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ORIGINAL PAPER

How do climate warming and plant species richness affect water use in experimental grasslands?

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Abstract Climate warming and plant species richness loss have been the subject of numerous experiments, but studies on their combined impact are lacking. Here we studied how both warming and species richness loss affect water use in grasslands, while identifying interactions between these global changes. Experimental ecosystems containing one, three or nine grassland species from three functional groups were grown in 12 sunlit, climate-controlled chambers (2.25 m^2 ground area) in Wilrijk, Belgium. Half of these chambers were exposed to ambient air temperatures (unheated), while the other half were warmed by 3°C (heated). Equal amounts of water were added to heated and unheated communities, so that warming would imply drier soils if evapotranspiration (ET) was higher. After an initial ET increase in response to warming, stomatal regulation and lower above-ground productivity resulted in ET values comparable with those recorded in the unheated communities. As a result of the decreased biomass production, water use efficiency (WUE) was reduced by warming. Higher complementarity and the improved competitive success of water-efficient species in mixtures led to an increased WUE in multi-species communities as compared to monocultures, regardless of the induced warming. However, since the WUE of individual species was affected in different ways by higher temperatures, compositional changes in mixtures seem likely under climatic change due to shifts in competitiveness. In conclusion, while increased complementarity and selection of water-efficient species ensured more efficient water use in mixtures than monocultures, global warming will likely decrease this WUE, and this may be most pronounced in species-rich communities.

Keywords Evapotranspiration · Global warming · Grassland species · Species richness · Water use efficiency

Abbreviations

ANCOVA	Analysis of co-variance
B	Biomass

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ET	Evapotranspiration
GLM	General linear model
h (subscript)	heated
mix (subscript)	mixture
mono (subscript)	monoculture
res (subscript)	residual
S	Species richness
SWC	Soil water content
T	Temperature
u (subscript)	unheated
WUE	Water use efficiency

Introduction

Global surface temperatures are projected to increase by 1.4–5.8°C by 2100, in reaction to the rising concentrations of greenhouse gases in the atmosphere (Houghton et al. 2001). Our understanding of the response of plant communities to a warming world has significantly increased due to a growing body of experimental and modelling studies, especially during the last 10 years. These studies span a variety of ecosystems and climatic regions (e.g. Llorens et al. 2003; Marchand et al. 2004), although most knowledge exists about warming effects on plant communities in temperate and polar regions. A warmer climate could cause productivity to increase in temperate grasslands through temperatures closer to the growth optimum (Thornley and Cannell 1997; Carter et al. 1997) and through a lengthening of the growing season (Myneni et al. 1997; Walther 2003). Unless precipitation increases, decreases in soil moisture caused by higher evapotranspiration (ET) may on the other hand lower plant production (Eatherall 1997; Saleska et al. 1999). Low soil water contents (SWCs) limit above-ground production rates mainly through decreases in stomatal conductivity, down-regulation of the photosynthetic machinery and increased allocation to roots (Chaves et al. 2002). Water relations are therefore a key determinant in the outcome of the plant communities' reaction to future higher temperatures and associated soil drying.

The issue of declining plant species numbers, another global change (Sala et al. 2000), has raised considerable interest as well, particularly in the past decade. The question whether species-poor ecosystems perform differently than species-rich systems has been the subject of numerous studies (for a review, see Hooper et al. 2005), but possible interactions with the ongoing climate warming have hardly been investigated. The two main mechanisms through which performance can be altered with changing species richness are complementarity and selection (Loreau and Hector 2001). More water is available for transpiration in species rich systems when complementarity between root systems allows for a higher total uptake ("overyielding"). Complementarity may therefore be more important when water is limiting, which is more likely when higher temperatures lower the SWC. Plant species that exhibit a high water use efficiency (WUE) may in many ecosystems be better adapted to a situation in which water is limited. Mixtures have a higher probability of containing such water-efficient species, and would therefore be better "insured" against drought than the average monoculture (Naeem and Li 1997).

We subjected artificially assembled grassland communities of different species richness to either ambient temperatures or temperatures that were continuously 3°C higher, as the combination of both global changes makes it possible to uncover interactions. To ensure that warming would also encompass lower SWCs if ET was higher, both the unheated and the heated communities received equal amounts of rainfall. In previous papers, we already reported on autumn physiology with the use of chlorophyll fluorescence (Gielen et al. 2005) and on the ecophysiology of the individual grassland species (Lemmens et al. 2006). The objective of the current study is to investigate water relations and changes therein resulting from warming and species richness loss. In particular, we hypothesise that exposing plant communities to higher temperatures may result in increased water loss and drier soils, which could trigger physiological and structural responses altering ET and WUE. We also hypothesise that WUE could be improved in mixtures compared to monocultures through complementarity and the selection of water efficient species. By alleviating or avoiding negative effects of drought in this way, we expect differences between the temperature treatments to be smallest at the highest richness level.

Material and methods

Study site and experimental set-up

This study was conducted at the Drie Eiken Campus of the University of Antwerp (Belgium, 51°09′ N, 04°24′ E), where an experimental platform containing 288 artificially assembled grassland model ecosystems was established in July 2003. The climate of north Belgium is characterised by mild winters and cool summers, with average annual air temperatures varying around 9.6°C, and mean monthly air temperatures between 2.2°C (January) and 17.0°C (July). Annual precipitation averages 776 mm, equally distributed throughout the year.

The platform consisted of 12 (10 from November 2003 on after destructively harvesting two) sunlit, climate-controlled chambers (2.25 m^2 ground area) facing south, half at ambient temperatures (unheated) and the other half continuously at ambient temperatures +3°C (heated). 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 aluminium-frame chambers were covered with a colourless polycarbonate plate (4 mm thick), and polyethylene film (200 μm thick) at the sides, both UV transparent and a total light transmission of 86%. Each chamber contained the same series of 24 different grassland communities of varying species richness (S): nine monocultures, nine S = 3 communities and six S = 9 communities. We opted for species from three functional groups, which were equally represented at each S 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.). These C3 species were 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, and different temperature and drought resistance. Species representative of the three functional groups, were used to create each of the S = 3 communities, with each species only placed together once with any other species. Out of these three potential sets of nine different S = 3 communities, only one randomly chosen set was used. Each of the six S = 9 communities had a different internal arrangement. Each community contained 30 individuals planted in a hexagonal grid at 4 cm distance, with interspecific interactions maximised. Similar plant densities were used in other experiments (e.g. Van Peer et al. 2004), and are deemed realistic for temperate grasslands. 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. Most of the species were flowering by August 2003. By November 2004, the average species richness had dropped to 2.8 and 8.0 in S = 3 and S = 9, respectively, due to interspecific competition. The soil used in the experiment (76.3% silt, 14.8% clay and 8.7% sand; field capacity 0.39 $m^3 m^{-3}$; pH 6.45, carbon content 1.6%) was collected from an agricultural field and sieved (0.5 mm mesh size) to remove stones and large organic material. No fertiliser was added to this rich agricultural soil. Plants were treated regularly to avoid fungal infection and insect damage, and weeding was done manually throughout the experiment. For further information regarding the experimental set-up, we refer to Lemmens et al. (2006).

Water supply

The containers containing the plant communities (24 cm inner diameter, 60 cm deep), were installed in the soil to ensure realistic soil temperatures. Water could freely drain from the containers, while capillary rise of soil water from deeper layers and lateral water inflow were prevented. Profile probe tubes (55 cm long) which fitted with a PR1 soil moisture sensor (Delta-T Devices Ltd, Cambridge, UK) were installed in two sets of 24 different communities across six chambers (three unheated, three heated) and outside in a breach filled with the same sieved (0.5 mm mesh size) clay/loam soil as inside. The quantities of water supplied to the unheated chambers were calculated weekly from the difference between the soil moisture outside and inside, to create the same SWC. The heated chambers received the same amount of water as the unheated chambers, so that any enhanced consumption would result in (aggravated) soil drought. The irrigation strategy was further improved to account for above-ground water run-off. Indeed, any precipitation on soils saturated with water would result in run-off, so this amount was not supplied as it would not increase SWC in the unheated chambers. This excess water would, however, increase SWC in the heated chambers if the soil there was not yet saturated. From June 2004 onwards, we therefore based the irrigation on precipitation data from a nearby weather station (Lint, Belgium, 51°07′ N, 04°29′ E) rather than on SWC, and corrected the precipitation data for the (observed) artificial enhancement of ET in the chambers. To this end, ET inside unheated chambers was calculated from SWC and the amount of administrated water, and the outside ET was calculated with Hamon's equation (Haith and Shoemaker 1987), based on day length, vapour pressure and air temperature. The difference in ET between these two (ΔET) was approximately constant during the first growing year (45 l m^{-2} month^{-1}), and was consequently used in the current experiment so that:

$$\text{Water supplied} = \text{Outside precipitation} + \Delta\text{ET} \quad (1)$$

These amounts of water were supplied to the communities in both temperature treatments, with an average frequency of three times a week. In May 2004, we supplied extra quantities of water (25 l m^{-2}) to the heated communities, to more fully assess the extent of any limitations of low SWC on ET.

Measurements

Soil water content, measured on average once a week (always before watering) from August 2003 until November 2004 with the PR1 sensor, was corrected using the calibration determined in the lab for the specific soil used. For every community, the average SWC of the four measurement depths (10, 20, 30 and 40 cm) was plotted as a function of time, and one linear regression per month was fitted through these points. To calculate ET, we used the principle:

$$\text{Total water loss} = \text{ET} + \text{leaching} = \text{precipitation} - \Delta\text{SWC} \quad (2)$$

ΔSWC is the amount of water gained or lost during a month, and was calculated from the slope of the regression line. Since soils were very rarely at field capacity due to relatively dry winter conditions, any water loss through leaching was limited. Furthermore, when SWC was higher than field capacity, we replaced these data by field capacity values in calculations. The influence of leaching in the calculations is therefore very limited, and the total water loss reflects the actual monthly ET well. Limiting the ET calculations to one monthly value improved accuracy, as in this way, fluctuations due to PR1 measurement errors (0.04 m^3 m^{-3}), were dampened.

Combining monthly ET with biomass measurements allowed to make an accurate estimation of WUE, expressed as grams of biomass produced per liter of water used. The WUE was calculated for three periods, with the end of each period determined by a biomass harvest: August–October 2003 (period one), November 2003–May 2004 (period two) and June–October 2004 (period three). Above-ground biomass was determined by cutting plant communities approximately 3.5 cm above the soil surface (to allow regrowth), drying the plant parts for at least 2 days at 70°C and weighing them. In half of the chambers, biomass was sorted by species within each community, while in the other half, biomass was collected per community only.

We assessed 'overyielding' of ET and WUE in mixtures as the difference between observed and expected values for either parameter. Positive values indicate overyielding (higher ET or WUE values in mixtures than expected based on weighted monoculture data), negative values indicate underyielding. The expected values for ET of a mixture, were calculated with the formula:

$$\Sigma_j[(\mathrm{ET}_{\mathrm{mono},j}/B_{\mathrm{mono},j}) \times B_{\mathrm{mix},j}] \tag{3}$$

with $\mathrm{ET}_{\mathrm{mono},j}$ the ET of species j in monoculture, and $B_{\mathrm{mono},j}$ and $B_{\mathrm{mix},j}$ the biomass of species j in monoculture and in mixture, respectively.

For WUE, which is independent of biomass, the expected values were calculated with the formula:

$$\Sigma_j[\mathrm{WUE}_{\mathrm{mono},j} \times B_{\mathrm{mix},j}/B_{\mathrm{mix}}] \tag{4}$$

with $\mathrm{WUE}_{\mathrm{mono},j}$ the WUE of species j in monoculture and B_{mix} the biomass of the mixture.

Meteorological data

Ambient temperatures during the three periods were approximately 1.1°C above normal, with the summer of 2003 being especially warm and also relatively dry and sunny. Precipitation amounts and hours of sunshine were near normal during the rest of 2003 and 2004 (data from the Royal Meteorological Institute of Belgium).

Statistical analysis

All statistics were performed using SPSS 10.0 (SPSS Science, Woking, UK). To test for effects of heating, species richness level, period, and interactions, we used general linear model (GLM) univariate analysis (with or without covariate, as explained in the results section) or repeated measures analysis where appropriate. The Tukey post-hoc test was used to separate multiple means. Testing whether the mean of a single variable differed from a constant was done with one-sample *t*-tests. Regressions between two variables were linear. Residual analysis was performed to detect relationships between the residuals of a regression (i.e. the observed values minus the values calculated using the equation of the regression) and an independent variable (see also Marchand et al. 2004). The significance level was 0.05. No transformations (logarithmical or otherwise) had to be made to meet the assumptions of the tests.

Results

Soil water content

Warming led to slightly drier soil conditions in heated communities in all periods ($P < 0.05$, repeated measures analysis), with an average SWC of 26.0 ± 1.5% (standard deviation) under heating and 27.3 ± 1.7% at ambient temperatures. Also S had a significant influence on SWC ($P < 0.05$), and post-hoc tests showed that monoculture soils (27.9 ± 2.0%) generally contained more water than S = 3 (26.0 ± 1.6%) and S = 9 (25.9 ± 1.5%) communities ($P < 0.05$). The non-significant interaction between temperature treatment and S indicated that the effect of S on SWC was similar in both heated and unheated communities.

Observed ET

There was a small increase in ET under warmer conditions ($P < 0.05$, repeated measures analysis), but the data (Fig. 1), together with the significant interaction between temperature treatment and month ($P < 0.001$), suggest that higher ET rates were constrained to the beginning of the experiment and to the one month in which we applied more water to the warmed communities. Evapotranspiration was affected by S ($P < 0.05$), with monocultures exhibiting the lowest ET rates ($P < 0.05$, post-hoc), although the effect was small during most months (not shown). Species richness effects on ET were similar in both temperature treatments, as their non-significant interaction indicated.

Observed WUE

Warming lowered the efficiency of water use ($P < 0.001$, repeated measures analysis), and the WUE was also affected by S ($P < 0.01$), with

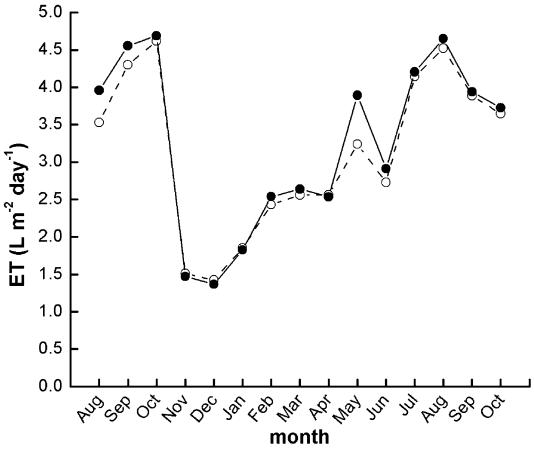

Fig. 1 Evapotranspiration (ET) in 2003 and 2004 at ambient temperatures (o and –) and ambient temperatures +3°C (• and —). Each symbol represents the average monthly ET of 24 different plant communities (all species richness levels combined)

multi-species communities consistently having higher WUE values than monocultures (post-hoc, $P < 0.05$). In the two later periods, the S effect seemed different in both temperature treatments, with the biggest WUE differences at S = 9, although no interaction between these two factors was detected. Because S had only small effects on ET, the WUE curves (Fig. 2a) reflect the community biomass, which showed a similar relationship with S (Fig. 2b).

We tested the relationship between WUE in unheated (WUE_u) and heated (WUE_h) monocultures with linear regressions, to find out whether species reacted similarly to future warmer conditions with regard to their WUE. The linear relationship between WUE_u and WUE_h was strong at the start of the experiment (period one: $R^2 = 0.92$, $P < 0.001$, slope = 0.80), but grew weaker with time (period two: $R^2 = 0.73$, $P < 0.01$, slope = 0.58; period three: $R^2 = 0.42$, $P < 0.05$, slope = 0.50), indicating increased interspecific variability. Some species, such as *Rumex acetosa*, were using water much less efficiently in warmer conditions (Fig. 3).

ET and SWC

To test how the communities' ET was affected by soil drying, we analysed effects of period, temperature treatment and S level on ET, with SWC as a covariate (ANCOVA). With period being by far the most significant factor influencing the SWC-ET relationship ($P < 0.001$), we analysed each period separately, and found no significant influences of either temperature treatment or S in periods two and three. In both these periods, ET dropped as SWC increased (linear regression, $P < 0.01$), although the biological significance of this trend is likely limited because of the high variation (Fig. 4c, d). In period one, temperature treatment was significant ($P < 0.001$) and by analysing both temperature treatments separately, S proved significant in the unheated communities ($P < 0.05$). Linear regression showed that under the current climate, ET decreased with drier soils in this period (Fig. 4a). This trend was observed at all S levels (Fig. 4b), but proved only significant at S = 3 ($P < 0.01$), although it was nearly significant at S = 1 and S = 9 ($0.05 < P < 0.10$).

To include the effect of temperature on ET, we calculated residuals (ET_{res}) and tested their relationship (linear regression) with monthly average air temperatures (T_{air}). In the first period, there was surprisingly a negative effect of T_{air} on ET_{res} at every S level in the unheated treatment, indicating that the SWC-ET relationship overestimated ET at high temperatures, and underestimated it at lower temperatures. A similar negative linear relationship between T_{air} and ET was found in the heated communities in period one ($P < 0.001$). In periods two and three, there was a positive effect of T_{air} on ET_{res} ($P < 0.001$; Fig. 5), so the opposite from what was observed in period one.

Overyielding in mixtures

In our assessment of overyielding of both ET and WUE, we found that there were statistically large differences between each of the three periods ($P < 0.001$, repeated measures analysis), and hence we treated each period separately in further analysis. We further separated the datasets based on whether effects of temperature treatment, S, or their interaction were significant. Results are shown in Table 1.

Mixtures generally used less water (lower ET) than expected from the monocultures of their

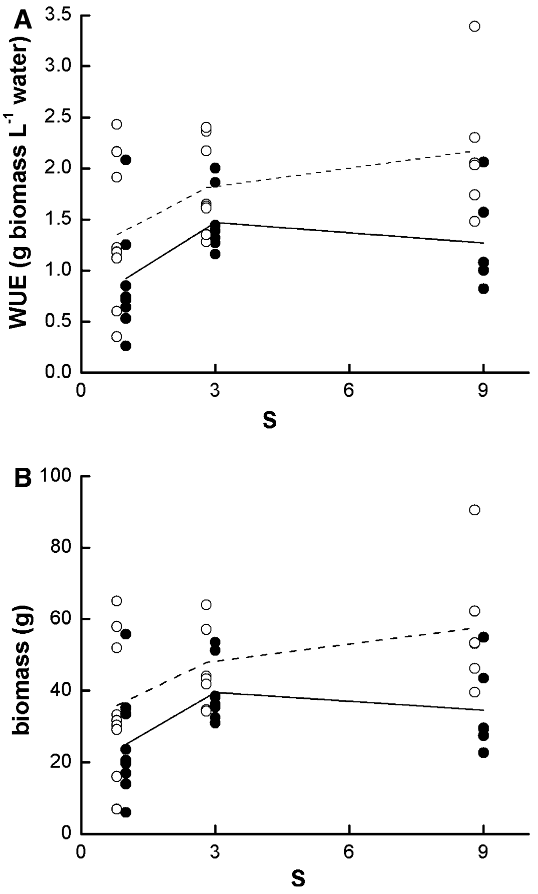

Fig. 2 (**A**) Water use efficiency (WUE) and (**B**) biomass, in period three (June–October 2004) for different species richness levels (S), at ambient temperatures (unheated, o) and ambient temperatures +3°C (heated, •). Each symbol represents a different community. Averages for unheated (–) and heated (—) communities are connected with a straight line. Symbols are slightly shifted along the horizontal axis with respect to S for clarity

composing species (Eq. 3), and they used the water more efficiently (higher WUE) than expected (Eq. 4).

Success in mixtures

We first tested whether a higher WUE would benefit species' competitive success in mixtures. The success was determined as follows: multiplying the observed "per-individual" biomass of a species in a mixture with 30 (i.e. the number of plants in a community), gives the biomass of that species if it were a monoculture. Subtracting the actual observed monoculture biomass from that value, dividing this by the observed monoculture biomass and subsequently multiplying this by 100, gives the percentage of biomass increase of that species in that particular mixture.

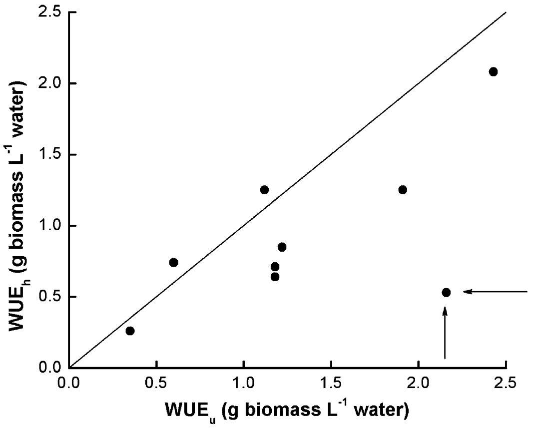

Fig. 3 Comparison of water use efficiency (WUE) of all nine monocultures at ambient temperatures (WUE$_u$) and at ambient temperatures +3°C (WUE$_h$) in period three (June–October 2004). The 1:1 line (—) is depicted, and *Rumex acetosa* is indicated by arrows

An ANCOVA with WUE as covariate showed a significant influence of period ($P < 0.01$), but not of temperature treatment or S. Linear regression of the whole dataset (Fig. 6) revealed significant positive relationships between WUE and the percentage of biomass increase in periods two and three ($P < 0.01$), but not in period one.

Discussion

Soil water content and observed ET

The warming treatment dried soils slightly, as expected, and the ET was somewhat increased by warming, although this increase was small or absent during most months (Fig. 1). Because the precipitation quantities were identical in both temperature treatments, it is not surprising that the ET under warmed conditions was higher only at the beginning of the experiment. Starting with similar moisture conditions in all soils, a new balance between water inputs and outputs was established after three months, resulting in drier soils in the warmed communities and ET rates

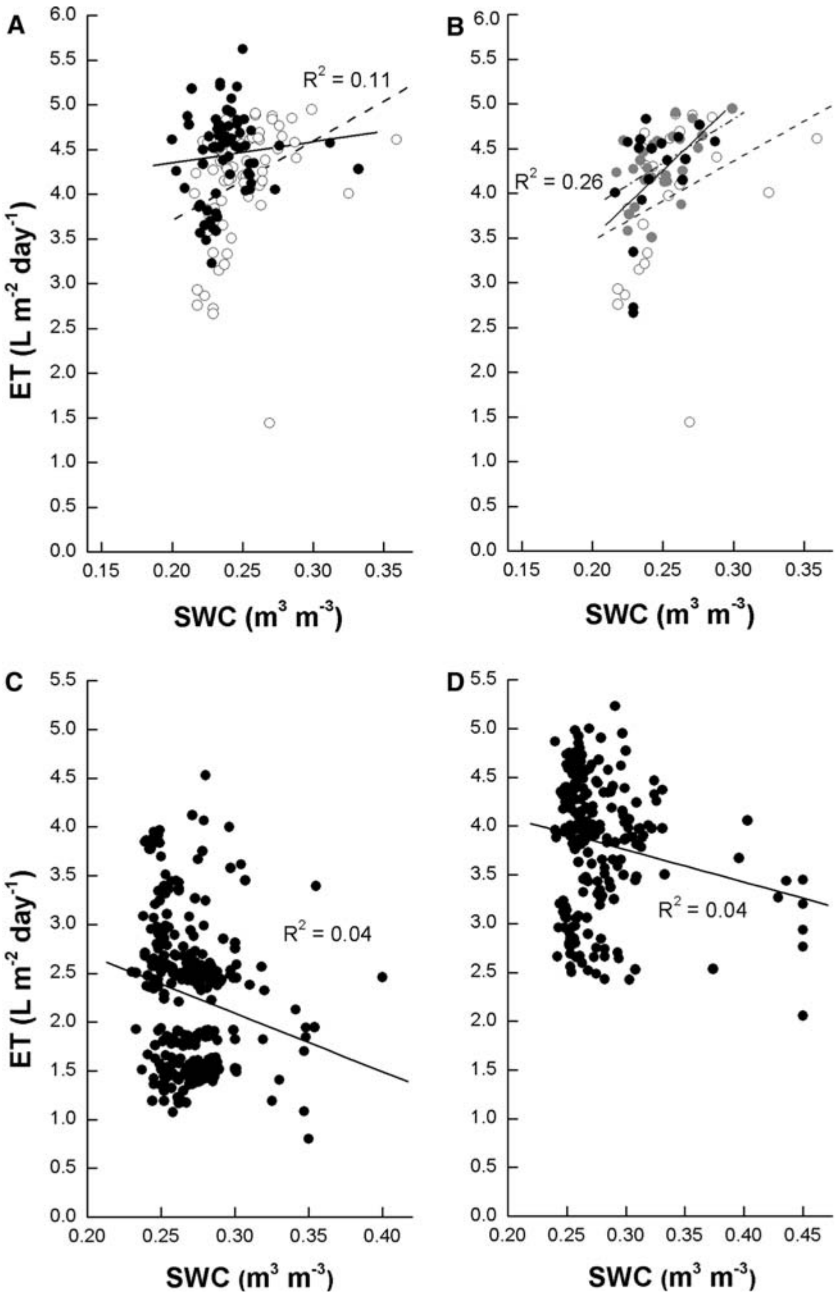

Fig. 4 Effect of soil water content (SWC) on evapotranspiration (ET) (**A**) in period one (August–October 2003) at ambient temperatures (o and –) and ambient temperatures +3°C (• and —); (**B**) in period one at different species richness (S) levels, S = 1 (o and –), S = 3 (• and -·-) and S = 9 (• and —), all at ambient temperatures; (**C**) in period two (November 2003–May 2004) and (**D**) in period three (June–October 2004), at ambient temperatures and ambient temperatures +3°C combined. Linear regressions are shown, and R^2 values indicated when regression is significant. See text for details

that were highly similar to those recorded in unheated communities (Fig. 1). Various plant responses exist in reaction to decreased soil moisture levels, but stomatal regulation is usually the first in the short term (Chaves et al. 2002). Indications for stomatal regulation were found in the same experiment, with Lemmens et al. (2006) reporting a lower stomatal conductance in heated as compared to unheated communities in the late summer of 2003. In the longer term, an increase in the proportion of roots to shoots is a drought avoidance strategy observed in some studies (Rodrigues et al. 1995; Xu and Zhou 2005). Biomass data from period one, however, indicated no such change in root to shoot proportion (De Boeck et al. in press). Both the absence of a groundwater table and the container size (60 cm depth) may have been responsible for this, by

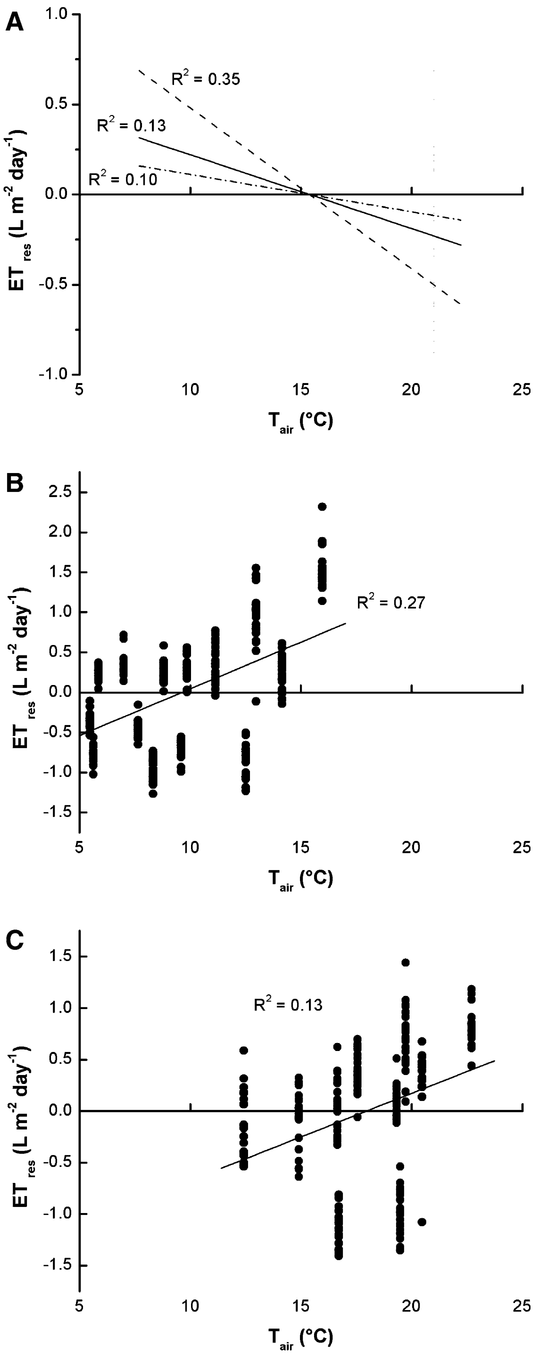

Fig. 5 Effect of monthly average air temperatures (T_{air})▶ on residual (observed–calculated) evapotranspiration values (ET_{res}), derived from the linear relationship between soil water content (SWC) and ET. (**A**) In period one (August–October 2003) at different species richness (S) levels, S = 1 (–), S = 3 (-·-) and S = 9 (—), all at ambient temperatures (only linear regressions with R^2 values are shown); (**B**) in period two (November 2003–May 2004) and (**C**) in period three (June–October 2004) at ambient temperatures and ambient temperatures +3°C combined, with linear regressions and R^2 values. See text for details

limiting the extra quantities of water that could be extracted from the soil through root proliferation. Data from later years should clarify whether shifts in the root to shoot proportion were absent also when the vegetation had further matured, although preliminary data from 2004 and 2005 (not shown) do not suggest any such shifts.

A decreased above-ground biomass was observed in warmed communities. This likely limited ET, as the transpiration of a stand is approximately proportional to its green mass (Larcher 2003). The possibility that decreases in the biomass alone were responsible for reducing ET, was rejected by the observation that significant ET increases occurred when the input of water in heated communities was increased (May of 2004). Lower above-ground productivity rates, together with stomatal regulation, therefore helped in saving water under warmer conditions and were thus an effective response mechanism to drought. Grassland productivity would therefore decline under future warmer conditions, unless precipitation is increased (Eatherall 1997).

Less water was present in soils of mixtures than in monocultures, which was associated with higher ET. This could indicate that below-ground complementarity in mixtures allowed multi-species communities to extract more water from the soil. The absence of differences in SWC between 3 and 9-species communities could mean that below-ground complementarity was not significantly increased between high richness levels (De Boeck et al. 2006). The SWC decrease and ET increase in multi-species communities may have been caused at least partially by the higher above-ground productivity in mixtures and the subsequent rise in transpirational surface.

Observed WUE

There was a clear trend for less efficient use of water in heated communities (Fig. 2a), which predominantly reflected a sharp biomass decrease (Fig. 2b), as differences in ET between both temperature treatments were small. The reduc-

Table 1 Results of one-sample *t*-tests (test value = 0) on overyielding data of evapotranspiration (ET, l m^{-2} day^{-1}; top) and water use efficiency (WUE, g biomass l^{-1} water; bottom)

Period	Data	ET overyielding	
		Average	Significance
1	All	–0.003	0.983
2	S = 3	–0.532	**0.006**
	S = 9	–0.437	**0.033**
3	S = 3	–2.578	**0.008**
	S = 9	–1.699	0.102
		WUE overyielding	
1	All	–0.141	0.086
2	Unheated, S = 3	**0.681**	**0.031**
	Unheated, S = 9	**1.508**	**0.006**
	Heated, S = 3	**0.634**	**0.005**
	Heated, S = 9	0.338	0.251
3	Unheated, S = 3	0.465	0.209
	Unheated, S = 9	**0.913**	**0.003**
	Heated, S = 3	0.108	0.667
	Heated, S = 9	–0.063	0.791

Data separated per temperature treatment (unheated or heated) and/or per species richness level (S) when indicated as significant by univariate analysis. Significant over- or underyielding ($P < 0.05$) is indicated in bold

tion of WUE was recorded at the leaf scale as well, both in 2003 (Lemmens et al. 2006) and in 2004 (Lemmens et al. unpublished). Because measurements indicated that both leaf-level transpiration and $P_{\max}$ were increased at least in some months due to warming (Lemmens et al. 2006; Lemmens et al. unpublished), we suggest that the observed biomass decrease was attributed to other causes than reduced photosynthesis through stomatal regulation alone. Fluorescence measurements suggest that the reduction in above-ground productivity at elevated temperatures may be explained in part by more intense midday stress causing down-regulation of photosystem 2 (Gielen et al. unpublished). Furthermore, Reichstein et al. (2002) noted that processes other than the classical stomatal control, such as stomatal patchiness (Beyschlag and Eckstein 1998), could affect water use. Indeed, if for example a larger proportion of sun leaves than shade leaves is inactive due to stomatal closure under heating, WUE of the canopy would be lower under these warmer conditions.

Water use efficiency generally increased with species richness. This was again a reflection of the relationship between S and biomass, as ET differed little between S levels. Complementarity in resource use in mixtures, which we found indications for regarding water extraction, is known to stimulate productivity (van Ruijven and Berendse 2005) and could have caused at least part of the increases in biomass and WUE. Selection may also play a role in this (Loreau and Hector 2001), and the generally positive relationship between WUE and success in mixtures (Fig. 6) indeed seems to confirm that hypothesis. The absence of such a relationship in period one is in agreement with the fact that no net selection effect on productivity was detected in this period (De Boeck et al. in press). Indeed, effects of species richness and competition often increase with time (van Ruijven and Berendse 2005), as seemingly confirmed in the current experiment and as highlighted by Fig. 6.

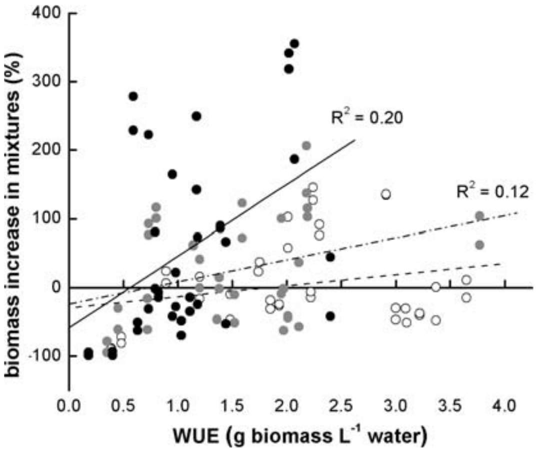

Fig. 6 Effect of monoculture water use efficiency (WUE) on species' competitive success in mixtures, expressed as a species' biomass decrease or increase in mixtures, compared to their monoculture (see text for details). Each symbol represents a species in a community of richness levels 3 or 9, at ambient temperatures and ambient temperatures +3°C combined, in period one (August–October 2003, o and –), two (November 2003–May 2004, • and -·-) or three (June–October 2004, • and —). Linear regressions are depicted and R^2 values indicated when regression is significant. See text for details

Impact of stresses on ET and WUE

In contrast with our original hypothesis, the difference in WUE between both temperature treatments, was generally more, rather than less, pronounced at S = 9 (Fig. 2a). Increased complementarity in multi-species communities together with the ‘‘insurance hypothesis’’, according to which communities are better protected against heat and drought when more species are present (Naeem and Li 1997), theoretically suggest a decreased negative impact of warming and associated soil drought at higher richness levels. The relatively short time frame of the current experiment limits the power of conclusions on this matter, however, although biomass data of 2005 (not shown) suggest a continuing trend.

In the summer and early autumn of 2003 (period one), ET was apparently lowered with increasing soil drought, although only significantly in unheated communities (Fig. 4a). This would indicate that increased levels of drought triggered stomatal responses, effectively lowering ET. The residual analysis surprisingly highlighted that high temperatures further reduced ET, in both temperature treatments. If sufficient water is available to plants, ET increases with air temperature (Allen et al. 2003). However, when water supply is limited, stomatal closure protects plants from excessive water loss, because the vapour pressure deficit in the air also increases with temperature (Larcher 2003). This further clarifies that water was in short supply in the summer and early autumn of 2003, limiting productivity as observed in other European studies that year (Ciais et al. 2005), but moreso in heated communities because these experienced higher drought and heat stress. In the two later periods, the trend of lower ET with declining SWC was not observed, suggesting that regulatory mechanisms were triggered less because drought and/or heat stress were not as acute as in 2003.

Overyielding

Mixtures used less water and used it more efficiently than predicted from monocultures, with the exception of period one (Table 1). As species proportions were included in the overyielding calculations, this further proves that complementarity increased with rising species richness, making mixtures more water efficient, regardless of the increased success of water efficient species. Apart from below-ground complementarity, as previously discussed, it is likely that above-ground complementarity also stimulated efficient water use. In multi-species communities, the three-dimensional space of the canopies is generally filled more completely (Cernusca 1976; Naeem et al. 1994). The decreased wind speed through these better-filled canopies limits ET (Larcher 2003), while photosynthesis is at the same time stimulated through increased total light interception. This may explain why biomass was higher in mixtures than in monocultures, while ET was only marginally increased.

Success in mixtures

Inherently water-efficient species generally improved their success in mixtures, and the importance of a high WUE was similar in warmed and control conditions. Nevertheless, because no single species significantly improved its WUE under heating (Fig. 3), we conclude that plants adapted to relatively warmer and drier conditions such as *Dactylis glomerata* and *Medicago sativa*, are not necessarily more efficient under those conditions. A similar conclusion was made in a study of Mahmoud and Grime (1976), in which species from productive habitats still outcompeted species from unproductive habitats when grown under low nutrient conditions. However, the WUE in heated compared to unheated conditions decreased more for some species than for others, indicating that relative shifts in competitive ability are likely, regardless of whether or not absolute WUE increases under warming. For example, *Rumex acetosa*, indicative of humid grasslands (Bruun and Ejrnæs 2000; Ejrnæs and Bruun 2000), had a markedly lower WUE and productivity in heated chambers. This shows that even relatively small changes in growing conditions (3°C warmer, slightly lower SWC) can alter the outcome of competitive interactions and ultimately the composition of plant communities.

Conclusion

In conclusion, this study provides new results about the effects of warming and species richness loss on water use in grasslands. The combination of higher temperatures and slightly increased soil drought lowered WUE mainly through decreased productivity. Higher complementarity and selection of water-efficient species led to more efficient water use in mixtures than in monocultures, although surprisingly, this did not lower the negative impact of warming and associated drier soils on WUE in multi-species communities. The findings in these experimental ecosystems provide insights into the complex interactions between two simultaneous global changes. Future studies should test whether our conclusions are equally valid in natural communities.

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