Complementarity among species in horizontal versus vertical rooting space

von Felten, S; Schmid, B
Complementarity among species in horizontal versus vertical rooting space

Abstract

Aims:
Many experiments have shown a positive effect of species richness on productivity in grassland plant communities. However, it is poorly understood how environmental conditions affect this relationship. We aimed to test whether deep soil and limiting nutrient conditions increase the complementarity effect (CE) of species richness due to enhanced potential for resource partitioning.

Methods:
We grew monocultures and mixtures of four common grassland species in pots on shallow and deep soil, factorially combined with two nutrient levels. Soil volume was kept constant to avoid confounding soil depth and volume. Using an additive partitioning method, we separated biodiversity effects on plant productivity into components due to species complementarity and dominance.

Important findings:
Net biodiversity and complementarity effects were consistently higher in shallow pots, which was unexpected, and at the low nutrient level. These two results suggest that although belowground partitioning of resources was important, especially under low nutrient conditions, it was not due to differences in rooting depths. We conclude that in our experiment (i) horizontal root segregation might have been more important than the partitioning of rooting depths and (ii) that the positive effects of deep soil found in other studies were due to the combination of deeper soil with larger soil volume.
Complementarity among species in horizontal versus vertical rooting space

Stefanie von Felten1,2,* and Bernhard Schmid1

1 Institute of Environmental Sciences, University of Zurich, 8057 Zurich, Switzerland
2 Institute of Plant Sciences, ETH Zurich, 8092 Zurich, Switzerland
*Correspondence address. Stefanie von Felten, Institute of Plant Sciences, ETH Zurich, LFW A2, Universitätsstrasse 2, 8092 Zürich, Switzerland. Tel: +41-44-632-31-90; Fax: +41-44-632-11-53; E-mail: stefanie.vonfelten@ipw.agrl.ethz.ch

Abstract

Aims
Many experiments have shown a positive effect of species richness on productivity in grassland plant communities. However, it is poorly understood how environmental conditions affect this relationship. We aimed to test whether deep soil and limiting nutrient conditions increase the complementarity effect (CE) of species richness due to enhanced potential for resource partitioning.

Methods
We grew monocultures and mixtures of four common grassland species in pots on shallow and deep soil, factorially combined with two nutrient levels. Soil volume was kept constant to avoid confounding soil depth and volume. Using an additive partitioning method, we separated biodiversity effects on plant productivity into components due to species complementarity and dominance.

Important findings
Net biodiversity and complementarity effects were consistently higher in shallow pots, which was unexpected, and at the low nutrient level. These two results suggest that although belowground partitioning of resources was important, especially under low nutrient conditions, it was not due to differences in rooting depths. We conclude that in our experiment (i) horizontal root segregation might have been more important than the partitioning of rooting depths and (ii) that the positive effects of deep soil found in other studies were due to the combination of deeper soil with larger soil volume.

Keywords: biodiversity effects • nutrient limitation • resource partitioning • root competition • soil depth

Received: 25 August 2007 Accepted: 7 December 2007

Introduction
Evidence for positive effects of species richness on ecosystem functioning has rapidly accumulated in recent years (as reviewed e.g. in Balvanera et al. 2006; Cardinale et al. 2006; Hooper et al. 2005; Kinzig et al. 2002; Loreau et al. 2002). In particular, plant species richness was shown to increase productivity in temperate grassland communities (e.g. Hector et al. 1999; Roscher et al. 2005; Tilman et al. 1996; van Ruijven and Berendse 2003). However, little is still known about the precise mechanisms that create this relationship and how environmental conditions can alter it.

In line with resource-based competition theory (e.g. Tilman et al. 1997), the positive effect of species richness on productivity (i.e. overyielding of mixtures) has largely been attributed to complementarity of species with regard to resource use. Through differences among species in nutrient uptake in space or time, species richness is thought to improve the resource use of mixtures. It is possible to statistically assess the importance of species complementarity as opposed to selection or dominance by additive partitioning (Fox 2005; Loreau and Hector 2001). While application of the method has shown that complementarity is an essential mechanism behind the diversity–productivity relationship (Cardinale et al. 2007; Loreau and Hector 2001; Roscher et al. 2005; Tilman et al. 2001; van Ruijven and Berendse 2003), it remains difficult to demonstrate which plant traits are actually involved in complementary resource use leading to overyielding in mixtures.

It has long been known that grassland species differ in root morphology including rooting depth (e.g. Cole and Holch 1941; Fitter 1986; Parrish and Bazzaz 1976; Weaver 1958), suggesting consequences for interspecific competition. McKane et al. (2002) found evidence for complementary use of nitrogen in arctic plant communities with respect to depth, chemical form and timing of uptake. Berendse (1981, 1982) demonstrated that a deep-rooting temperate grassland
species benefits from deeper soil when growing in mixture with a shallow-rooting species. The effect of increased soil depth was larger in unfertilized than in fertilized soil, indicating that different environmental aspects (soil depth and nutrients) interact and that species complementarity may be more relevant under limiting conditions. However, in other studies, increased soil fertility enhanced the effect of diversity on productivity in experimental plant communities (Fridley 2002, 2003; He et al. 2002; Reich et al. 2001).

A recent study showed that increased biotope space in terms of soil depth and volume linearly increased biodiversity effects due to increased complementarity of species (Dimitrakopoulos and Schmid 2004). However, in this experiment as well as in Berendse’s (1981, 1982), soil volume and soil depth were confounded, and it remains unclear whether the result was due to increased soil depth or soil volume and the nutrients within. More precisely, did deep soil benefit mixtures by allowing differences in rooting depths per se, thereby relaxing interspecific competition, or rather by providing a higher total amount of nutrients, explored by those species able to grow deeper roots?

In this study, we tested whether soil depth and nutrients— independent of soil volume—positively affect the diversity-productivity relationship in mixtures of four common grassland plant species. We hypothesized that (i) deep soil would allow for enhanced complementarity between plant species with respect to rooting depth and (ii) that this may be more pronounced under limiting nutrient conditions. As a consequence, we expected both increased productivity and increased complementarity in mixtures grown on deep soil compared with those on shallow soil.

Materials and Methods

Experimental design

Our experiment was conducted in the experimental garden of the Institute of Environmental Sciences of the University of Zurich (Switzerland). We established monocultures and mixtures of four perennial species and grew these in four different soil environments. Two different soil depths (‘shallow’ and ‘deep’) were combined with two nutrient levels (‘unfertilized’ and ‘fertilized’). The plant species used were Arrhenaterum elatius (L.) P. Beauv. ex J. and C. Presl (tall grass, relatively deep roots), Holcus lanatus L. (shorter grass, shallower roots), Leucanthemum vulgare Lam. (tall rosette herb, rather shallow, mainly adventitious roots) and Plantago lanceolata L. (shorter rosette herb, deep tap root). We chose these plant species because of their high abundance in natural grasslands of the region, high germination rate, ability to form monocultures and potential for belowground niche separation (Dimitrakopoulos and Schmid 2004). They represent different functional types and show between-species variation in maximum rooting depth and both above- and belowground biomass distribution (Grime et al. 1988; Kutschera and Lichtenegger 1982). The mean depth of root biomass in a previous field study was 2.9, 1.6, 1.5 and 3.2 cm for A. elatius, H. lanatus, L. vulgare and P. lanceolata, respectively (see Dimitrakopoulos and Schmid 2004). We did not include legumes because of their potential to alter nitrogen dynamics. Two replicates of each monoculture and eight replicates of the four-species mixture were grown for each environment in a full-factorial design (n = (2 monocultures × 4 species + 8 mixtures) × 4 environments = 64).

Our pots were designed to allow different rooting depths with a constant soil volume to avoid confounding of depth and volume. Deep pots were 46.4 cm deep by 19.5 cm wide, while shallow pots were 18.8 cm deep by 31 cm wide. Pots were made from polyvinylchloride (PVC) tubes with a punched PVC bottom attached (with five holes of 1 cm diameter). To allow proper drainage of the soil, we added 1.1 of gravel at the bottom of each pot, resulting in a gravel layer of 1.3 and 3.4 cm height in shallow and deep pots, respectively. The gravel was covered with a separating fleece to prevent the soil from clogging the gravel. Pots were then filled with a 1:1 mixture of natural grassland soil and sand (13 l), to which we evenly mixed 14.3 g Osmocote fertilizer (Osmocote mini, 18% N + 6% P + 12% K, Scotts, De Meern, The Netherlands) for all fertilized soils, corresponding to 12 g N per m (assuming a light interception area of 0.215 m per pot based on the spacing of pots). The grassland soil here was relatively nutrient rich, so the combination of adding sand and fertilizer was chosen to work with two nutrient levels, with the lower one poorer than the original soil.

In early June 2004, we grew seedlings of each species in small pots (3 cm diameter, 4.6 cm depth) in the experimental garden. After 25 days, ~10 cm tall seedlings were transplanted to the experimental pots (rosettes of L. vulgare were shorter). To even out size differences and to prevent strong transpiration directly after transplantation, a leaf was cut or trimmed from large seedlings. To avoid confounding soil depth and planting density, we constrained the planted area in the shallow but wide pots to the size of that in deep but narrow pots by covering the outer ring of the upper surface with a flat PVC ring. Per pot, 12 seedlings (three per species in mixtures) were planted in an inner ring of four and an outer ring of eight seedlings. In the mixtures, seedlings were randomly assigned to planting positions with one individual of each species present in the inner ring and two in the outer ring. The 64 pots were randomly assigned to positions within two blocks, each containing half of the replicates. Pots were watered daily with a constant amount of water except on rainy days (automated irrigation system) and weeded regularly.

Measurements

Five weeks after transplanting, on 2–4 August 2004, we measured the ‘extended’ height of all individuals (distance from soil surface to uppermost leaf tip or inflorescence tip, when stem and leaves were pulled up to form a straight, vertical line). On 4–5 August 2004, we cut plants at 5 cm height to
mimic mowing, sorted the cut plant material to species and measured its biomass after drying at 80°C. In five pots, one individual of *H. lanatus* had died and was replaced. Twelve weeks after transplanting, we again measured individual plant height and fully harvested aboveground biomass on 16–19 September 2004. Plants were cut at ground level, and the biomass of each plant individual was measured (*n = 64 × 12 = 768*) after drying at 80°C. Thereafter, pots were kept in the garden and soil cores (4.8 cm diameter, extending to the bottom) were taken in the centre of each pot on 13–15 December 2004. Cores were cut into horizontal slices of 5 cm, and after washing the roots in a 1-mm sieve, total root biomass in each slice was determined after drying at 80°C.

To avoid root development becoming pot bound, the duration of our experiment was kept relatively short. Inspection of soil volumes at the end of the experiment confirmed that roots reached all parts of the pots but had not yet accumulated along the pot walls and bottoms.

**Data analysis**

We used general linear models to analyse shoot and root biomass (combined from both harvests) and summarized the results in analysis of variance (ANOVA) tables (according to Schmid *et al.* 2002). For the analysis of shoot biomass at the pot = community level (*n = 64*), we fitted the following terms: (i) block, (ii) nutrients, (iii) soil depth, (iv) species richness, (v) monoculture species (species composition term fitted after species richness, mixtures have equal composition) and (vi) several interaction terms (Table 1). The same model was used for root biomass (Table 1), except that the term block was omitted, as root samples were taken only in one block (*n = 32*). To test whether vertical root distribution differed between monocultures and mixtures, we evaluated for each pot the proportion of roots present in each 5-cm soil layer. For the deep pots, the two adjacent soil layers were pooled, resulting in four layers per pot (as in shallow pots). The proportion of root biomass within each layer was analysed depending on (i) nutrients, (ii) total soil depth, (iii) actual soil depth (layer), (iv) species richness, (v) monoculture species and (vi) all interaction terms.

In addition, we analysed biomass at the population level (species within pots, *n = 160*, Table 2). Because the biomass of a species was based on *n = 12* individuals in monoculture but only *n = 3* individuals in mixture, we used mean individual plant biomass as response (to avoid confounding of species richness and abundance). For the same reason, we used a weighing variable in the ANOVA with value 1 and 0.25 for populations in monoculture and mixture, respectively. Terms that varied at the pot level (block, nutrients, soil depth, species richness and species in monocultures) were tested against the between-pot variation, terms that varied at the population level against the residual variation. We did not detect problems with autocorrelated residuals within pots (in mixtures, biomass values for all four species were used in the analysis). Residuals were never positively correlated between species, rather slightly negative autocorrelations lead to slightly inflated error terms and thus conservative tests for population-level effects.

To test whether effects of biodiversity on aboveground productivity in the mixtures changed with soil depth and nutrient level, we used the additive partitioning method of Loreau and Hector (2001). This method allows to partition the net effect (NE) of biodiversity into a complementarity effect (CE) due to niche separation or facilitative interaction of species and a selection effect (SE) due to dominance of species with particular traits. For each of the four identical mixtures per environment in each block, the four monocultures from the same block and treatment were used in the calculations. In addition, we used the tripartite partitioning method of Fox (2005) to further subdivide SE into a dominance effect (DE), strictly analogous to natural selection, and a trait-dependent complementarity effect (TDCE), attributable to species complementarity. We also calculated the relative yield total (RYT) for each mixture, which is the sum of every species’ yield in mixture divided by its yield in monoculture (de Wit and van den Bergh 1965; Loreau 1998). For the different components of the biodiversity effects and the RYT of the mixtures (*n = 32*), we fitted a general linear model including the terms (i) overall mean, (ii) block, (iii) nutrients, (iv) soil depth and (v) nutrient × soil depth interaction (Table 3). The NE was also calculated for

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>d.f.</th>
<th>Shoot biomass (% SS)a</th>
<th>Root biomass (% SS)b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Block</td>
<td>1</td>
<td>0.27</td>
<td>*</td>
</tr>
<tr>
<td>Nutrients</td>
<td>1</td>
<td>84.89 ***</td>
<td>25.57 ***</td>
</tr>
<tr>
<td>Soil depth</td>
<td>1</td>
<td>1.57 ***</td>
<td>5.19 ***</td>
</tr>
<tr>
<td>SR</td>
<td>1</td>
<td>0.10</td>
<td>16.57 ***</td>
</tr>
<tr>
<td>MS</td>
<td>3</td>
<td>2.11 ***</td>
<td>23.60 ***</td>
</tr>
<tr>
<td>Nutrients × soil depth</td>
<td>1</td>
<td>6.49 ***</td>
<td>11.36 ***</td>
</tr>
<tr>
<td>Nutrients × SR</td>
<td>1</td>
<td>0.11</td>
<td>6.48 ***</td>
</tr>
<tr>
<td>Soil depth × SR</td>
<td>1</td>
<td>0.16 **</td>
<td>0.075</td>
</tr>
<tr>
<td>Nutrients × MS</td>
<td>3</td>
<td>0.65 **</td>
<td>4.88</td>
</tr>
<tr>
<td>Soil depth × MS</td>
<td>3</td>
<td>0.94 ***</td>
<td>1.28</td>
</tr>
<tr>
<td>Nutrients × soil depth × SR</td>
<td>1</td>
<td>0.00</td>
<td>1.21</td>
</tr>
<tr>
<td>Nutrients × soil depth × MS</td>
<td>3</td>
<td>0.77 ***</td>
<td>0.49</td>
</tr>
<tr>
<td>Residuals</td>
<td>43/12a</td>
<td>1.94</td>
<td>3.30</td>
</tr>
</tbody>
</table>

To account for differences in total root biomass from soil cores due to different soil depths, total root biomass per pot was estimated (rule of proportion), and used as response.

a % SS indicate increases in multiple R² (explained variance) due to the addition of this term to the model. Marginally significant terms are indicated by bold (*P < 0.1*), significant terms by asterisks: *P < 0.05, **P < 0.01, ***P < 0.001. Note that the full model explains >95% of the total variance for both variables (% SS residual < 5).

b The residual degrees of freedom for above- and belowground biomass were 43 and 12, respectively, as belowground biomass was analysed for one block only.

SR, species richness; MS, monoculture species; SS, sums of squares.
belonging to only present part of their results (Moran 2003).

Fertilization generally increased root biomass in absolute terms, but the effect depended on soil depth, species richness and the identity of monoculture species (see two-way interactions in Table 1, Fig. 1). In mixtures and in monocultures of A. elatius and Plantago lanceolata, the response to soil depth and nutrients was similar for root and shoot biomass. By comparison, root biomass of H. lanatus and L. vulgare was rather constant, resulting in a clear decrease in the root:shoot ratio at high nutrient level. Under high nutrient conditions, root biomass was consistently smaller in deep than in shallow soil, whereas under low nutrient conditions there were only slight and inconsistent differences in root biomass between soil depths.

All species extended their roots to the lowest soil layer in monoculture, independent of soil depth (Fig. 2). However, root distributions differed significantly among monocultures [depth × monoculture species interaction, % sums of squares (SS) = 2, P = 0.045]. The mean depth of root biomass was 9.0, 9.5, 6.9 and 9.9 cm for A. elatius, H. lanatus and L. vulgare and P. lanceolata, respectively. In agreement with the data used for species selection, L. vulgare was the most shallow-rooted species, and P. lanceolata the most deep-rooted species. In deep soil, the mean depth of root biomass increased for all species (by 57, 57 103 and 123%, same order as above). This indicates that rooting depths are plastic, particularly those of the two herbaceous species. In all four environments, root biomass decreased with soil depth (% SS = 75, P < 0.001). In mixtures, a larger proportion of root biomass than in monocultures was found in deeper soil layers (depth × species richness interaction, % SS = 1, P = 0.033, Fig. 2).

### Shoot biomass of populations

The shoot biomass at the population level (mean individual plant biomass per species and pot) was increased by

---

**Table 2** ANOVA for whole-season aboveground (shoot) biomass of populations based on biomass of individual plants

<table>
<thead>
<tr>
<th>Line</th>
<th>Source of variation</th>
<th>d.f.</th>
<th>Errora</th>
<th>Shoot biomassb (% SS)c</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Block</td>
<td>1</td>
<td>16</td>
<td>0.22</td>
</tr>
<tr>
<td>2</td>
<td>Nutrients</td>
<td>1</td>
<td>16</td>
<td>69.10 ***</td>
</tr>
<tr>
<td>3</td>
<td>Soil depth</td>
<td>1</td>
<td>16</td>
<td>1.28 ***</td>
</tr>
<tr>
<td>4</td>
<td>SR</td>
<td>1</td>
<td>16</td>
<td>0.09</td>
</tr>
<tr>
<td>5</td>
<td>MS</td>
<td>3</td>
<td>16</td>
<td>1.72 ***</td>
</tr>
<tr>
<td>6</td>
<td>Species in mixture</td>
<td>3</td>
<td>17</td>
<td>1.49 *</td>
</tr>
<tr>
<td>7</td>
<td>Nutrients × soil depth</td>
<td>1</td>
<td>16</td>
<td>5.28 ***</td>
</tr>
<tr>
<td>8</td>
<td>Nutrients × SR</td>
<td>1</td>
<td>16</td>
<td>0.09</td>
</tr>
<tr>
<td>9</td>
<td>Soil depth × SR</td>
<td>1</td>
<td>16</td>
<td>0.13</td>
</tr>
<tr>
<td>10</td>
<td>Nutrients × MS</td>
<td>3</td>
<td>16</td>
<td>0.53 *</td>
</tr>
<tr>
<td>11</td>
<td>Soil depth × MS</td>
<td>3</td>
<td>16</td>
<td>0.76 **</td>
</tr>
<tr>
<td>12</td>
<td>Nutrients × species in mixture</td>
<td>3</td>
<td>17</td>
<td>0.90</td>
</tr>
<tr>
<td>13</td>
<td>Soil depth × species in mixture</td>
<td>3</td>
<td>17</td>
<td>2.34 **</td>
</tr>
<tr>
<td>14</td>
<td>Nutrients × soil depth × SR</td>
<td>1</td>
<td>16</td>
<td>0.00</td>
</tr>
<tr>
<td>15</td>
<td>Nutrients × soil depth × species in mixture</td>
<td>3</td>
<td>17</td>
<td>0.47</td>
</tr>
<tr>
<td>16</td>
<td>Pot residuals</td>
<td>46</td>
<td></td>
<td>2.21</td>
</tr>
<tr>
<td>17</td>
<td>Species residuals</td>
<td>84</td>
<td></td>
<td>13.38</td>
</tr>
</tbody>
</table>

*a The line number of the error term used for the calculation of F-values.

b % SS indicate increases in multiple R2 (explained variance) due to the addition of this term to the model. Significant terms are indicated by asterisks: *P < 0.05, **P < 0.01, ***P < 0.001. Note that the full model explains >85% of the total variance (% SS residual < 15).

c Observations from monocultures and mixtures are given weight 1 and 0.25, respectively.

SR, species richness; MS, monoculture species; SS, sums of squares.
fertilization (Fig. 3, Table 2). At the high nutrient level, it was higher in shallow than in deep soil. The four species responded differentially to soil depth both in monoculture (soil depth × monoculture species interaction) and in mixture (soil depth × species in mixture interaction). On shallow, unfertilized soil all species profited from growing in mixture (Fig. 3). In the other environments, there was at least one species that grew better in monoculture.

Biodiversity effects

Composed of a positive complementarity effect (CE) and a negative selection effect (SE), the net effect of biodiversity (NE) on aboveground productivity was only marginally positive overall (Fig. 4, Table 3). NE was higher on shallow soil than on deep soil, mainly due to higher CE, and marginally higher in unfertilized soil than on fertilized soil (Table 3). The negative SE was strongest in deep soil at the high nutrient level (nutrients × soil depth interaction) because in this environment one species, *L. vulgare*, had much less biomass in mixtures than expected from its monoculture yields in this environment. The tripartite partitioning method of Fox (2005) revealed that SE almost exclusively reflected the dominance effect (DE), whereas trait-dependent complementarity (TDCE) was negligible. Therefore, we only show SE in Fig. 4. Values of the relative yield total (RYT, Fig. 4, rightmost panel) were significantly larger than 1 overall and like NE higher on shallow than deep soil and at low than high nutrient level. Only on deep soil at high nutrient level, RYT was lower than 1 (0.997).

The NE of biodiversity on belowground productivity was positive overall (% SS = 64, P < 0.001) and was strongest in

Table 3 Analyses of variance for the net effect of biodiversity (NE), the effects of complementarity (CE) and selection (SE), as well as for the relative yield total (RYT)

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>d.f.</th>
<th>NE (%)</th>
<th>CE (%)</th>
<th>SE (%)</th>
<th>RYT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>1</td>
<td>7.75</td>
<td>14.57</td>
<td>23.63</td>
<td>19.89</td>
</tr>
<tr>
<td>Block</td>
<td>1</td>
<td>13.04</td>
<td>14.48</td>
<td>0.80</td>
<td>4.84</td>
</tr>
<tr>
<td>Nutrients</td>
<td>1</td>
<td>7.97</td>
<td>3.72</td>
<td>18.11</td>
<td>11.88</td>
</tr>
<tr>
<td>Soil depth</td>
<td>1</td>
<td>11.98</td>
<td>8.91</td>
<td>10.67</td>
<td>10.67</td>
</tr>
<tr>
<td>Soil depth × nutrients</td>
<td>1</td>
<td>0.09</td>
<td>0.15</td>
<td>10.65</td>
<td>4.14</td>
</tr>
<tr>
<td>Residuals</td>
<td>27</td>
<td>59.16</td>
<td>58.18</td>
<td>41.63</td>
<td>48.59</td>
</tr>
</tbody>
</table>

a Data analysed were whole-season aboveground biomass values from n = 32 four-species mixtures (eight per environment) and corresponding sets of n = 32 monocultures (two per species in each environment).

b % SS indicate increases in multiple R2 (explained variance) due to the addition of this term to the model. Marginally significant terms are indicated by bold (P < 0.1), significant terms by asterisks: *P < 0.05, **P < 0.01, ***P < 0.001. Note that the full model explains 41, 42, 58 and 51% of the total variance for NE, CE, SE and RYT, respectively.

SS, sums of squares.

Fig. 1 Mean biomass per pot (in g) for the four-species mixture and the monocultures of *Arrhenaterum elatius*, *Holcus lanatus*, *Leucanthemum vulgare* and *Plantago lanceolata* when grown in pots of different soil depth and nutrient level. The error bars (right panel, top and bottom) show 2 standard errors of the difference between means (SED) and apply to all top and bottom panels, respectively.

fertilization (Fig. 3, Table 2). At the high nutrient level, it was higher in shallow than in deep soil. The four species responded differentially to soil depth both in monoculture (soil depth × monoculture species interaction) and in mixture (soil depth × species in mixture interaction). On shallow, unfertilized soil all species profited from growing in mixture (Fig. 3). In the other environments, there was at least one species that grew better in monoculture.
fertilized soil, amounting to 12.2, 15.6, 6.1 and 0.3 g for fertilized deep, fertilized shallow, unfertilized deep and unfertilized shallow soils, respectively. Overyielding of mixtures was transgressive (most productive mixture with more roots than most productive monoculture) in all but the unfertilized shallow environment.

The between-species variance component for plant height in mixtures at the first (second) harvest increased from 44.9 (34.5) cm in deep soil at low nutrient level to 58.5 (37.1) cm in deep soil at high nutrient level to 119.8 (59.3) cm in shallow soil at low nutrient level and 128.2 (108) cm in shallow soil at high nutrient level. This indicates stronger differentiation of plant heights between species in mixtures on shallow than on deep soil and also at high compared with low nutrient level.

**Discussion**

**Biodiversity effects and soil depth**

The results of this experiment do not support our first hypothesis that deep soil should enhance niche complementarity among species with respect to rooting depth and should thus increase CEs in mixtures. If soil depth had been important for the partitioning of rooting depth among species, we should have found higher values of CE and RYT in deep soil than in shallow soil, particularly under low nutrient conditions,

---

**Fig. 2** Mean proportion of root biomass in layers of 5-cm thickness in monocultures (n = 4, one per species) and four-species mixtures (n = 4) grown in pots of different soil depth at low (a) and high (b) nutrient level. The error bar (lower right panel) shows 2 standard errors of the difference between means (SED) and applies to all panels.

**Fig. 3** Mean biomass per individual (in g) for *Arrhenatherum elatius*, *Holcus lanatus*, *Leucanthemum vulgare* and *Plantago lanceolata* when grown in monoculture and in the four-species mixture in pots of different soil depth and nutrient level. The error bar (right panel) shows 2 standard errors of the difference between means (SED) and applies to all panels.
where belowground resource partitioning is presumably more important (e.g. Harpole and Tilman 2007). Contrary to our expectation, CE and RYT were highest in mixtures grown on shallow, unfertilized soil.

Our results contrast with those of Berendse (1982) and Dimitrakopoulos and Schmid (2004), where RYT or biodiversity effects increased with soil depth, respectively. However, in both of these experiments, soil depth was confounded with soil volume and nutrients within that volume. In Berendse (1982), competition with Anthoxanthum probably caused the deep rooting plant Plantago to access nutrients in lower soil layers that were not accessed in monoculture. Our results suggest that increasing soil depth without increasing the soil volume is not sufficient to increase CE or RYT. In addition to larger CE and RYT, we also found larger between-species variance components for plant height on shallow soil compared with deep soil. This suggests increased complementarity and partitioning of light aboveground, possibly reflecting increased resource partitioning belowground.

### Biodiversity effects and nutrients

The higher RYT and CE in unfertilized than fertilized soil agree with our second hypothesis and the findings of Berendse (1982). Limiting nutrient conditions may have increased belowground partitioning of resources between species. This is in line with Harpole and Tilman (2007) and the theory of greater niche dimensionality at low nutrients, while fertilization may decrease the number of limiting resources. This is a potential mechanism to explain the coexistence of higher species numbers in nutrient-poor as opposed to nutrient-rich grasslands, although this is still debated (Craine 2005). Positive effects of fertilization on RYT (Fridley 2003; He et al. 2002; Reich et al. 2001) may be explained by enhanced light partitioning (Fridley 2003). However, we found overyielding in root biomass of mixtures which was most pronounced under fertilized conditions. While root:shoot ratios in most of the monocultures were reduced by fertilization, they remained nearly constant in mixtures, suggesting considerable interspecific competition below ground. Thus, possibly supported by the mowing treatment, light partitioning was probably less important than belowground partitioning in our experiment. Even so, belowground partitioning seems not due to different rooting depths of species.

#### Explanations for complementarity on shallow soil

Despite good evidence for niche differences in rooting depth from other studies (Berendse 1982; Fargione and Tilman 2005; Fitter 1986; Mamolos et al. 1995; McKane et al. 1990; McKane et al. 2002; Parrish and Bazzaz 1976, e.g.), our results suggest that other mechanisms may also affect complementarity and overyielding of mixtures. Indeed, we found a more constant vertical distribution of roots in mixtures compared with monocultures (Fig. 2), but it was similar in deep and shallow soil. So, how can the positive effect of shallow soil on CE and RYT be explained?

We speculate that root foraging was less efficient in deep soils than in shallow soils. The larger the soil volume that roots can access, the more nutrients are available. Although soil volume was kept constant in our experiment, and all species were able to extend their roots to the bottom of all soils (monoculture), it was probably more costly for plants to exploit the space and resources at the bottom of deep soils. This suggests that in deep soil the disadvantage of shallow rooters was not compensated for by the corresponding advantage of deep rooters. Whereas in shallow pots plants can exploit rather spherical volumes, plants in deep pots are forced to exploit volumes of oblong shape with a less optimal root length to rooting space ratio. Horizontal constriction in deep pots may have intensified competitive interactions between plants, particularly at the early stage of root growth when all species had roots in the topsoil only. In our experiment, some species in deep soils had larger individual aboveground biomass in monocultures than in mixtures (H. lanatus and L. vulgare when fertilized, H. lanatus and A. elatius when unfertilized). This suggests that interspecific competition was stronger than intraspecific competition.

There is evidence that rooting space per se can be regarded as a soil resource (McConnaughay and Bazzaz 1991). Together with resource-independent space requirements due to root morphology and development, plants may primarily compete for space rather than nutrients (McConnaughay and Bazzaz 1992). In their review, Schenk et al. (1999) depict various examples for the defence of space through horizontal as well
as vertical root segregation between individuals of the same or different species. Species that use resources efficiently and conservatively may profit from active root segregation as more prodigal species are unable to access the resources within the defended area. To explain the high CE in mixtures on shallow soil, root segregation would have to be stronger between species than within species and require the recognition of alien roots. Finally, if such territoriality matters, it is plausible that a rather spherical volume of soil is easier to defend than more derived shapes or in other words that horizontal segregation of roots is less costly than vertical segregation.

Potential caveats of our experiment
Some caveats regarding the employed design and duration of the experiment should be mentioned. First, although deep soil (>17.5 cm) did not enhance partitioning of resources here, the importance of vertical partitioning within shallow soil (<17.5 cm) is unknown. In addition to volume, this could explain the disagreement with Dimitrakopoulos and Schmid (2004) where soil depth ranged from 5 to 15 cm. Second, a number of studies have shown that pot geometry, and in particular the surface area/depth (S/D) ratio of pots, affects plant growth (Campbell et al. 1985; Hanson et al. 1987). In line with Dominguez-Lerena et al. (2006), these studies suggest that intermediate values of S/D are optimal, probably through a trade-off between limited aeration and leaching out of nutrients from the rooting zone (mineral deficiencies) in deep pots and increased evapotranspiration in shallow pots. We cannot exclude that part of the positive effect of shallow pots we saw in our study relate to this trade-off. However, as the additional surface area of shallow pots was covered, differences in evapotranspiration and aeration of the soil should be very small. And, although with an S/D of 6.3 and 39.0 for deep and shallow pots, respectively, our deep pots might be closer to the optimum than the shallow pots, the latter supported higher CE and RYT. Third, we do not have data on root length or absorptive surface area of roots, which would be more directly related to nutrient acquisition than root biomass that was measured here. And last, our experiment was of relatively short duration. Running the experiment over a longer period of time might have changed the outcome of below- and aboveground competition in mixtures versus monocultures. However, belowground competition (mainly addressed here) is likely to occur earlier than aboveground competition (Fitter 1986). Also, roots were well distributed across the soil profile in all soils, and prolongation of the pot experiment might have distorted the results due to pot-bound foraging behaviour of roots.

Conclusions
From our results, we conclude that increased soil depth without simultaneously increased soil volume does not result in enhanced belowground complementarity of species. The positive effects of increased soil depth on complementarity and RYT found in other studies, e.g. by Berendse (1982) and Dimitrakopoulos and Schmid (2004) might have been due to the combination of increased soil depth and volume. Finding the largest biodiversity effects in shallow pots at low nutrients suggests (i) that belowground partitioning of resources was important and (ii) that horizontal rather than vertical root segregation between roots of different species might have occurred. In line with our results, we would expect a larger effect of species richness on productivity in natural grasslands where the same soil volume is distributed over a shallow, stone-free rather than a deep, rocky profile.

Acknowledgements
Many thanks to Eva Vojtech and Virgine Boreux for their help with harvests and measurements, as well as Kurt Bössiger for his support with the construction of the pots. Furthermore, we thank Panayiotis Dimitrakopoulos, Hans de Kroon, Kelly D.M. McConnaughey, Robert B. McKane, Jasper van Ruijven, H. Jochen Schenk and two anonymous reviewers for valuable comments on the manuscript. Funding was provided through the University of Zürich and the Swiss National Science Foundation (grant no. 31-65224-01 to B.S.).

References
von Felten & Schmid  |  Complementarity among species in rooting space  


