of spike and blade of the uppermost leaf of equal height; 3 = spike overtops blade of the uppermost leaf conspicuously, not flowering; 4 = flowering with visible anthers. The number of spikelets per spike was counted. The prevailing flowering stage of the flowering shoots was calculated as mode, whereas numbers of spikelets per spike were averaged per cultivar for each plot. All flowering shoots per sample were pooled to determine dry mass after drying at 70 °C for 48 h. Vegetative tillers were harvested between 14 and 19 August 2003 before the second mowing. Ten randomly selected tillers were cut off near the base, dead leaf material was separated, and samples were weighed after drying. The obligate vernalization for flower induction in L. perenne ensured that cultivars could be unequivocally assigned to their sub-plots during the first and the second growing season of the experiment. Populations consisted of the initially sown cohort because inflorescence development started almost exclusively after the first winter period.

Data analyses

Data were analysed using S-Plus® 7.0 (Insightful Corp., 2005). Because the experimental factors species number and functional group number are linked in The Jena Experiment (Roscher et al., 2004), it is not possible to completely disentangle their relative effects in a statistical analysis. Therefore, general linear models were used with sequential sum of squares (type I SS) following the a priori hypotheses of the experiment which addresses: sown species richness (partitioned into log-linear term and deviation from linear); functional group richness (partitioned into linear term and deviation from linear); and cultivar identity and interactions between cultivar and the experimental factors. Additionally, alternative statistical models were fitted that included separate contrasts for the presence/absence of each functional group and their interaction with Lolium cultivars to test for effects of particular functional groups. Block identity was incorporated as a factor to remove variance caused by a gradient of soil conditions in the experimental field (Roscher et al., 2004), but a block effect could also result from a specific order of plot management (weeding, mowing) or the block-wise organization of data collection. Because of the split-plot design of the Lolium cultivar experiment, the diversity terms (species richness, functional group richness, presence of particular functional groups) were tested at the between-plot level, while Lolium cultivars and their interaction with diversity terms were tested at the within-plot level. The following response variables were analysed: population size (number of individuals in the first and relative population growth in the second year, respectively), mean size of plant individuals (average number of tillers per tussock in the first and relative change in the second year, respectively), mean dry mass per vegetative tiller and per flowering shoot, flowering stage and number of spikelets per spike in the second year. In addition, the product of population size (number of plant individuals) and plant individual biomass (average tussock size multiplied by average dry mass per vegetative tiller) were used to estimate population performance (= estimated yield) from the second-year data. Analyses of variables characterizing generative reproduction included plots with one, four and 16 species (in total 46 plots), because these variables were not measured in all plots. By contrast, analyses of other variables were based on all species-richness levels (in total 82 plots). When necessary, the response variables were log-transformed to homogenize variances. Multiple comparisons (e.g. between specific cultivars) were not performed after the global split-plot analysis of variance because this study was mainly interested in the estimation of the relative contribution of the different explanatory factors, especially of the genetic variation between all the cultivars, to the different response variables. The hypotheses tested in the split-plot analysis of variance are well-defined a priori hypotheses and a Bonferroni-type correction was therefore not applied (Quinn and Keough, 2002).

The sequential sums of squares were used to estimate the amount of variation explained (= fraction of total sum of squares) by cultivar identity, the diversity treatments (sum of variance explained by community species richness and functional group richness) and differences among the cultivars in their response to the diversity treatments (interaction of cultivar identity x diversity treatments). For these calculations, a reduced data set of 46 plots (only mixtures with one, four and 16 species) was used for all variables to make the analyses comparable.

In addition, the performance (= estimated yields) of Lolium cultivars in mixtures ($P_{\text{mix}}$) was compared with the performance expected from the average of the four replicated monocultures of the respective cultivar ($P_{\text{mono}}$). Monoculture performance was corrected for sowing density (monocultures, 1100 Lolium individuals m$^{-2}$; mixtures, 100 Lolium individuals m$^{-2}$ and 1000 plant individuals of other species). A ratio ($P_{\text{mix}}/P_{\text{mono}}$) > 1 indicates that performance in mixtures was better than performance in monocultures, while ($P_{\text{mix}}/P_{\text{mono}}$) < 1 suggests the opposite.

Coefficients of variation (CVs) were computed across all experimental communities for estimated yield and its components to compare the extent of variation within each Lolium cultivar induced by different environments in species mixtures. Coefficients of variation across cultivars in each plant community were used to assess whether this variation changes in relation to community species richness.
**RESULTS**

*Population size*

*Lolium* cultivars differed significantly in the number of established individuals (Table 2). Initial population size in individual cultivars, measured 10 weeks after sowing, decreased with a log-linear increase of sown species richness (Fig. 1A) and was affected by the presence of particular functional groups. While the presence of tall herbs positively affected the establishment success of *Lolium* cultivars, the overall negative effects of other grasses in the experimental communities on initial population sizes depended on cultivar identity (significant interaction term; Table 2). Relative growth rates of populations estimated 1 year later decreased with a log-linear increase of community species richness and were often negative in mixtures with higher species richness (Table 2 and Fig. 1B). Thus, the negative effects of species richness on population sizes of *Lolium* cultivars increased from the first to the second year. However, relative population growth rates and the size of the species richness effect differed significantly among cultivars. Additionally, effects of the presence of legumes on changes in population size varied with *Lolium* cultivar identity.

*Individual plant size*

The mean number of tillers per tussock in the year of establishment (2002) and relative growth of plant individuals 1 year later (2003) differed significantly among *Lolium* cultivars. Tussock size and relative growth decreased with increasing community species richness, but the size of this effect varied among cultivars (Table 2 and Fig. 1C, D). On average, relative growth rates of *Lolium* tussocks were positive in one- to 16-species communities, whereas in communities made up of the full complement of 60 species *Lolium* tussocks had fewer tillers in the second year compared with the first year. In addition, it was observed that the number of tillers per tussock developed during the first growing season was positively affected by the presence of tall herbs, whereas the presence of legumes had negative effects on the average number of tillers per tussock. The presence of small herbs had significant positive effects on the relative growth of plant individuals in the second year (Table 2).

Average size of individual tillers (= tiller dry mass) in the second year differed significantly among *Lolium* cultivars. However, a log-linear increase of community species richness had negative effects on tiller dry mass in all cultivars (Table 2 and Fig. 1E).

*Estimated yield per population*

Estimated yield per population in the second year of the study (= number of plant individuals × average number of tillers × average tiller dry mass) depended on *Lolium* cultivar identity. Although all cultivars sustained a decrease of estimated yield with increasing species richness of the mixtures, the size of this effect varied between different *Lolium* cultivars (Table 2 and Fig. 1F).

*Reproductive growth*

Being exposed to low temperatures and short days during winter, nearly all surviving tillers of the first growing season induced inflorescence development in the spring of the second growing season. The mean flowering stage before the first mowing differed significantly among *Lolium* cultivars (Table 3 and Fig. 2A). The presence of legumes delayed inflorescence development of *L. perenne* independent of cultivar identity. Although dry mass of flowering shoots varied considerably among cultivars, overall negative effects of increasing community species richness were found on dry mass of flowering shoots (Table 3 and Fig. 2B). The average number of spikelets per spike differed significantly among cultivars (Fig. 2C). The negative effect of legume presence on the number of spikelets (Table 3) disappeared when the flowering stage was entered as covariate (analysis not shown). Thus, this effect was attributable to differences in the developmental stage of the spikes in mixtures with/without legumes. Community species richness had no effect on the flowering stage or the number of spikelets per spike.

*Relative population performance of cultivars in mixtures compared with cultivar monocultures* ($P_{\text{mix}}/P_{\text{mono}}$)

The performance (= estimated yield) of *Lolium* cultivars in mixtures ($P_{\text{mix}}$) was compared with expected values derived from the average of the respective cultivar monocultures ($P_{\text{mono}}$). All cultivars reached an average $P_{\text{mix}}/P_{\text{mono}} > 1$ (inter-specific competition < intra-specific competition) in mixtures with one or two other species, although large variation within the species-richness levels was found. The ratio $P_{\text{mix}}/P_{\text{mono}}$ decreased below or only slightly exceeded one (inter-specific competition > intra-specific competition) in mixtures with four and more other species. Averaged across all mixtures, the relative performance differed to some degree among *Lolium* cultivars (see Table S2 in Supplementary Information, available online).

Coefficients of variation (CVs) of the ratio $P_{\text{mix}}/P_{\text{mono}}$ across cultivars were calculated for each mixture. These CVs increased significantly with species richness ($F_{1,77} = 24.67, P < 0.001$; Fig. 3), indicating a higher variation in competitive responses of *Lolium* cultivars with increasing inter-specific competition. *Lolium* cultivars were ranked for each mixture according to their relative population performance. The proportion of occurrences of these ranks is shown for all cultivars in Fig. 4 and illustrates that no cultivar performed best in all mixtures, but that each of them achieved its best performance in particular mixtures.

The magnitude of variation in population performance (= estimated yields) across all species mixtures, expressed as CV, was compared with the CVs of single components of population performance to inspect whether cultivars differed with respect to the traits responsible for the variation in estimated yields (Fig. 5A). CVs of population performance ranged from 0.99 to 1.59 for the 15 cultivars. In all cultivars, the largest variation across experimental mixtures was found for the size of the plant individuals (CV = 0.87 ± 0.14 s.d.), while coefficients of variation were lower for
Table 2. Summary of statistical analyses of measures of vegetative growth in relation to the experimental treatments

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>No. of individuals</th>
<th>Rel. population growth</th>
<th>Tussock size</th>
<th>Rel. tussock growth</th>
<th>Dry mass per tiller</th>
<th>Population performance</th>
</tr>
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<tr>
<td></td>
<td>d.f.</td>
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<tr>
<td>Block</td>
<td>3</td>
<td>227.5 0.90</td>
<td>8.13 2.62</td>
<td>0.42 0.45</td>
<td>8.89 2.28</td>
<td>6457.4 6.42***</td>
</tr>
<tr>
<td>SR (log-linear)</td>
<td>1</td>
<td>2981.9 11.82*** ↓</td>
<td>40.71 13.11*** ↓</td>
<td>9.04 9.80** ↓</td>
<td>104.13 26.70*** ↓</td>
<td>13247.5 13.18*** ↓</td>
</tr>
<tr>
<td>Deviation from SR</td>
<td>4</td>
<td>303.8 1.20</td>
<td>4.26 1.37</td>
<td>0.95 1.03</td>
<td>5.38 1.38</td>
<td>694.5 0.69</td>
</tr>
<tr>
<td>FG (linear)</td>
<td>1</td>
<td>68.2 0.27</td>
<td>4.08 1.32</td>
<td>0.56 0.61</td>
<td>4.34 1.11</td>
<td>2.6 &lt;0.01</td>
</tr>
<tr>
<td>Deviation from FG</td>
<td>2</td>
<td>257.8 1.02</td>
<td>7.71 2.48</td>
<td>0.66 0.71</td>
<td>3.80 0.97</td>
<td>815.2 0.81</td>
</tr>
<tr>
<td>Grasses</td>
<td>1</td>
<td>2030.9 8.97*** ↓</td>
<td>1.35 0.43</td>
<td>1.66 1.82</td>
<td>14.70 3.93</td>
<td>3055.6 3.13</td>
</tr>
<tr>
<td>Legumes</td>
<td>1</td>
<td>1.5 &lt;0.01</td>
<td>6.47 2.12</td>
<td>5.47 6.39*** ↓</td>
<td>11.03 2.91</td>
<td>3114.1 3.20</td>
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<tr>
<td>Small herbs</td>
<td>1</td>
<td>2.3 &lt;0.01</td>
<td>5.29 1.72</td>
<td>2.09 2.31</td>
<td>18.27 4.95†</td>
<td>412.3 0.41</td>
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<tr>
<td>Tall herbs</td>
<td>1</td>
<td>1945.3 8.54*** ↑</td>
<td>0.66 0.21</td>
<td>6.26 7.41*** ↑</td>
<td>14.01 3.73</td>
<td>316.9 0.31</td>
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<td>Plot</td>
<td>70</td>
<td>252.2 3.10</td>
<td>0.92</td>
<td>3.90</td>
<td>1005.1 39.59</td>
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<tr>
<td>Cultivar</td>
<td>14</td>
<td>1462.3 74.54***</td>
<td>2.36 12.63*** ↑</td>
<td>0.34 15.22*** ↑</td>
<td>1.93 6.73*** ↑</td>
<td>6978.1 55.70***</td>
</tr>
<tr>
<td>Cultivar × SR (log-linear)</td>
<td>14</td>
<td>29.1 1.48</td>
<td>0.64 3.41*** ↑</td>
<td>0.05 2.09**</td>
<td>1.30 4.52*** ↑</td>
<td>70.1 0.56</td>
</tr>
<tr>
<td>Cultivar × deviation from SR</td>
<td>56</td>
<td>21.2 1.08</td>
<td>0.28 1.47*</td>
<td>0.03 1.24</td>
<td>0.52 1.81*** ↓</td>
<td>160.7 1.28</td>
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<tr>
<td>Cultivar × FG (linear)</td>
<td>14</td>
<td>23.9 1.22</td>
<td>0.20 1.05</td>
<td>0.03 1.44</td>
<td>0.19 0.67</td>
<td>118.9 0.95</td>
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<tr>
<td>Cultivar × deviation from FG</td>
<td>28</td>
<td>22.2 1.13</td>
<td>0.18 0.96</td>
<td>0.02 1.09</td>
<td>0.27 0.94</td>
<td>83.7 0.67</td>
</tr>
<tr>
<td>Cultivar × grasses</td>
<td>14</td>
<td>39.2 2.03†</td>
<td>0.29 1.55</td>
<td>0.02 0.74</td>
<td>0.17 0.60</td>
<td>45.0 0.36</td>
</tr>
<tr>
<td>Cultivar × legumes</td>
<td>14</td>
<td>20.7 1.06</td>
<td>0.33 1.77*</td>
<td>0.02 0.79</td>
<td>0.32 1.12</td>
<td>101.0 0.80</td>
</tr>
<tr>
<td>Cultivar × small herbs</td>
<td>14</td>
<td>15.8 0.80</td>
<td>0.06 0.32</td>
<td>0.01 0.46</td>
<td>0.37 1.31</td>
<td>137.3 1.10</td>
</tr>
<tr>
<td>Cultivar × tall herbs</td>
<td>14</td>
<td>20.3 1.03</td>
<td>0.11 0.60</td>
<td>0.01 0.52</td>
<td>0.15 0.53</td>
<td>87.3 0.70</td>
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<tr>
<td>Residuals</td>
<td>1022</td>
<td>19.6 0.19</td>
<td>0.02 0.29</td>
<td>125.3 2.21</td>
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</table>

Due to the split-plot design of the experiment, species richness (SR) and functional group richness (FG) were tested at plot level, while cultivar and the interaction with the diversity treatments (SR, FG) were tested against the residuals. Note, that the terms for the presence of functional groups (grasses, legumes, small herbs, tall herbs) and their interaction with cultivar idenity were added alternatively in additional models. The analyses included mixtures of all species-richerst levels (n = 82). Listed are the mean sums of squares (MS), F ratios (F) and the level of significance, where *P ≤ 0.05, **P < 0.01 and ***P < 0.001. Arrows indicate significant increase (↑) or decrease (↓) of the measures with community species richness or presence of particular functional groups.
population size (CV = 0.50 ± 0.10 s.d.) and average dry mass per tiller (CV = 0.43 ± 0.08 s.d.). CV of population performance was linearly correlated with CVs of individual performance components. The significant negative correlation between CVs of population performance across mixtures and average relative population performance per cultivar ($P_{\text{mix}}/P_{\text{mono}}$) indicated that variation was particularly high in cultivars with a low average performance (Fig. 5B).

Relative importance of genetic variation

Although the diversity treatments (in particular species richness) and cultivar identity significantly affected the variables measured to characterize the performance of *Lolium* cultivars, the fraction of explained variation differed strongly (Fig. 6). Variation in characteristics of generative reproduction (flowering stage, number of spikelets per spike) was largely attributable to *Lolium* cultivar identity. Cultivar identity also explained a large percentage of variation in the biomass of vegetative tillers and reproductive shoots, but diversity treatments also accounted for a significant proportion of variation in these variables (see also Tables 2 and 3). By contrast, higher percentages of variation in population sizes and the size of individual plants ($=$ tussock size) were explained by effects of the experimental treatments, although the establishment success
Due to the split-plot design of the experiment, species richness (SR) and functional group richness (FG) were tested at plot level, while cultivar and the interaction with the diversity treatments (SR, FG) were tested against the residuals. Note, that the terms for the presence of functional groups (grasses, legumes, small herbs, tall herbs) and their interaction with cultivar identity were added alternatively in additional models. The analyses included mixtures with one, four and 16 species only (n = 46). Listed are the mean sums of squares (MS), F ratios (F) and the level of significance, where *P ≤ 0.05, **P < 0.01 and ***P < 0.001. Arrows indicate significant decrease (↓) of the measures with community species richness or presence of particular functional groups.

(Initial population sizes) varied considerably depending on cultivar identity. For all measured variables, differences among cultivars in response to the diversity treatments accounted for 3–6% of variation.

**DISCUSSION**

*Effects of plant community diversity on cultivar performance*

The results show that increasing species richness of the experimental communities had overall negative effects on the performance of 15 cultivars of *L. perenne*. These findings are in accordance with previous studies that reported that the positive biodiversity–productivity relationship at the community level is not necessarily related to a higher productivity of individual species in plant communities of increasing species richness (e.g. Naeem et al., 1996; Tilman et al., 1997; van Ruijven and Berendse, 2003; Roscher et al., 2007).

Plant species productivity is controlled via plant species density and plant individual size. Although density–size regulations have often been studied in populations of individual species (see Harper, 1977), the relationship between plant species density and plant individual size has attracted less attention in plant communities of higher species richness so far (but see Roscher et al., 2007). Studies with transplanted individuals in The Jena Experiment (Scherber et al., 2006; Mwangi et al., 2007) and at the Swiss BIODEPTH site based on a similar pool of grassland species (Diemer and Schmid, 2001) found no or negative effects of plant species richness on plant individual biomass. The present analysis of *L. perenne* sown with the same initial density in all experimental communities provided evidence for the occurrence of negative effects of plant species richness on plant performance at the population level (number of established and surviving individuals) and at the level of individual plants (tussock size, dry mass of vegetative tillers and flowering shoots). However, the negative effects of plant species richness were most pronounced with respect to the number of tillers per individual (Fig. 5A). Density experiments with *L. perenne* demonstrated that plant individual size of this species with a highly organized modular growth is predominantly determined by variation in tiller number, rather than by tiller size (Kays and Harper, 1974). *Lolium perenne* has a high tolerance to grazing and cutting. However, it is well known that lower light availability is mainly responsible for reduced tillering and increased leaf death in dense monocultures of perennial ryegrass, while growth control by nutrient availability is more important in stands of lower density (Ong, 1978; Simon and Lemaire, 1987). Previous biodiversity experiments have shown that more species-rich plant communities may utilize resources more completely by intercepting more light and taking up more nutrients (e.g. Spehn et al., 2005). Closed vegetation canopies are characterized by a gradient in light quantity (photon flux density) as well as changes in light quality (ratio of red to far red light) in the lower canopy levels (Monsi and
Saeki, 1953; Jones, 1992). Lower red/far-red ratios change the phytochrome status and lead to a reduction in tillering rate in *L. perenne* (Deregibus *et al.*, 1983). Self-shading at the tiller base in dense monoculture swards induces a reduction of tillering accompanied by an increasing weight of individual tillers in perennial ryegrass (Kays and Harper, 1974). In the present study, the reduced formation of tillers with increasing community species richness and in plant communities with legumes was probably due to shading by taller, more competitive species and could not be compensated for by an increased size in individual tillers. Smaller plant individuals have a lower chance of survival. Therefore, the more pronounced negative effects of plant species richness and legume presence on population sizes of *L. perenne* cultivars in the second year were not surprising. In particular, at the highest species-richness levels, a high mortality of *Lolium* individuals was found, suggesting a regulation of species diversity by competitive interactions.

The present study of *L. perenne* populations only included the first two years after the establishment of the experiment. A decrease in plant individual size with increasing plant species richness could in the longer term result in the extinction of *L. perenne* in these communities, as it is associated with a reduced seed production, and establishment from seed is the predominant mechanism of regeneration in this grass species. The negligible effects of the diversity treatments on the number of spikelets per spike imply that the number of seeds produced is mainly controlled by the number of flowering shoots per individual plant. Possible direct effects on seed quality could not be tested because differences in seed maturation prevented the development of ripe seeds in all cultivars before mowing. Generally, modules (≈ tillers) of *L. perenne* complete their life cycle with the development of an inflorescence, and smaller individuals thus inevitably produce a smaller number of flowering shoots. In addition, it was observed that the presence of legumes affected reproduction by a delay in inflorescence development. Spike emergence in *L. perenne* is influenced by the prevailing temperature conditions during floral development and stem elongation (Camlin, 1975). Thus, the effect of legumes on flowering stages could also be due to modified microclimatic conditions in dense plant canopies.

**Phenotypic plasticity**

Although the present study showed that the general outcome of competition across a species-richness gradient in different multi-species assemblages was similar for all cultivars tested, a significant proportion of variation in the variables measured was accounted for by cultivar identity.
Fig. 4. Ranking of *L. perenne* cultivars corresponding to their relative performance ($P_{\text{mix}}/P_{\text{mono}}$) in species mixtures ($n = 82$) in the second year of the experiment (2003). Ranks from 1 to 15 are given starting with the best cultivar in a mixture. Ranks between 2 and 5, 6 and 10 and 11 and 14 are summarized in the graph. The proportional occurrence of these ranks was calculated across all mixtures. Cultivars were arranged from left to right according to their overall relative success in species mixtures.

Fig. 5. Coefficient of variation (CV) of population performance across all species mixtures in relation to (A) CVs of individual traits, and (B) in relation to relative population performance ($P_{\text{mix}}/P_{\text{mono}}$; mean ± 1 s.e.), for 15 *L. perenne* cultivars in the second year of the experiment (2003). Pearson correlation coefficients are given, where *$P \leq 0.05$, **$P < 0.01$ and ***$P < 0.001$. (Fig. 6). In addition, the strength of the response to the species-richness gradient and the presence of particular functional groups in the experimental plant communities differed to some degree among cultivars (Table 2). It is well known that the degree of phenotypic plasticity in response to environmental conditions varies amongst individuals of a species and between traits (Schmid, 1992; Sultan, 1995; West-Eberhard, 2003). In plants, plasticity is often lower in association with reproductive structures than in association with vegetative growth characteristics (Schmid, 1992). In the present study, variables measured at the shoot level appeared to underlie stronger developmental constraints and had the lowest variation. In particular, variation in characteristics of generative reproduction (flowering stage, number of spikelets per spike) and biomass of individual shoots could largely be attributed to differences among cultivars and depended less on the resident plant community. Active phenotypic plasticity is a well-known mechanism of resource-use optimization to
enhance plant performance. Therefore, a negative correlation between the degree of variation in population performance across the experimental mixtures and the average population performance of individual cultivars (Fig. 5B) suggests that this variation was not due to a plastic adaptation but caused by a passive reduction (‘non-adaptive’ plasticity) because of growth limitation (Scheiner, 1993; van Kleunen and Fischer, 2005; Ghalambor et al., 2007).

The importance of genetic variation

A number of studies have shown that competitive interactions among plant species favour particular genotypes and lead to a decline in genetic variation within plant populations, while a heterogeneous biotic environment has positive effects on within-species diversity (e.g. Turkington and Harper, 1979; Aarssen and Turkington, 1985; Kelley and Clay, 1987; Taylor and Aarssen, 1990; Lütscher et al., 1992; Vavrek, 1998). Only recently, Fridley et al. (2007) demonstrated that plant genotype performance is influenced by the genetic identity of neighbouring species, but that relationships among genotypes of different species may change with environmental conditions. The insurance hypothesis of Yachi and Loreau (1999) stresses the idea that species diversity provides greater guarantee of ecosystem functioning because species respond differently to environmental fluctuations. While previous studies on genotype-specific responses were mostly conducted in species-poor model systems, the present study with different cultivars of *L. perenne* across communities of different species richness and composition is consistent with the view that genetically based differences within species determine competitive ability in specific environmental situations or in communities with different competitors and that such differences may thus contribute to ecosystem stability (Aarssen 1989, 1992; Hughes and Stachowicz, 2004; Vellend, 2006; Whitlock et al., 2007). However, the increasing variation of relative population performance across cultivars with increasing plant community diversity (Fig. 3) supports the hypothesis that only a subset of genotypes of a species is able to coexist with a diverse community of competing species (Vellend and Geber, 2005).

In conclusion, differences in plant genetic constitution provide a basis for larger phenotypic variation and may increase competitive ability even in a subordinate species. However, the genotype-specific responses to selection pressure caused by plant communities of different species composition imply that diversity in biotic environments is important for the maintenance of genetic diversity of a species.

**SUPPLEMENTARY INFORMATION**

Supplementary Information is available online at http://aob.oxfordjournals.org/ and consists of details of the 82 species mixtures, including numbers of species sown and functional groups (Table S1), and relative performance of *Lolium* cultivars calculated as the ratio of the performance in mixture ($P_{mix}$) to the performance expected from the average of the four replicated monocultures of the respective cultivar ($P_{mono}$; Table S2).

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**LITERATURE CITED**


