

# Combined effects of climate warming and plant diversity loss on above- and below-ground grassland productivity

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## Abstract

Projections of global change predict both increases of the surface temperature and decreases of biodiversity, but studies on the combined impact of both on terrestrial ecosystems are lacking. We assessed the impact of these two global changes on above- and below-ground productivity of grassland communities. Experimental ecosystems containing one, three or nine grassland species were grown in 12 sunlit, climate-controlled chambers in Wilrijk, Belgium. Half of these chambers were exposed to ambient air temperatures, while the other half were warmed by 3 °C. Equal amounts of water were added to heated and unheated communities, so that any increases in evapotranspiration due to warmer conditions would result in a drier soil. Warming led to a decreased productivity of both above-ground plant parts (−18%) and roots (−23%), which coincided with a significantly lower soil water content. Complementarity in resource use and/or facilitation slightly enhanced above-ground productivity in multi-species communities, regardless of the induced warming. Interactive effects between temperature treatment and species richness level were found below-ground, however, where warming nullified the positive effect of richness on root productivity. Future warmer conditions could further increase losses of productivity associated with declining species numbers.

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## 1. Introduction

Both the effects of declining species richness and of climate warming on plant communities have received widespread scientific attention, but studies on the combined effects of those global changes are lacking. Climate is projected to warm significantly as a result of increasing greenhouse gas concentrations (IPCC, 2001a). Higher temperatures will likely affect plant communities in a number of ways. Direct responses to climate warming can occur through altered reaction kinetics, which could lead to increased primary production especially in

regions where carbon assimilation is limited by low temperatures, although adaptive responses could counter this reaction (Larcher, 2003). It is also likely that warming will affect a number of phenological processes (Walther, 2003). Indirect effects of temperature increase include changes in resource availability and competitive interactions (De Valpine and Harte, 2001). Especially decreases in soil water content (SWC) caused by higher evapotranspiration may significantly affect ecosystem response, for example by a decrease of plant biomass production as observed in several studies (Eatherall, 1997; Saleska et al., 1999).

Plant diversity is declining worldwide (Sala et al., 2000), and its effects on ecosystem functioning have been investigated in numerous experiments during the past decade (e.g. Hector et al., 1999; van Ruijven and Berendse, 2005). In many of these studies, productivity increased with species richness, though no or

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idiosyncratic responses have also been found (Schmid et al., 2002). The underlying mechanisms have been hotly debated (Hooper, 1998; Huston et al., 2000), and Loreau and Hector (2001) have formulated an equation to quantify effects of complementarity and selection, the two main mechanisms involved in determining the effects of species richness on community productivity (Loreau, 2000).

The present study investigates the effects of climate warming, declining species richness and possible interactions between these two global changes on productivity of grassland communities. Synthesized model ecosystems in climatized chambers were used, and both above-ground and below-ground plant productivity was studied. Few studies incorporate root biomass in productivity analyses, though it makes up an important part of the total plant biomass, and this could alter relationships between species richness and productivity (Liira and Zobel, 2000). Moreover, examining root biomass could reveal the presence of spatial below-ground complementarity, which is often associated with higher species richness (Wardle and Peltzer, 2003; Silvertown, 2004). The soil drying, often associated with elevated temperatures, may lead to a change in root distribution (e.g. less roots near the surface, Asseng et al., 1998).

The response to climate warming and plant diversity loss combined may be different than could be expected from the single responses to each of these two global changes. We studied whether there was overyielding (Hector, 1998) in multi-species communities and how it was influenced by the induced warming. We expected above-ground productivity to be limited more by drought in heated communities, but interactions of lower SWC with increased species richness are difficult to predict (Van Peer et al., 2004). For example, if enhanced productivity in species-rich communities increases canopy transpiration, then soil drying may occur more frequently, which would counteract the productivity enhancement (Pfisterer and Schmid, 2002) unless complementarity improves access to water. Root/shoot allocation patterns and their response to environmental changes can vary widely (Zobel and Zobel, 2002), though an increased root/shoot ratio could be a reaction to increased soil drought in a warmer environment (Chaves et al., 2002). Increased competition for water due to warming may at the same time enforce increased niche separation between plants by inducing species to root at different depths (Wardle and Peltzer, 2003). This mechanism could result in increased complementarity in heated communities, and a different root distribution pattern. Warming may also change the magnitude and direction of the selection effect, as drought stress and higher temperatures could change the success of the individual species in the community, for example by increasing the competitive success of less productive species (Grime, 2001), or of species with a more southern range. Similarly, certain species may see their competitive success markedly lowered when the imposed climate change exceeds their tolerance limits, thereby committing them to (local) extinction. There are studies that have already documented such climate-driven extinctions (Klein et al., 2004), and further climate change is expected to increase the rate of ongoing biodiversity loss (Thomas et al., 2004).

## 2. Materials and methods

### 2.1. Experimental platform and design

In July 2003, an experimental platform containing 288 artificially assembled grassland model ecosystems was established at the Drie Eiken campus of the University of Antwerp (Belgium, 51°09'N, 04°24'E). Annual precipitation at this location is 776 mm on an average, equally distributed over the year. Average annual air temperatures ( $T_{\text{air}}$ ) vary around 9.6 °C. The platform consisted of 12 sunlit, climate-controlled chambers, facing south. The distances between the chambers were maximised to avoid mutual shading. The interior surface area was 150 cm × 150 cm, the height at the north side 150 cm and at the south side 120 cm. The chambers were covered with a colourless polycarbonate plate (4 mm thick), while the sides were made of polyethylene film (200 µm thick), both UV transparent.

Six of the 12 chambers were exposed to the ambient  $T_{\text{air}}$  (unheated chambers), while the other 6 (heated chambers) were continuously heated 3 °C above fluctuating ambient values. Each chamber had its individual air control group with an electrical heating battery, and was linked to a central refrigeration unit by isolated pipes. The conditioned air was evenly distributed throughout the chambers by means of aerators with regulated flow. The air temperature was measured with a combined humidity–temperature sensor (Siemens, type QFA66, Germany) and the photosynthetically active radiation (PAR) with a quantum sensor (SDEC, type JYP1000, France), inside each chamber as well as outside. All measurements were monitored and logged every half hour on a computer with GE Fanuc Cimplicity software 150 I/O (Scada, MA, USA). During this first growing season (planting to harvest), the average temperature of the unheated chambers was equal to the ambient  $T_{\text{air}} + 0.80 \pm$  (S.D.) 1.12 °C, the average temperature of the heated chambers to the ambient  $T_{\text{air}} + 3.51 \pm$  (S.D.) 1.19 °C. The average instantaneous difference between heated and unheated chambers was 2.71 °C, so 0.29 °C lower than targeted. This was partly caused by the fact that the temperature range of climatisation was between –35 and +35 °C, whereas during 34 h in the exceptionally hot 2003 growing season, maximum temperatures exceeded 32 °C. At these times, heating provided less than the targeted 3 °C difference between both temperature treatments.

### 2.2. Grassland communities

Each chamber contained the same set of 24 different grassland communities in containers (grey PVC-tubes of 24 cm inside diameter and 60 cm height), filled with sieved soil (76.3% loam, 14.8% clay and 8.74% sand; field capacity 0.39 m<sup>3</sup> m<sup>-3</sup>; pH 6.45) collected from a 25-year-old maize field in Brabant (Central Belgium). To avoid unnatural soil temperatures in the containers (which would strongly influence below-ground activity), they were buried into the surrounding soil. Holes were plugged into the lids sealing the bottom of the containers to ensure that water could drain freely. Anti-root mats prevented roots from

growing outside the containers. Soil water from deeper layers (which would cause unwanted changes in the container's water regime), was prevented from entering the containers by installing drainage pipes underneath the chambers. A layer of gravel and coarse sand was placed between the containers and the drainage pipes to prevent capillary rise. Waterproof plastic was buried into the soil at the sides of the chambers to prevent lateral water inflow.

During summer and autumn 2003, water conditions in the unheated chambers were kept equal to outside. Profile probe tubes (28 mm diameter, 554 mm length) which fitted with the PR1 soil moisture sensor (Delta-T Devices Ltd., UK) were installed in 48 communities (the set of 24 different communities in both temperature treatments spread throughout several chambers), as well as outside in a breach filled with the same sieved soil as inside. The quantities of water administered to the unheated chambers were calculated weekly from the difference between the soil moisture outside and inside, to maintain the same average SWC. The heated chambers received the same amount of water as the unheated chambers, so that any enhanced water consumption would result in (aggravated) soil drought.

The plant communities were created using nine grassland species, selected according to three criteria: presence in temperate grasslands, perennial life cycle, and preference for clay or loam soil. Species were chosen with different productivity, temperature resistance and drought resistance. Three levels of species richness ( $S$ ) were created:  $S=1$ , 3 and 9. The use of different species combinations at each species richness level,

made it possible to statistically separate the effect of species richness from species composition. The initial range of functional characteristics of plant species influences the ability of complementarity and positive interactions (Loreau and Hector, 2001). We consequently opted for species from three functional groups, which were equally represented at each species richness level: three grass species (*Dactylis glomerata* L., *Festuca arundinacea* Schreb., *Lolium perenne* L.), three N-fixing dicots (*Trifolium repens* L., *Medicago sativa* L., *Lotus corniculatus* L.), and three non-N-fixing dicots (*Bellis perennis* L., *Rumex acetosa* L., *Plantago lanceolata* L.). Furthermore, each plant community at  $S=3$  and 9 had the same number of species from each of the three functional groups, which were each represented by the same number of individuals as much as possible. Each chamber contained the nine possible  $S=1$ , nine  $S=3$  and six  $S=9$  communities, but at different locations in the different chambers. Each of the six  $S=9$  communities had a different internal arrangement. Three different species, each representative of a different functional group, were used to create each of the  $S=3$  communities, with the specific combinations being random (though each species was used the same number of times). Each community contained 30 individuals planted in a hexagonal grid at 4 cm distance, with interspecific interactions maximised by avoiding clumping (Fig. 1). Prior to planting in the containers in June 2003 (which took approximately 3 weeks), the plants were sown in small seedling pots in April 2003. No fertiliser was added, and plants were treated regularly to avoid fungal infection and insect damage. Weeding was done manually throughout the experiment.

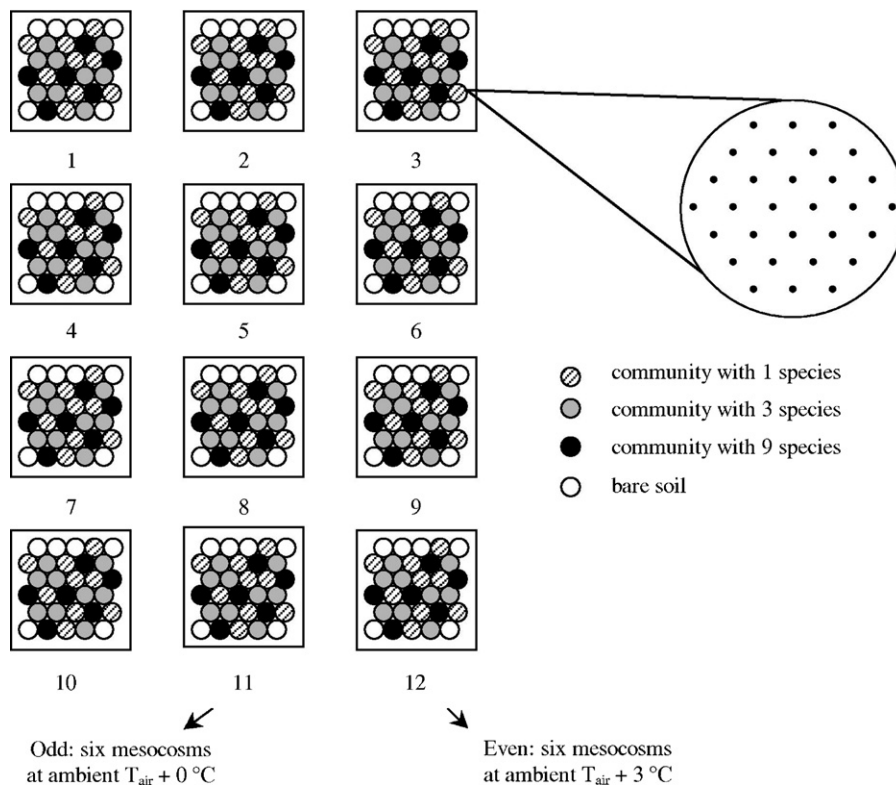


Fig. 1. Design of the 12 climate-controlled chambers. See text for details.

### 2.3. Measurements

To determine above-ground biomass, plants were cut 3.5 cm above the soil surface in all containers between 27 and 31 October 2003. In six chambers (three heated and three unheated) biomass was sorted by species within community, while the biomass of the other six chambers was collected per community. Plant material was then dried (1 week at 70 °C) and weighed. From two chambers (one heated, one unheated), the remaining above-ground biomass (stubble biomass = 0–3.5 cm height) was collected, sorted by species and community, and dried (at least 2 days at 70 °C). These two chambers were then dismantled and all containers were removed. Soil slices (4 cm thick) were cut out of the soil cylinder at depths of 1–5, 6–10, 13–17, 23–27, 35–39 and 48–52 cm. The soil samples were subsequently dried during 2 weeks at 80 °C to prevent root decomposition. Later, roots were carefully separated from the soil slices manually, dried (2 days at 70 °C) and weighed.

Below-ground biomass was reconstructed from slice biomass by regression (the curve chosen per community to best fit the root distribution), followed by a calculation of root biomass for every millimeter along the depth profile and subsequent addition of these 1-mm biomass values. Total community biomass was the sum of above-ground, stubble and interpolated root biomass determined in the two destructively harvested chambers. Root/shoot ratios were calculated from below-ground and above-ground biomass including stubble in these same two chambers. Selection and complementarity effects were calculated for six chambers (those in which above-ground biomass was determined up to the species level) using the additive partitioning method formulated by Loreau and Hector (2001).

Soil water content (see “Grassland communities”) was measured on 15 days between August and October 2003. Readings were taken at four depths (10, 20, 30 and 40 cm).

### 2.4. Statistical analysis

All statistics were performed using SPSS 10.0 (SPSS Science, Woking, UK). To test for effects of heating, species richness level, depth in soil (for SWC), rooting depth and interactions, we used general linear model (GLM) univariate analysis or repeated measures analysis where appropriate. Post hoc tests (Games-Howell or Fisher’s LSD) were used to separate multiple means. To compare means of two groups of cases, either independent or paired *t*-tests were performed. Testing whether the mean of a single variable differed from a constant was done with one-sample *t*-tests. Finally, the linear association between two variables was tested with Pearson’s two-tailed correlation. No transformations (logarithmical or otherwise) had to be made to meet the assumptions of the tests.

## 3. Results

### 3.1. Chamber effects

Univariate analysis of above-ground community biomass, with chamber number as random factor, revealed a “chamber

effect” on heated chambers ( $p=0.031$ ), but not on unheated chambers ( $p=0.217$ ). Since the communities were not all planted (or harvested) on the same date, we cancelled out the “growing days” effect by dividing community biomass ( $\text{g m}^{-2}$ ) by the number of days from planting to harvest in each chamber. With productivity expressed as  $\text{g m}^{-2} \text{day}^{-1}$ , chamber effects were no longer significant (heated:  $p=0.294$ ; unheated:  $p=0.059$ ).

### 3.2. Soil water content

Heating lowered average SWC by  $5.2 \text{ m}^3 \text{ m}^{-3}$  during the measurement period ( $p<0.001$ , repeated measures analysis), and the drying effect became more important as the season progressed, as also indicated by the significant interaction between measurement day and temperature treatment ( $p<0.001$ ) and our post hoc analysis (Fig. 2a). Soil water content decreased with increasing  $S$  ( $p<0.001$ ), and this decrease was sharpest from monocultures to multi-species communities, as post hoc analysis revealed a significant difference between  $S=1$  and both  $S=3$  and  $9$  (both  $p$  values  $<0.05$ ), but not between  $S=3$  and  $9$  ( $p=0.507$ ). The non-significant interaction between temperature treatment and  $S$  ( $p=0.766$ ) indicated that the effect of  $S$  on SWC was similar in both heated and unheated communities. The soil contained the most water at 10 cm depth ( $p<0.001$ , post hoc tests), and the absence of significant interactions of depth with either temperature treatment,  $S$ , or both together (all  $p$  values  $>0.75$ ), showed that the influence of depth on SWC remained constant under different global change scenarios (Fig. 2b and c).

### 3.3. Above-ground productivity

Increased temperatures significantly reduced above-ground productivity ( $p<0.001$ , univariate analysis), from an average of  $1.715 \text{ g m}^{-2} \text{day}^{-1}$  (unheated) to  $1.405 \text{ g m}^{-2} \text{day}^{-1}$  (heated) (Fig. 3a). The effect of species richness was also significant ( $p=0.021$ ), although post hoc tests showed that only at  $S=3$ , productivity was significantly higher than at  $S=1$  ( $p=0.031$ ). There were no significant interactive effects ( $p=0.806$ ), so the absolute influence of species richness on above-ground productivity was the same in both temperature treatments.

A detailed assessment of overyielding (univariate analysis) showed that neither complementarity nor net selection differed significantly between  $S=3$  and  $9$ , nor between temperature treatments (all  $p$  values  $>0.3$ ). No significant interactions were found either (both  $p$  values  $>0.8$ ). One-sample *t*-tests on the entire dataset revealed that net selection ( $-0.013 \text{ g m}^{-2} \text{day}^{-1}$ ) was not significantly different from 0 ( $p=0.622$ ), but there was a significant small positive complementarity effect ( $+0.127 \text{ g m}^{-2} \text{day}^{-1}$ ;  $p=0.026$ ). The individual species’ above-ground biomass responses to warming and  $S$  are shown in Fig. 4.

### 3.4. Below-ground productivity

In the analysis of below-ground biomass (reconstructed from slice biomass), we used a repeated measures GLM to increase

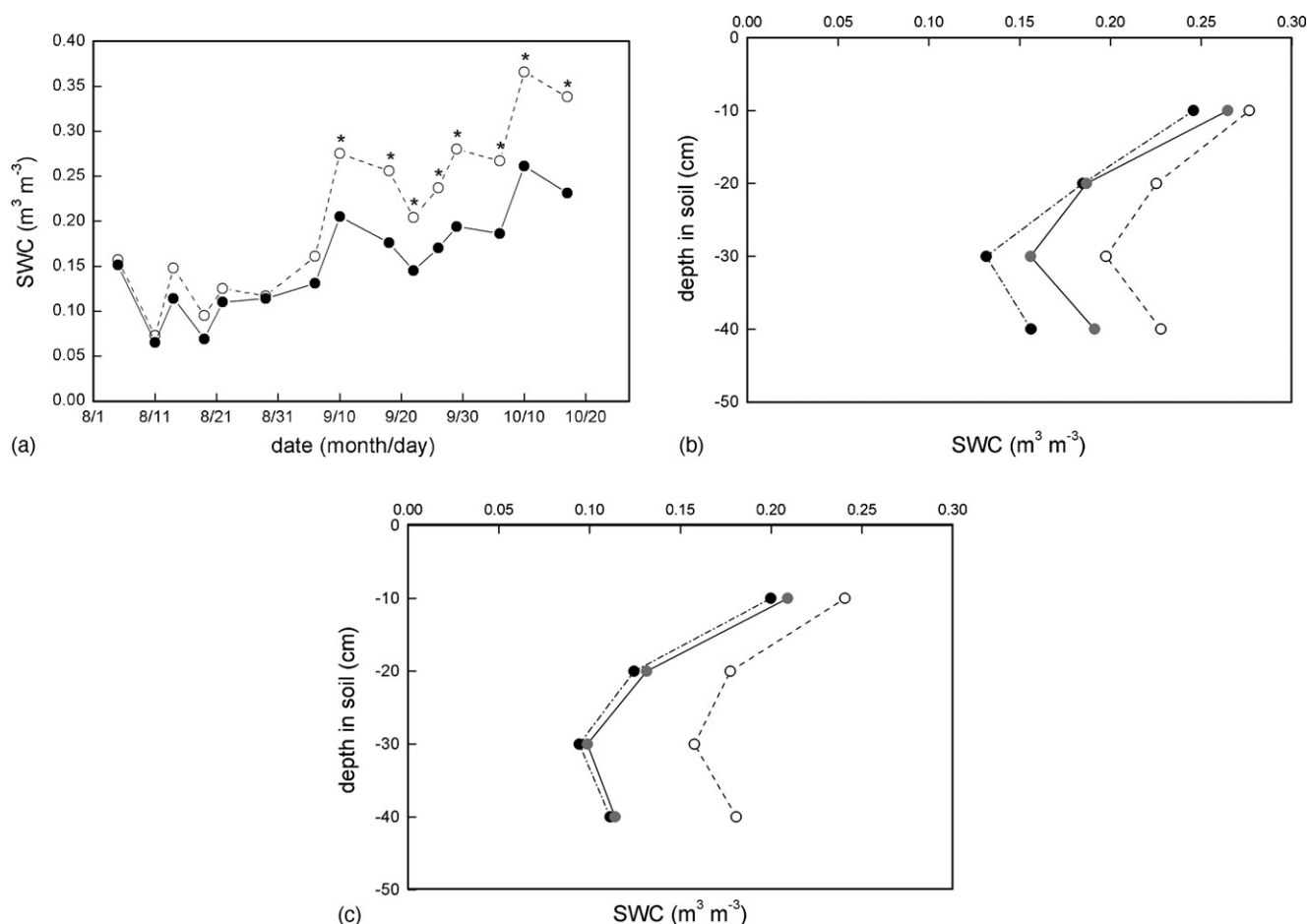


Fig. 2. Soil water content (SWC). (a) At different measuring dates in 2003, in communities at ambient temperatures ( $\circ$ ) and ambient temperatures + 3 °C ( $\bullet$ ). Each symbol represents the average SWC of 24 different plant communities (all species richness levels ( $S$ ) combined), measured at four soil depths. Significant SWC differences between the two temperature treatments ( $p < 0.05$ , Fisher's LSD test) are indicated by an asterisk. (b) At different depths, in unheated and (c) heated communities at  $S = 1$  ( $\circ$ ),  $S = 3$  ( $\bullet$ ) and  $S = 9$  ( $\bullet$ ). Each symbol represents the average SWC of measurements from August till October.

statistical power, linking each unheated community with its counterpart in the heated chamber. This technique is also referred to as 'matched-pairs analysis' and allows us to include the information held within the differences imposed by experimental conditions, between highly similar subjects (e.g. Mokhtarian et al., 2002). The communities that were linked in the current experiment almost exclusively differed in the applied temperature treatment, as planting and harvesting date, light regime, the identity of neighbouring communities, and other possible confounding factors, were almost identical.

We found a significant and negative effect of warming on root productivity ( $p = 0.001$ ), which dropped from  $1.645$  to  $1.259 \text{ g m}^{-2} \text{ day}^{-1}$ , but no effect of species richness ( $p = 0.386$ ). There was, however, an interaction between the two factors ( $p = 0.049$ ), indicating that the warming effect on below-ground productivity varied with species richness. Fig. 3b shows that this interaction was caused most likely caused by a difference at  $S = 9$ .

The use of slice productivity (= productivity of the roots in the soil slices) in statistical analysis for studying root profiles, is correct only when total slice and total (reconstructed) root productivity are well correlated, which was the case in our data

with  $r = 0.98$  and  $0.96$  for unheated and heated, respectively ( $p < 0.001$  in both cases). In the analysis of slice productivity (repeated measures GLM), we found that both warming and soil depth negatively influenced root productivity ( $p < 0.001$  for both) (Fig. 5), but effects of species richness were not significant ( $p = 0.077$ ). However, warming and  $S$  interacted significantly ( $p = 0.001$ ), and when applying a univariate analysis on each temperature treatment separately, we found an effect of  $S$  on root productivity in unheated ( $p = 0.006$ ) but not in heated communities ( $p = 0.721$ ). So the trend towards higher productivity at  $S = 9$ , observed in unheated communities when studying total root productivity (Fig. 3b), became significant in the slice productivity analysis. This is likely due to the addition of soil depth as an explanatory variable, thereby decreasing error variance and increasing statistical power. Depth and  $S$  did not interact significantly ( $p = 0.649$ ), implying that  $S$  modified root-productivity to the same extent at every depth. Warming did influence the below-ground profile, however, as temperature treatment and depth interacted with each other ( $p < 0.001$ ). This was caused by a lower root productivity in the shallow soil layers of heated communities (Fig. 5a). A significant three-way interaction between depth,  $S$  and temperature treatment was

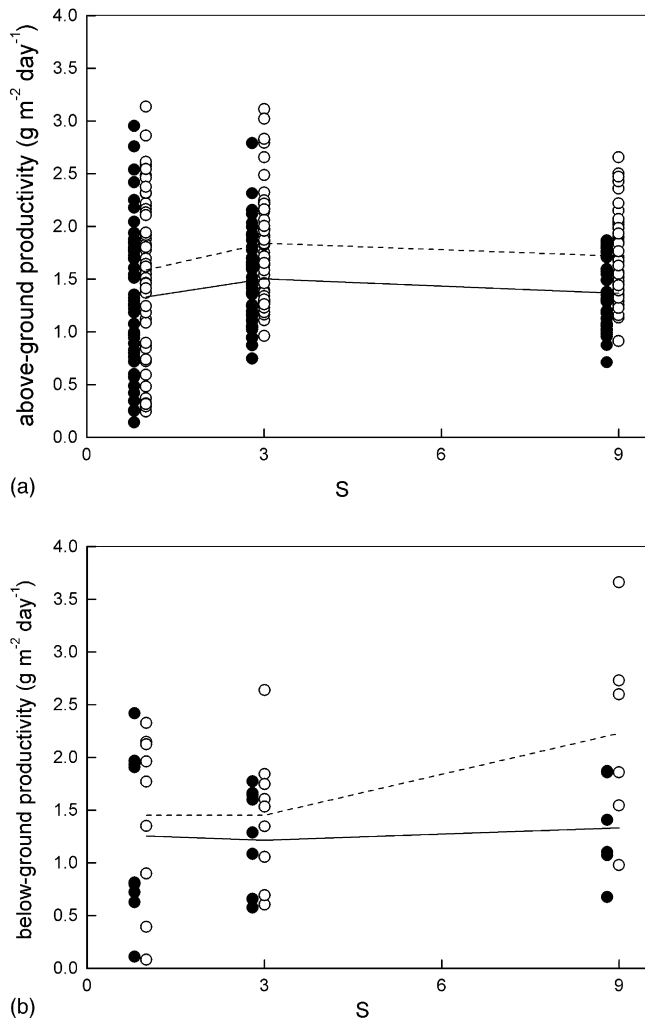


Fig. 3. Community productivity for different species richness levels ( $S$ ), at ambient temperatures ( $\circ$ ) and ambient temperatures  $+3\text{ }^{\circ}\text{C}$  ( $\bullet$ ). (a) Above-ground productivity (plant parts above 3.5 cm). (b) Root productivity. Each symbol represents a different community. Averages for unheated (---) and heated (—) communities are connected with a straight line. Symbols are slightly shifted with respect to  $S$  for clarity.

detected ( $p=0.016$ ), meaning that the influence of warming on the root profile changed with species richness (Fig. 5b and c): in unheated communities, root productivity was higher in shallow soil layers at  $S=9$ , while this effect was absent in heated communities. When using the relative root productivity (percentage of roots at each depth), all studied interactive effects were no longer significant (all  $p$  values  $>0.05$ ).

### 3.5. Total productivity

Climate warming significantly decreased total productivity ( $p=0.003$ , repeated measures GLM), from on average  $3.679$  (unheated) to  $3.174\text{ g m}^{-2}\text{ day}^{-1}$  (heated). Neither species richness ( $p=0.663$ ) nor its interaction with temperature treatment ( $p=0.122$ ) was significant. The effect of the increased root productivity at  $S=9$  (Fig. 3b) was still visible (Fig. 6a), but statistically hidden because of the high variance. The analysis of above-ground productivity in the two chambers in which

the roots were harvested, indicated that the weak  $S$  effect could not be detected there. The productivity decrease caused by the warming was significant, though lower than average ( $0.088$  as compared to  $0.310\text{ g m}^{-2}\text{ day}^{-1}$ ). So even though effects of both warming and species richness were less pronounced in these two chambers, effects of both temperature treatment and  $S$  on below-ground productivity could still be observed.

### 3.6. Root/shoot ratio

Root/shoot ratios did not differ significantly with temperature treatment ( $p=0.265$ , repeated measures GLM) nor with species richness ( $p=0.738$ ), and were  $0.788$  on an average. No interaction was detected ( $p=0.733$ ). One remarkable outlying result occurred at  $S=9$ , however (Fig. 6b), and an independent samples  $t$ -test revealed a significant difference in the root/shoot ratio between both temperature treatments at this richness level ( $p=0.017$ , two-tailed). The higher root productivity in unheated  $S=9$  communities caused the average root/shoot ratio to increase to  $1.020$ .

## 4. Discussion

Exposing 144 experimental plant communities to higher ambient temperatures led to an overall decline in productivity, both above- and below-ground. We hypothesize that much of this decline resulted from increased drought stress, with SWC being generally lowered by heating. The small and non-significant SWC differences between temperature treatments in August (Fig. 2a) were likely caused by the fact that all soils contained the same amount of water at the start of the experiment, in combination with the limited potential for further temperature-driven SWC decreases in the already very dry soil. Nevertheless, in the already hot and dry European 2003 summer, even small reductions of soil moisture levels like those reported in the current study, would have further increased plant stress levels (Ciais et al., 2005). Plants respond to drought in a number of ways, which have been reviewed by Chaves et al. (2002) and which generally have a negative influence on carbon assimilation and growth (Haupt-Herting and Fock, 2002; Shah and Paulsen, 2003). Stomatal closure is one of the early reactions to water deficit, and evidence of increased drought-induced stomatal regulation was found in the heated communities of the present experiment (Lemmens et al., 2006). Another possible response to drought is the promotion of root growth (Chaves et al., 2002), though neither absolute nor relative (root/shoot) increases in below-ground productivity of heated communities were found in our study. Research by Dubrovsky et al. (1998) suggested that rapid drying, as often occurs near the soil surface, would hamper root growth more than gradual drying, as occurs usually in deeper soil layers. Asseng et al. (1998) found a similar result in their experimental study, where root growth was reduced under water deficit in the top 30 cm, while the root system in the deeper soil profile (between 30 and 60 cm) continued to grow. In the present study, less roots were indeed found near the surface in heated communities (Fig. 5a), but as our study of relative root productivity showed, this decrease was proportionate in deeper layers.

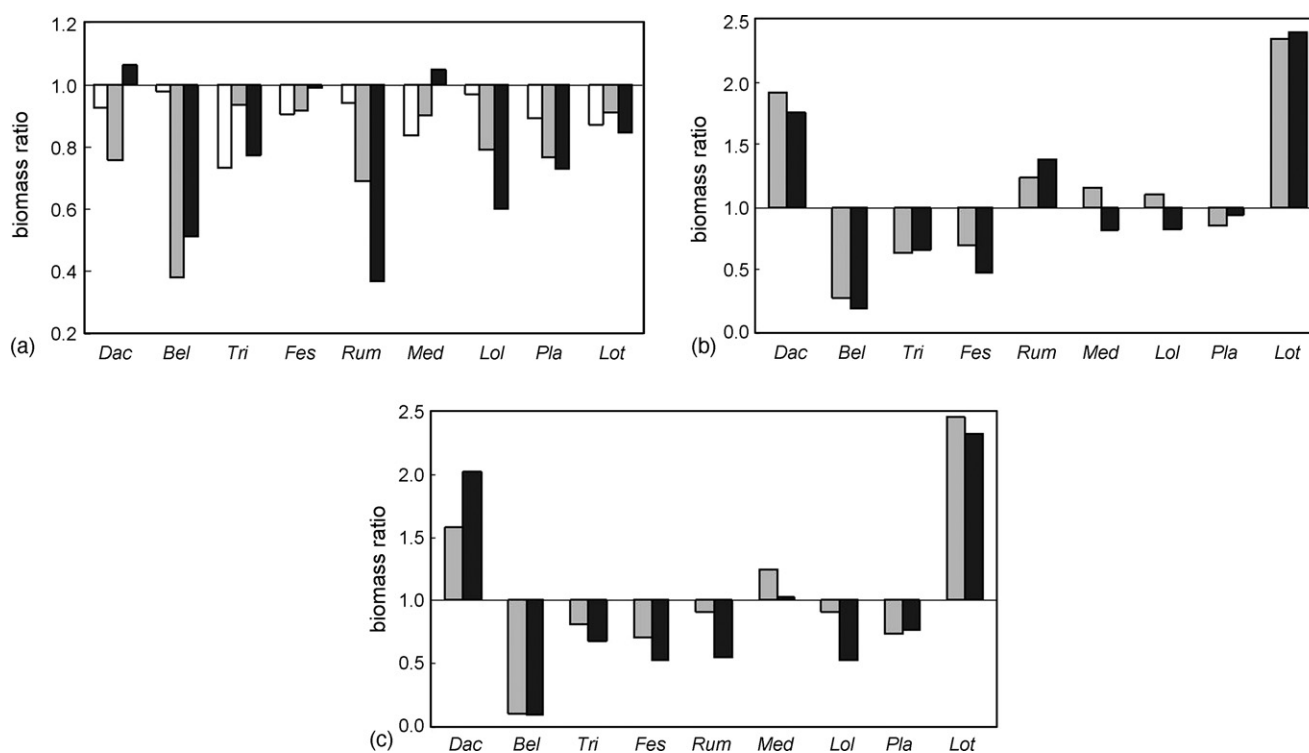


Fig. 4. Individual species productivity. (a) Reaction to warming: ratio of productivity in heated to unheated one- (□), three- (■) and nine-species (■) communities. (b) Reaction to co-occurrence with other species: productivity in three- and nine-species communities relative to monocultures (■ and ■, respectively) in unheated and (c) heated communities. Species: *Bellis perennis* (Bel), *Dactylis glomerata* (Dac), *Festuca arundinacea* (Fes), *Lolium perenne* (Lol), *Lotus corniculatus* (Lot), *Medicago sativa* (Med), *Plantago lanceolata* (Pla), *Rumex acetosa* (Rum) and *Trifolium repens* (Tri).

We found that most soil water was present at 10 cm depth, a result of the experimental set-up which precluded the water table from exerting unwanted outside influence. During a long dry period with rare rain showers, as was the case in the 2003 summer, this is not unrealistic, as we found the same general soil water profile outside (data not shown). Because all new water entered from above, rapid drying in the shallow soil layers was less probable and this may have prevented relative decreases in productivity near the soil surface, as described in the studies of Dubrovsky et al. (1998) and Asseng et al. (1998).

Apart from indirect effects of warming through aggravated soil drought, plants also respond directly to increased temperatures. Edwards et al. (2004) found that soil warming (without accompanying lowering of SWC) led to a reduction in root number and mass due to increased root death, especially during autumn. Furthermore, fluorescence measurements made in the same plant communities that were used in the current study, suggest more intense midday stress at elevated temperatures, causing down-regulation of photosystem 2 (Gielen et al., unpublished). Our productivity data may confirm both of these findings, but it is difficult to separate the effects of temperature increase and drought. Although warming could also benefit plant growth and carbon assimilation, these positive effects dominate in winter and spring (Walther, 2003), whereas in summer, the negative influences of supra-optimal temperatures and low SWC would dominate. Over the course of a full year, we could therefore see a different reaction to increased temperature than what we observed in this ‘de novo’ grassland, planted in spring.

Increased heat and drought could favour species that use water more efficiently, often at the cost of productivity (Grime, 2001). Though shifts were observed in the individual species’ success in heated as compared to unheated communities (Fig. 4a), no net selection effect was detected. Any increased success of more drought resistant species did therefore not lead to decreases of community productivity. *F. arundinacea*, *D. glomerata* and *M. sativa*, species that occur also in warmer regions, seemed to be least affected by the imposed heating. In contrast, *R. acetosa*, indicative of humid grasslands (Bruun and Ejrnæs, 2000; Ejrnæs and Bruun, 2000), and *B. perennis*, had a markedly lower productivity in heated chambers. Our data therefore seem to support the prediction that species adapted to a warmer climate will improve their competitive success in temperate regions under climatic change, at the cost of certain local species who may disappear from the community, hence lowering species richness (IPCC, 2001b; Thomas et al., 2004). However, because the time-frame of the current study is limited, the power of predicting species-specific responses from these data is limited as well.

Overall, species richness did not influence productivity majorly. The significant effect of *S* on above-ground productivity seemed to be attributed to an increase of biomass in the  $S=3$  communities compared to the monocultures. Complementarity in resource use and/or facilitation were the drivers of this enhanced productivity, though the effect remained relatively small and no differences were detected between  $S=3$  and 9, neither for productivity nor for complementarity. In other words, it

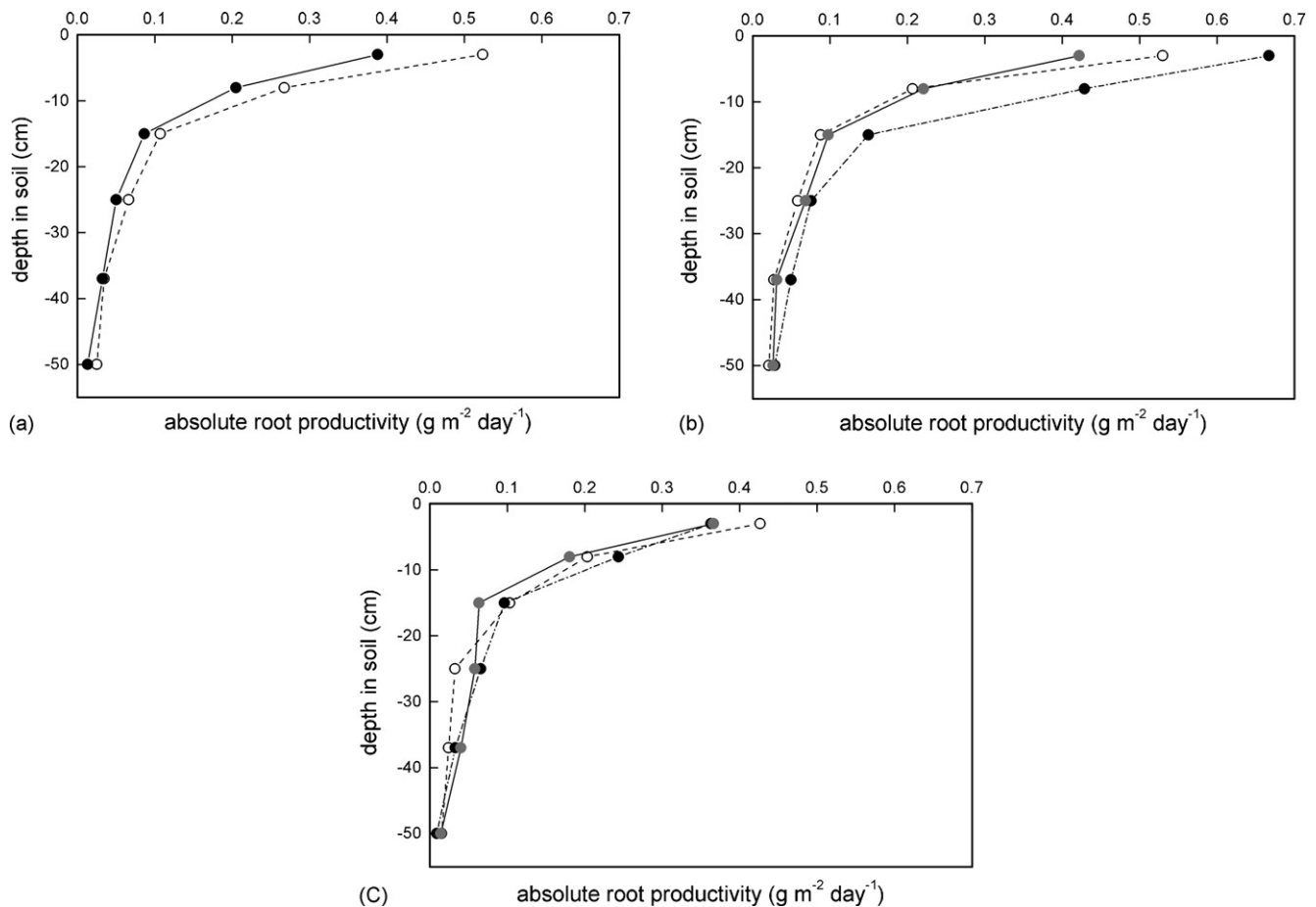


Fig. 5. Root productivity in 4-cm soil slices centered at different depths in the soil. (a) Comparison of root distribution at ambient temperatures ( $\circ$ ) and at ambient temperatures  $+3\text{ }^{\circ}\text{C}$  ( $\bullet$ ). Each symbol represents the averaged root productivity of soil slices from 24 different plant communities. (b) Comparison of root distribution in unheated and (c) heated one- ( $\circ$ ), three- ( $\bullet$ ) and nine-species ( $\bullet$ ) communities. Each symbol represents the averaged root productivity of soil slices from six ( $\bullet$ ) or nine ( $\circ$  and  $\bullet$ ) plant communities.

is not unlikely that the response leveled off. Soil moisture data show that more water was taken up in multi-species systems than in monocultures, which could be caused by increased below-ground complementarity and/or the observed greater biomass. The absence of a net selection effect was already obvious from our study of individual species success on the same communities. Two species with an average monoculture biomass (i.e. *Dactylis glomerata* and *L. corniculatus*, see Lemmens et al., 2006), were especially successful in multi-species communities, while the success of other species in mixtures generally declined (Fig. 4b and c). In their biodiversity experiment at Cedar Creek (USA), Lambers et al. (2004) also observed that the overyielding species were not the most productive in monoculture. There were indications for an effect of  $S$  below-ground, but also here, no strong trends were observed. Root/shoot ratios may be altered by competition with other species. For example, asymmetrical above- and below-ground responses of grassland species productivity to changing species number were found by Wardle and Peltzer (2003), resulting in a decreased root/shoot ratio in multi-species communities. No such general change in root/shoot ratio was found in our experiment, suggesting a symmetrical above/below-ground response to changes in  $S$ .

The generally moderate species richness effects in this study were likely caused by the fact that the model ecosystems were still in their establishment stage in this first experimental year. The net effect of species richness in grassland ecosystems can increase with time, as observed by van Ruijven and Berendse (2005). In addition, the homogeneous soil at the start of the experiment could have affected niche separation below-ground. Several studies (Mou et al., 1995; Nobel, 1997; Wardle and Peltzer, 2003) suggest that plants can shift their rooting patterns when co-occurring with other species to avoid competition, but competition for nutrients is generally lower in homogeneous soil environments (Hutchings et al., 2003). Perhaps the lack of change of root distribution with  $S$  was indicative for such a limited below-ground niche segregation.

We found significant interactions between temperature increase and species richness level, but only below-ground and concentrated at  $S=9$ . Whereas root productivity did not change with  $S$  in heated communities, it did so in unheated communities due to an increase at  $S=9$ , most of which occurred in the top 15 cm of the soil (Fig. 5c). Why was root productivity and the associated root/shoot ratio higher in unheated nine-species communities? Drought stress may limit niche separation in heated



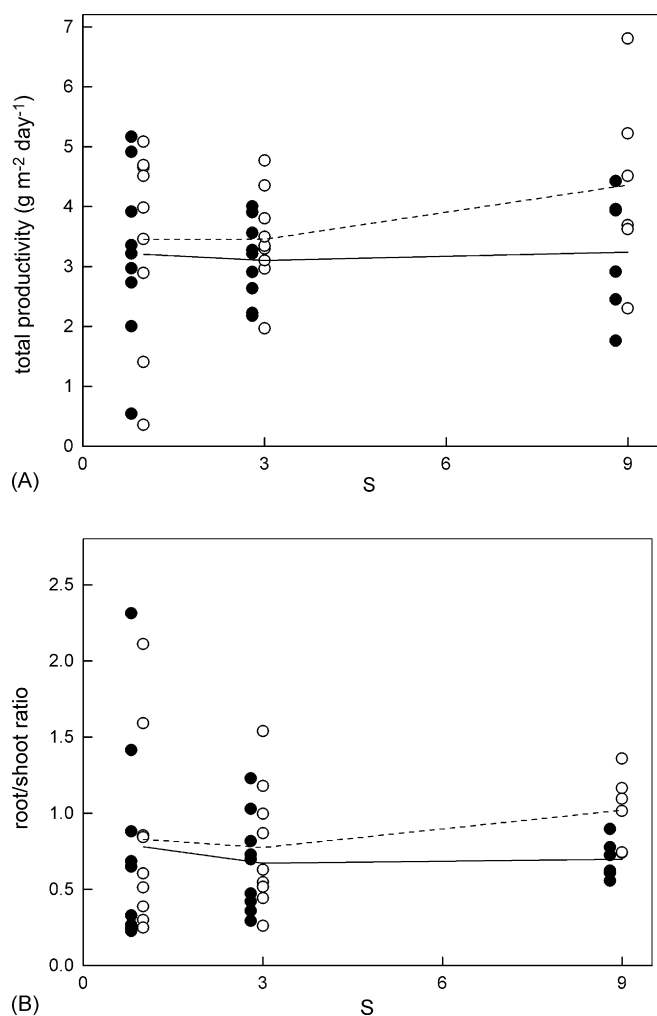


Fig. 6. (a) Total productivity (above-ground including stubble + below-ground) and (b) root/shoot ratio for different species richness levels ( $S$ ), at ambient temperatures ( $\circ$ ) and ambient temperatures  $+3\text{ }^{\circ}\text{C}$  ( $\bullet$ ). Each symbol represents a different community. Averages for unheated (---) and heated (—) communities are connected with a straight line. Symbols are slightly shifted with respect to  $S$  for clarity.

multi-species ecosystems as a result of decreased plant vigour (Wardle and Peltzer, 2003). However, we detected no change in root production in the three-species communities between both temperature treatments, while any limitations of root segregation would be expected also at that richness level as drought stress was aggravated to the same extent by warming at all  $S$  levels. Another possibility is that the shallow soil layers dried out more in heated  $S=9$ , compared to unheated  $S=9$  communities, thereby decreasing root productivity in these layers. Soil water data suggest no such effect, as the three-way interaction between depth, temperature treatment and  $S$  was not significant (see also Fig. 2b and c). Perhaps the observed difference in root productivity between nine-species communities in both temperature treatments was caused by the disproportionate (negative) effect of one species. This could be the case if: (1) such a species would be less successful in heated ecosystems, and (2) if its contribution in three-species systems was lower than at  $S=9$ , or, if its below-ground overyielding remained undetected at  $S=3$

because not all possible species combinations were made at this richness level (as opposed to  $S=9$ ). When comparing data from individual species above-ground biomass and community root biomass at all  $S$  levels (data not shown), we find that *R. acetosa* may have caused such an effect. As separating root biomass of multiple species in the same communities is extremely difficult, testing this hypothesis is problematic. Studies of below-ground biomass in later years of the current experiment should reveal whether the effect at  $S=9$  is ephemeral. To our knowledge, no studies have thusfar investigated interactive effects of warming and species richness, precluding comparisons with other studies.

## 5. Conclusion

In this first growing season, the decrease in productivity caused by warming was striking. Effects of species richness were less pronounced, though we found evidence of complementarity in resource use and/or facilitation. Interactive effects between the two simulated global changes were observed below-ground, for which we proposed several possible explanations. Future research should shed more light on this, as well as provide answers with regard to the response of other processes to combined increased temperature and declining species richness levels, such as plant phenology, carbon fluxes, soil carbon turnover and resource use efficiency.

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