View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Ghent University Academic Bibliography

Functional Ecology 2004
18, 769–778

Species richness and susceptibility to heat and drought extremes in synthesized grassland ecosystems: compositional *vs* physiological effects

L. VAN PEER,* I. NIJS,*† D. REHEUL‡ and B. DE CAUWER‡

**Research Group Plant and Vegetation Ecology, Department of Biology, University of Antwerp, Campus Drie Eiken, Universiteitsplein 1, B-2610 Wilrijk, Belgium, and ‡Department of Plant Production, Faculty of Agricultural and Applied Biological Sciences, University of Ghent, Coupure Links 653, B-9000 Ghent, Belgium*

Summary

1. We investigated effects of declining plant species richness (*S*) on resistance to extremes in grassland communities.
2. Synthesized model ecosystems of different *S*, grown outdoors in containers, were exposed to a stress peak combining heat and drought. The heat wave was induced experimentally by infrared irradiation in free air conditions.
3. Before the heat wave, the more species-rich communities produced more biomass as a result of a large and positive complementarity effect that outweighed a small negative selection effect.
4. Water use during the heat wave was likewise enhanced by *S*, which could not be attributed to dominance of 'water-wasting' species. Instead, water consumption at high *S* exceeded that expected from changes in community biomass and biomass composition. The observed enhancement of resource (water) acquisition under stress with increasing *S* therefore probably originated from complementarity.
5. Despite enhanced water use in the more diverse communities, plant survival was significantly less, affecting all species alike. Physiological stress, recorded as photochemical efficiency of photosystem II electron transport, was significantly greater. Before the heat wave, the changes in biomass composition that coincided with increasing *S* did not favour species that would later prove intrinsically sensitive or insensitive.
6. Complementarity in resource use for biomass production had a cost in terms of reduced survival under stress, despite the likelihood of complementarity in water acquisition during exposure. The greater loss of individuals from the more diverse grasslands suggests enhanced risk of local extinction.

Key-words: complementarity, sampling effect, stress resistance, survival, water use

Functional Ecology (2004) **18**, 769–778

Introduction

Extreme climatic events are predicted to increase in frequency and/or severity along with global warming. For example, during the 21st century heat waves would become more common almost everywhere (McCarthy *et al*. 2001). Few records exist on ecosystem impact of extreme heat; they indicate that more damage can be inflicted relative to milder, but year-round, increases in surface temperature, especially when accompanied by drought (Nijs *et al*. 1996b; White *et al*. 2000). Most studies on extremes in plants have focused on molecular mechanisms underlying survival, such as synthesis of thermotolerance-related proteins, often with a view to diminishing yield loss in single species (Howarth 1991). However, mortality being species-specific, severe stress can also alter community structure and local plant diversity (Tilman & El Haddi 1992; Buckland *et al*. 1997; White *et al*. 2000). Loss of diversity may, in turn, affect community resistance to extremes (Frank & McNaughton 1991; McGrady-Steed, Harris & Morin 1997; Naeem & Li 1997), which opens the possibility of positive feedback accelerating diversity decline. Evaluating this risk requires insight into how stress physiology and community structure interact, but the rare and ephemeral nature of natural extremes limits opportunities for investigation.

In the context of accelerating local and global change, the ability to withstand stress without loss of function (resistance), or to recover rapidly from stress (resilience), are increasingly important ecosystem traits (Virginia &

© 2004 British Ecological Society

†Author to whom correspondence should be addressed. E-mail: ivan.nijs@ua.ac.be

Wall 2001). Both these components of stability appear to be determined by traits of the dominant plant species. Productive communities generally have high resilience and low resistance, the opposite being true for communities of slow growers (MacGillivray & Grime 1995; Díaz 2001). Pfisterer & Schmid (2002) found that the mere presence of some species – as opposed to dominance – renders grassland communities more or less resistant (for example, legume presence reduced resistance; *Poa pratensis* presence enhanced it). Long-lived organs and low rates of nutrient turnover, in particular, appear to improve plant resistance. Within-plant trade-offs between these traits and low potential growth rate lead to a slow recovery after damage, and consequently to poor resilience (MacGillivray & Grime 1995).

Links between resistance or resilience to extremes and community properties, on the other hand, are still poorly understood. High species richness might increase the probability that a single – or a set of – drought/heat-adapted species is present in the community, ensuring sustained functioning and species persistence (Bengtsson 1998; Yachi & Loreau 1999; Nijs & Impens 2000a). Similarly, the presence of species critical to the resumption of activity following extreme stress might be more guaranteed in species-rich systems, and enhance resilience (Hooper *et al*. 2002). Both types of 'insurance' or buffering against environmental fluctuation would lead to more predictable aggregate properties of communities or ecosystems (Loreau *et al*. 2001). However, when Pfisterer & Schmid (2002) exposed the synthesized grassland plots at the Swiss site of the Biodepth experi--ment to prolonged drought, they observed decreased rather than increased resistance at greater plant diversity (resistance being expressed as absolute loss of biomass; diversity had no influence on relative loss). An alternative mechanism linking diversity to resistance could ensue from dominance of productive species in species-rich mixtures (sampling effect: Huston 1997; Tilman, Lehman & Thomson 1997). If inherent ecophysiological or morphogenetic traits make these fast growers more susceptible to extremes, then community resistance might decline concurrently. Yet another link may lie in the interspecific differences between plants, for example in rooting depth, phenology or physiological traits. Hooper (1998) suggested that interspecific differences would allow multispecies communities to utilize available resources more fully through complementarity. As stress can reduce availability of resources (such as water), greater complementarity might help species-rich communities cope better with extremes. Note, however, that Pfisterer & Schmid (2002) found indications for reduced niche complementarity under prolonged drought. Finally, studies on effects of species richness on resistance should also consider pathways that involve changes in stress physiology at the community or ecosystem scale (for example in canopy conductance, leaf and soil water potentials, synthesis of heat-shock proteins, etc.). For instance, if enhanced productivity in more species-rich grasslands (cf. Hector *et al*. 1999) increases canopy transpiration, then soil drying might occur more frequently, unless access to water is improved through spatial or temporal complementarity. To our knowledge, no studies have investigated the balance of these processes under normal rainfall regimes – let alone under extreme stress – and how individual species contribute to them.

A future climate with more extremes may exacerbate the global biodiversity crisis by enhancing local extinction of species, but the impact cannot be estimated reliably from the few available studies on past extremes in specific biomes. Moreover, risk estimates of diversity loss pertaining to enhanced extreme events will not be very informative unless we can also substantiate which species are being removed, rather than just how many, because species that are occasional or even rare today may thrive and take over the role of abundant species as the environment gradually changes (Chapin *et al*. 1998). The current study investigates effects of declining plant diversity on resistance to extremes by exposing synthesized grassland ecosystems of different species richness to a stress peak combining heat and drought. The heat wave was induced experimentally by infrared irradiation in free air conditions. Mortality, gap pattern formation and recovery dynamics of these communities were reported by Van Peer *et al*. (2001). Here we seek to disentangle the changes that occur in community structure, as species richness increases, from those that occur in ecosystem physiology. The following hypotheses were tested: (1) Are species-rich communities less sensitive to extremes (have higher survival) than species-poor communities? (2) Can differences in sensitivity be traced to changes in water consumption or water availability? (3) With increasing species richness, does species composition shift towards more conservative species with regard to transpiration, or towards species with higher survival regardless of water use? (4) Do effects of species richness on survival vary with species, and which type of species are most likely to be lost? (5) Can physiological stress indicators at the leaf scale (photosynthetic rate, chlorophyll fluorescence) explain mortality?

Materials and methods

EXPERIMENTAL SET-UP AND PLANT MATERIAL

Model ecosystems of different species richness (S) were synthesized from a species pool of eight predominantly perennial, temperate Gramineae: *Lolium perenne* L. cv. Paddock (Lp); *Festuca arundinacea* L. cv. Barcel (Fa); *Poa pratensis* L. cv. Julia (Pop); *Festuca rubra* L. cv. Ensylva (Fr); *Bromus catharticus* L. cv. Banco (Bc); *Dactylis glomerata* L. cv. Athos (Dg); *Phleum pratense* L. cv. Erecta (Php); and *Lolium multiflorum* L. cv. Meryl (Lm). All these species co-occur in European grasslands, although the Mediterranean species Bc is rarely found with Lp and Php (D.R., personal observation). We composed 24 different communities to create four S levels: eight monocultures

© 2004 British Ecological Society,

(Lp, Fa, Pop, Fr, Bc, Dg, Php, Lm); eight mixtures of two species (Lp–Fa, Pop–Fr, Bc–Dg, Php–Lm, Lp–Fr, Pop–Dg, Bc–Lm, Fa–Php); four mixtures of four species (Lp–Fa–Pop–Fr, Bc–Dg–Php–Lm, Lp–Fa–Php–Lm, Pop–Fr–Bc–Dg); and the mixture of eight species (Lp–Fa–Pop–Fr–Bc–Dg–Php–Lm) replicated four times with a different spatial arrangement. To separate effects of species richness from effects of species composition, we applied the following assemblage rules: (a) all species occur in equal proportions (whole experiment, every S level, within each community); (b) all different neighbourships occur in equal proportions (whole experiment, every S level, within each community); and (c) species mixtures at a given S level differ maximally (minimal number of species in common). Intraspecific neighbourships were excluded (no spatial aggregation). For criterion (b) we considered only nearest (orthogonal) neighbours; criterion (c) was aimed at maximal representation of all possible species interactions. The alternative technique of random drawing of species from a total pool (Naeem *et al*. 1996) was not used because, with a limited series of communities, equal representation of the species at every S is not guaranteed (for example, by chance, species Lm could be drawn much more frequently than species Fa).

The plants were sown in small pots placed outdoors between 15 and 29 April 1997. They were transplanted between 11 and 20 May to plastic containers (26·0 × 15·5 and 14·2 cm deep) also placed outdoors, filled with steam-sterilized and fertilized sandy loam. The soil surface was covered by a metal grid of 40 square cells (3·5 × 3·5 cm each), forming a matrix of eight rows by five columns with one plant per cell. Measurements on individual plants were taken only from the 18 core plants (6 × 3) of each container to minimize edge effects. The soil was kept close to field capacity by daily irrigation until a drought period was started by withholding water, coinciding with a simulated heat wave (5–18 August 1997, see below). Ten days before the heat wave, the stands were cut to have, at the start of exposure, leaf area index and evapotranspiration values intermediate to those of recently cut and fully regrown vegetation. Fertilizer was supplied after the cut (7·88 g N m^{-2} as NH_4NO_3; 2·49 g K m^{-2} as K_2O; 8·29 g P m^{-2} as P_2O_5). We exposed three replicate sets of the 24 different communities to the stress, and regularly switched container positions within each set. Due to time constraints, all data were collected on one set of 24 communities that was randomly drawn from the three replicate sets. Harvested above-ground biomass (>3·5 cm) was collected separately by species and by community, and was oven-dried for 48 h at 80 °C. Effects of S on biomass were partitioned in a complementarity and a selection effect, according to Loreau & Hector (2001).

SIMULATION OF HEAT WAVE

During the stress period, canopy temperature was increased by infrared irradiation in the absence of enclosure (Free Air Temperature Increase system, FATI; see Nijs *et al*. 1996a for description and performance). To create realistic temperature extremes we analysed daily maximum temperature (T_{max}) between 1968 and 1995 from the meteorological station at Ukkel, 45 km from our study site in Antwerp. Based on the frequencies of 20-day periods (anticipated duration of drought cycle) with average T_{max} above given thresholds, we aimed for a temperature increment of 8 °C, which increases the current long-term average T_{max} in August of 21 °C to 29 °C. The latter is close to the most extreme average T_{max} of all 20-day warm periods in the record (31 °C). Despite this target increment, the heat wave was ended at about 50% mortality in order to have sufficient survivors left for analysis.

Each of the three sets of 24 plant communities was irradiated by a separate FATI unit. The canopy temperature (T_c) of a fourth set of 24 communities, not subjected to the heat wave, was used as a baseline for heating (this set was not a control treatment; its sole purpose was to quantify the temperature extreme). FATI units consisted of a frame with six 1500 W infrared lamps, suspended 1·2 m above the ground, homogeneously irradiating a 1·2 × 1·2 m^2 area. On the unheated set a dummy construction was placed with empty lamp enclosures. During the experiment, type-T thermocouples (Stork Intermes, Antwerp, Belgium) measured abaxial leaf temperature (T_l at 5 cm height at five different locations); air temperature (same height but centre position only, shielded from direct sunlight); and soil temperature (centre position at 5 cm depth). Canopy temperature was measured with non-contact IR semiconductors with a 90° view angle placed 60 cm above the plots (Stork Intermes), providing average T_c for the 24 communities below the sensor. A data logger (DL3000/SA, Delta-T, Burwell, UK) registered the readings every 10 min, with all temperatures measured in the same two sets (the unheated and one of the heated sets). During the stress, average daily maximum T_l in the heated treatment was 5·0 ± SD 0·8 °C (n = 14) higher than in the unheated one, while average daily maximum T_c was increased 3·3 ± 0·9 °C (n = 14). Average instantaneous warming was 3·7 ± 0·2 and 3·3 ± 0·2 °C during the day (T_l and T_c, respectively, n = 756) *vs* 5·5 ± 0·1 and 6·0 ± 0·2 °C during the night (n = 1260). Higher night-time increments arise from stomatal closure, which reduces the fraction of the added infrared dissipated as latent heat in transpiration. Because a natural heat wave occurred during the stress period, ambient average maximum T_c in August exceeded 21 °C. Therefore an increment <8 °C was sufficient to increase maximum T_c to around 29 °C. In addition, the heating was switched off when ambient maximum T_c in the unheated communities exceeded 29 °C (7 out of 14 days), to avoid unrealistic stress in the heated communities. Daily maximum T_c realized during the stress was 28·6 ± 0·5 °C (n = 14).

During heating, irrigation was withheld and transparent polyethylene shelters above the vegetation

© 2004 British Ecological Society,

eliminated precipitation without obstructing direct solar radiation. Changes in gravimetric soil water content between the beginning and end of the stress period were calculated from the difference in container mass, and were expressed as water consumption as a fraction of the total soil water available in the containers at saturation.

PHOTOSYNTHESIS

Light-saturated net rates of leaf photosynthesis ($P_{n,max}$) were measured in the laboratory with an infrared gas analyser (LCA3, ADC, Hoddesdon, UK), at ambient atmospheric CO_2 concentration and a photosynthetic photon fluence rate (PPFR) of 1175 μmol m^{-2} s^{-1} over the waveband 400–700 nm (SOL 500/III sun-simulator, Hönle, Germany). Air temperature was 28·0 ± 0·7 °C and vapour pressure deficit of the air was between 0·8 and 1·1 kPa. Middle sections of youngest fully expanded leaves were used (each species in each community).

CHLOROPHYLL FLUORESCENCE

Extreme heating can damage plant tissues in general, and irreversibly disrupt chloroplast membranes in particular (White *et al*. 2000). Chlorophyll fluorescence measurements were taken in the laboratory with a chlorophyll fluorometer (PAM, Walz, Effeltrich, Germany) on days 1, 3, 7 and 9 of the stress period. Leaves were illuminated with a fibre-optic cable at a distance of 9 mm under a 60° angle, with modulated measuring light at a frequency of 600 Hz, switched to 20 kHz during saturation pulses (internal halogen lamp, PPFR 9600 μmol m^{-2} s^{-1} for 1·4 s). Light-adapted response was recorded after 5 min illumination (SOL 500/III lamp, Hönle) at 500 μmol m^{-2} s^{-1} in one leaf for each species in each community. Steady-state fluorescence (F_t) and maximal fluorescence after the saturation pulse ($F_{m'}$) were used to estimate the relative quantum efficiency of PSII photochemistry as $\phi = (F_{m'} - F_t)/F_{m'}$. Air temperature and vapour pressure deficit were similar to those during photosynthesis measurements.

SURVIVAL

To estimate the resistance of the stands (deviation from the condition prior to stress, Pimm 1984; in this case 100% live plants), we determined if individual plants survived regrowth after irrigation had been restored at the end of the heat wave. For a given *S* level, survival is expressed either by species (all model ecosystems in which a species occurs combined) or by community (average survival in all model ecosystems of that *S* level).

DATA ANALYSIS

Data were analysed statistically with one-way or two-way ANOVA to account for *S* and/or species effects, with analysis of covariance (ANCOVA) to account for effects of water use, or with linear or non-linear regression.

Changes in the species' biomass owing to growth in mixture were calculated as:

$$(B_{mix,j} - B_{mono,j})/B_{mono,j} \qquad \text{eqn 1}$$

with $B_{mix,j}$ and $B_{mono,j}$ the observed biomasses per plant of species *j* in mixture and in monoculture, respectively. We also calculated the extent to which the actual water use of a mixture deviated from the water use that could be expected based on the biomass and the intrinsic (monoculture) water use of the individual species of that mixture:

$$(W_{o,i} - W_{e,i})/W_{e,i} \qquad \text{eqn 2}$$

with $W_{o,i}$ the observed and $W_{e,i}$ the expected water use of mixture i. The expected $W_{e,i}$ was calculated from the monoculture water use of the species composing the mixture, weighted by their biomass:

$$\Sigma_j[(W_{mono,j}/B_{mono,j}) \times B_{mix,j}] \qquad \text{eqn 3}$$

with $W_{mono,j}$ the observed water use of species *j* in monoculture. If the water consumption of a mixture is explained entirely by its biomass composition and the intrinsic water use of each species, then $(W_{o,i} - W_{e,i})/W_{e,i}$ equals zero.

Evenness of the survival proportions (*P*) of the species within a community was quantified as $G' = 1 - G$, with *G* the Gini index (Weiner & Solbrig 1984):

$$G = \frac{\sum_{i=1}^{S}\sum_{j=1}^{S}|P_i - P_j|}{2S^2\bar{P}}. \qquad \text{eqn 4}$$

The G' evenness index varies between 0 (minimal evenness) and 1 (maximal evenness).

Results

Before the stress, average above-ground biomass was significantly greater in the more species-rich communities (Fig. 1a: linear regression of biomass per plant on *S*, all species in all communities combined, $F_{1,70} = 19·12$, $P < 0·0001$, $r^2 = 0·21$). Also, the range of the species' productivities increased with *S* (Fig. 1a), reflecting increased within-community compositional unevenness. Expressed per unit ground area, average above-ground biomass was increased from 260 (monocultures) to 465 g m^{-2} (eight-species mixtures). Greater productivity coincided with slightly more tillers per plant (11·2 at $S = 8$ *vs* 9·1 at $S = 1$), but this effect was not significant (ANOVA, $F_{3,57} = 0·50$, $P = 0·684$). Rather than promoting dominance of productive species, *S* alleviated particularly the suppression of slower growers (Fig. 1b). This was confirmed by a small negative selection effect, which was outweighed by a much larger positive complementarity effect (Fig. 2:

© 2004 British Ecological Society,

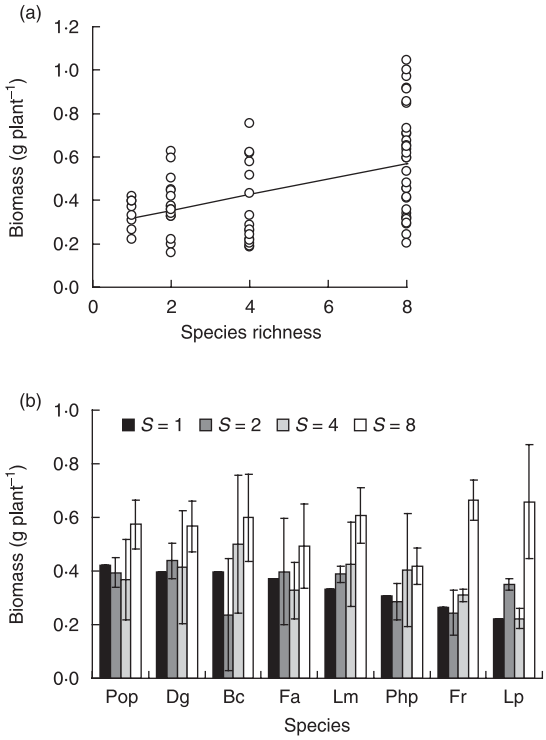

Fig. 1. (a) Above-ground biomass per plant before the onset of stress as a function of species richness (S). Each symbol represents a different species in a different mixture. (b) Average above-ground biomass per plant ±1 SE, by species and by S. Species were ranked according to productivity in monoculture ($S = 1$). Lp, *Lolium perenne*; Fa, *Festuca arundinacea*; Pop, *Poa pratensis*; Fr, *Festuca rubra*; Bc, *Bromus catharticus*; Dg, *Dactylis glomerata*; Php, *Phleum pratense*; Lm, *Lolium multiflorum*.

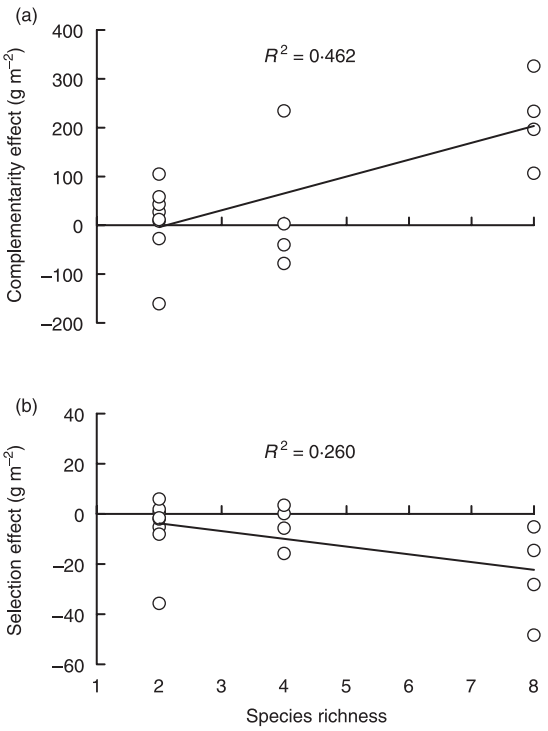

Fig. 2. Partitioning of biomass increase with species richness into (a) complementarity and (b) selection effects. Each symbol represents a different community.

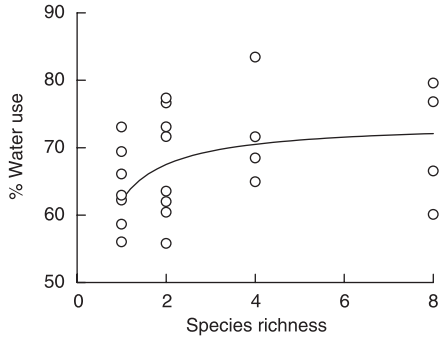

Fig. 3. Water consumption (Y) as a fraction of the total soil water available at saturation, recorded at the end of the stress period, as a function of species richness (S). Each symbol represents a different species mixture. The curve was fitted with $Y = (abS)/(aS + b)$.

linear regression, $F_{1,14} = 11{\cdot}999$ and $P = 0{\cdot}004$ for complementarity, $F_{1,14} = 4{\cdot}930$ and $P = 0{\cdot}043$ for selection). The sum of selection and complementarity match the aforementioned absolute increase in community biomass of 205 g m^{-2} (465–260 g m^{-2}).

By the end of the stress, the species-rich communities had consumed significantly more water than their species-poor counterparts (Fig. 3: non-linear regression of proportional water use on S, $F_{2,22} = 882{\cdot}40$, $P < 0{\cdot}0001$, $r^2 = 0{\cdot}20$). This might arise from (i) the greater biomass; (ii) a greater representation in the community of species that intrinsically use more water; or (iii) other influences of S on evapotranspiration (for example, changes in stomatal density or conductance, changes in canopy light profile, etc.). Hypothesis (i) was tested by regressing proportional water use on community biomass (all S levels combined). No significant fraction of the variance in water use could be explained by biomass ($F_{1,22} = 1{\cdot}304$, $P = 0{\cdot}266$), although S significantly increased both biomass and water use. Lack of significance might be due to water use saturating at low S, while biomass continued to increase. Hypothesis (ii) requires that the species vary in intrinsic (or potential) water use to begin with, which is evident from Fig. 4 (monocultures). During the heat wave the most 'water-wasting' species, *Lolium perenne*, consumed 51·2% more water than the most conservative species, *Lolium multiflorum*. Moreover, by the end of the stress evapotranspiration had almost stopped in some species (Fig. 4b), while others continued to draw water from the soil (Fig. 4a). To test hypothesis (ii), we investigated whether the changes that occurred in the species' biomass owing to growth in mixture, as calculated with equation 1, were related to the intrinsic (monoculture) water consumption of these species. No significant relationship was found [ANCOVA of $(B_{mix,j} - B_{mono,j})/B_{mono,j}$ with S as fixed factor and water use as covariate; $F_{1,60} = 0{\cdot}140$, $P = 0{\cdot}710$ for water use]. In other words, before stress the biomass of the 'water-wasting' and more conservative species was influenced in the same way by S, so neither type of species was favoured. The S effect in the above analysis was

© 2004 British Ecological Society,

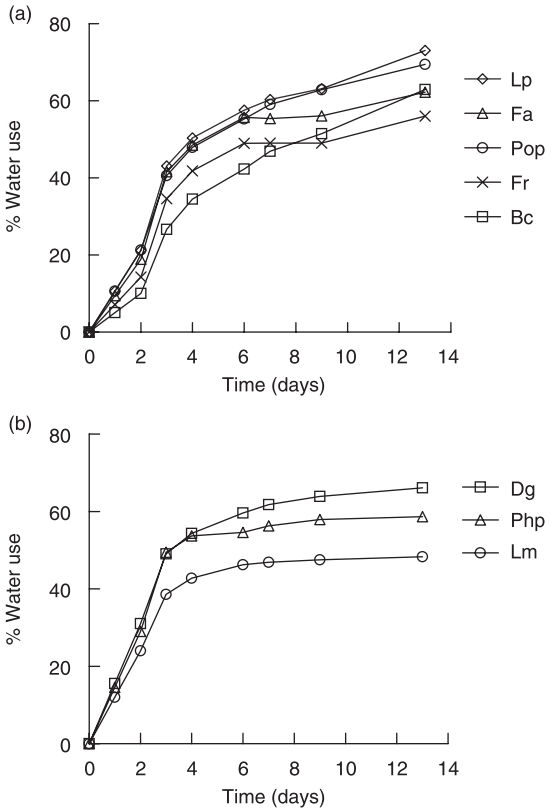

Fig. 4. Water consumption as a fraction of total soil water available at saturation in the monocultures as a function of time after onset of stress. Each symbol represents the water use of a different species. (a) Species still consuming water by the end of the stress; (b) species no longer consuming water. See Fig. 1 for species binomials.

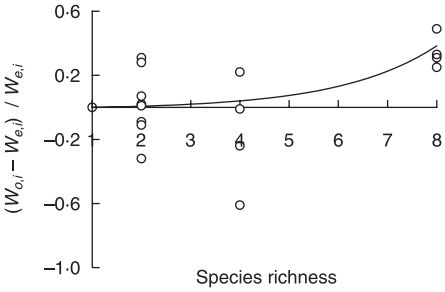

Fig. 5. Deviation from expected water use in the mixtures (Y) expressed as a function of species richness (S). Deviations were calculated as equation 2; expected $W_{e,i}$ was calculated as equation 3. Each symbol represents a different species mixture. The curve was fitted with $Y = a + be^{cS}$.

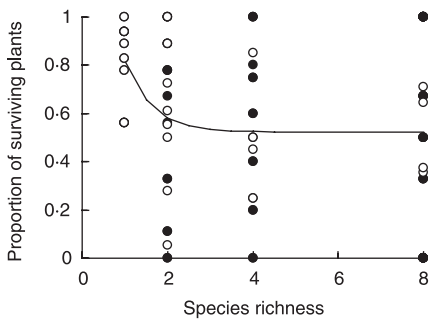

Fig. 6. Proportion of surviving plants (Y) at the end of the heat wave as a function of species richness (S). Closed symbols represent survival by species, open symbols by community. Curve fitted with $Y = a + be^{-cS}$.

significant and positive ($F_{2,60} = 7{\cdot}192$, $P = 0{\cdot}002$). As the covariate effect (water use) was not, this merely reflects that the relative biomass enhancement, owing to growing in the mixture, increased with S, in agreement with Fig. 1. Hypothesis (iii) was tested by calculating for each mixture the deviation of its actual water use from the water use that could be expected based on the biomass and the intrinsic (monoculture) water use of the individual species of that mixture (equation 2). In our synthetic communities, this ratio increased significantly with S (non-linear regression, $F_{3,14} = 3{\cdot}66$, $P = 0{\cdot}039$, $r^2 = 0{\cdot}34$), reaching +0·39 at $S = 8$ (Fig. 5). Plant species richness thus modified the intrinsic water consumption of the species, in support of hypothesis (iii).

In keeping with the greater water use, the more species-rich mixtures exhibited reduced survival (significant non-linear regression of proportion surviving plants on S, $F_{3,42} = 14{\cdot}30$, $P < 0{\cdot}0001$, $r^2 = 0{\cdot}11$; Fig. 6). Nevertheless, the regression of survival on community biomass, all S levels combined, was not significant ($F_{1,22} = 1{\cdot}488$, $P = 0{\cdot}235$), possibly owing to survival declining asymptotically already at low S, whereas biomass continued to increase linearly up to $S = 8$. Note that, contrary to the mixtures, none of the monocultures had survival below 0·50. In an ANCOVA of survival with S as fixed factor and water use as covariate, differences between S levels could not be explained by water use ($F_{1,19} = 0{\cdot}057$, $P = 0{\cdot}814$), nor were there S effects additional to the covariate ($F_{3,19} = 1{\cdot}511$, $P = 0{\cdot}244$), probably because variation of survival with S was limited to $S < 2$ (Fig. 6). A two-way ANOVA of survival with S and species as fixed factors yielded a significant and negative single-factor effect of S ($F_{3,42} = 14{\cdot}04$, $P < 0{\cdot}0001$), but no significant S–species interaction ($F_{1,39} = 0{\cdot}35$, $P = 0{\cdot}558$), indicating that S reduced survival to a similar absolute extent in all species (Fig. 7a). This suggests that (i) increases in S drive some species closer to local extinction than others, in particular those already strongly affected by the extreme in monoculture (e.g. *Festuca rubra*); and (ii) more species are threatened compared with the monocultures. The latter is also expressed in the more uneven survival proportions of the species observed in the more diverse communities (significant non-linear regression of the G' evenness index on S, $F_{3,13} = 156{\cdot}71$, $P < 0{\cdot}0001$, $r^2 = 0{\cdot}08$; Fig. 7b).

Although reduced survival in the more species-rich systems coincided with greater water use, this does not exclude other causes of mortality from having been involved. Changes in biomass composition with S before stress was imposed may have affected survival if these changes favoured either intrinsically stress-sensitive or insensitive species (regardless of water use). For every species in the eight-species mixtures, we therefore plotted the change in biomass relative to its

© 2004 British Ecological Society,

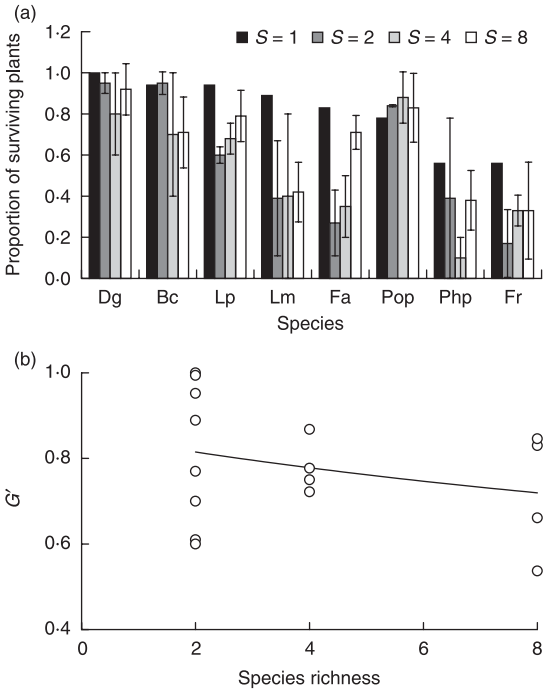

Fig. 7. (a) Mean ± 1 SE of proportion of surviving plants at the end of the heat wave for each species at each diversity level. Species ranked according to survival in monoculture (see Fig. 1 for species binomials). (b) G', evenness index of survival proportions of species within a community as a function of species richness (S). Curve fitted with $G' = a + be^{-cS}$.

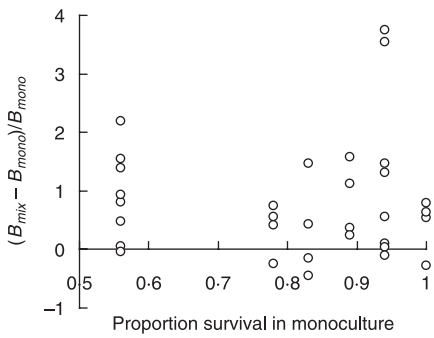

Fig. 8. Change in biomass per plant in the eight-species mixtures relative to the monocultures, calculated using equation 1, as a function of the proportion of survivors in monoculture (sensitivity to stress). Each symbol represents a different species in a different mixture.

monoculture (equation 1) against the proportion of survivors in that monoculture, a proxy for stress sensitivity (Fig. 8). Biomass responses to S before the stress were not related to survival proportion [ANOVA of $(B_{mix,j} - B_{mono,j})/B_{mono,j}$, $F_{5,58} = 0{\cdot}91$, $P = 0{\cdot}481$]. Increasing S therefore favoured stress-sensitive and stress-insensitive species alike.

Finally, we examined whether survival at community scale could be explained by physiological stress indicators in individual leaves. Surprisingly, initial light-saturated net rate of leaf photosynthesis ($P_{n,max}$) sampled before the stress was, on average, 12% greater in the most diverse mixtures compared with the monocultures

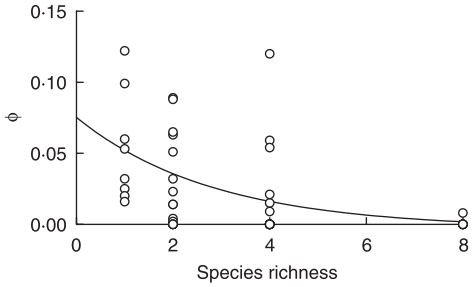

Fig. 9. Photochemical efficiency of PSII photochemistry (ϕ) at the end of the stress as a function of species richness (S). Each symbol represents a different species in a different mixture. Curve fitted with $\phi = a + be^{-cS}$.

(ANOVA of $P_{n,max}$ with fixed factor S, $F_{3,70} = 3{\cdot}46$, $P = 0{\cdot}021$). Following the onset of the heat wave, $P_{n,max}$ declined exponentially in all species, reaching zero within 5 days (not shown). Net photosynthetic rate was thus not an adequate measure for detecting influences of S on stress physiology. By contrast, ϕ values were still positive by the end of the heat wave, at which time they decreased exponentially with S (non-linear regression, $F_{3,45} = 10{\cdot}65$, $P < 0{\cdot}0001$, $r^2 = 0{\cdot}17$; Fig. 9). However, a plot of survival against ϕ at the end of the heat wave, all diversity levels combined, revealed no significant patterns (not shown), so the predictability of survival from this biophysical stress measure was poor. In fact, among the species with zero ϕ, several even survived well.

Discussion

The outcome of this heat-shock experiment corroborates the suggestion that ecosystem resistance of grasslands under severe climatic stress – measured as survival – declines with increasing species richness. Pfisterer & Schmid (2002) observed similar results for resistance to biomass loss, in synthesized grasslands exposed to a prolonged 8-week drought. Both studies point to diversity-dependent production underlying the response, but the proposed mechanisms differ. Pfisterer & Schmid (2002) argued that, because more species-rich systems have a greater chance of including species growing well under unperturbed conditions, they may also have a greater chance of losing this greater growth potential under drought (which they refer to as the 'opposite insurance hypothesis'). This would explain why productive systems suffer more from perturbation than those that are less productive. In our model grasslands, which faced a short but potentially lethal stress peak, loss of resistance (survival) at greater species richness probably originated from diversity-dependent water consumption (greater water use in the more diverse mixtures). Both mechanisms – although operating at different timescales – point to a trade-off between production and stability.

In contrast to these findings, earlier studies on less manipulated grasslands have associated greater plant

© 2004 British Ecological Society,

diversity with improved resistance to drought (Frank & McNaughton 1991; Tilman & Downing 1994; Tilman 1996), but the experimental designs used in those studies have been questioned (Givnish 1994; Huston 1997). Bias might arise from confounding resource availability with species richness (for example, if richness differences originate from differences in soil fertility), or species composition with species richness (for example, if richness is characterized by different species, i.e. species replacement occurs), both of which were avoided here by varying *S* as an independent factor in model ecosystems.

Some studies have used artificial assemblages of microbial communities in microcosms. These experiments have demonstrated enhanced predictability of respiration (McGrady-Steed *et al*. 1997), greater community biomass (Naeem & Li 1997), and more resistance to species loss (Petchey *et al*. 1999) when species richness was increased. Discrepancies with our experiment may be because the microcosms in those studies were not exposed to extremes, but were either left undisturbed or exposed to only moderate warming, such that stochastic rather than deterministic processes governed the response. Moreover, the mechanism of diversity-dependent transpiration that was responsible for reduced survival in our grassland systems obviously cannot operate in (aquatic) microcosms. In one study with grassland microcosms (Wardle *et al*. 2000), effects of plant diversity on resistance were idiosyncratic, reflecting the complex and varied nature of the effects of the component species of the community on ecosystem stability. Possibly the use of functional group richness, rather than of species richness, was responsible for the contrast with the negative effects of richness on resistance in the current experiment and in the study by Pfisterer & Schmid (2002).

Despite the knowledge gained from experiments with controlled assemblages, some ecosystem features are less realistically mimicked by this technique, for example age structure of populations (too homogeneous); heterogeneity of the physical environment (too uniform); 'history' of the experimental objects (too young); and species evenness (maximized). Setting these features to fixed levels keeps the experiment manageable, but complicates the extrapolation of results. Furthermore, maximizing evenness (same population size for all species) implies that stochastic processes (linked to population size) are not addressed, whereas they are often central in single-population extinction models (Soulé 1987). Even so, synthetic assemblage remains one of the few tools for isolating 'pure' influences of diversity from influences of its extrinsic determinants, such as disturbance regime or site history. Consequently, to interpret diversity-resistance trends in natural or less controlled systems, insights from studies such as ours cannot stand alone, but need to be combined with knowledge on how resistance is affected by factors that covary with diversity.

By the end of the heat wave in our experiment, the more species-rich communities had consumed more soil water and exhibited more severe stress in both soil and plant (lower soil water content and ϕ), which coincided with increased plant mortality. The fact that biomass was increased at the same time suggests that mortality increases were mediated by enhanced leaf area before the stress. Although species-rich communities have a greater probability of including potentially fast-growing species, the species that were least productive in monoculture were promoted most at the higher richness levels (Fig. 1b). A negative sampling effect (Fig. 2b) confirms that biomass was not increased by dominance of productive species, as proposed by Huston (1997) and Tilman *et al*. (1997). One property that could – theoretically – make less productive species more responsive to *S* than more productive species, is faster resource uptake rate (for example faster specific uptake rate of nitrate or ammonium by roots), provided this is outweighed by lower resource-use efficiency in the same species (neither was measured here; see model of Nijs & Impens 2000b). Koch *et al*. (1991) reported a fivefold range in root specific uptake rate of nitrogen across species. According to the aforementioned model, higher specific uptake rates would allow slow growers to absorb more nutrients per plant when grown in a mixture than they would normally do in monoculture, at the expense of species with lower specific uptake rates. In monoculture these higher uptake rates would remain unexpressed because interspecific root competition is absent, causing productivity simply to mirror resource-use efficiency (assumed to be smaller in the slow growers under this hypothesis). Although such a mechanism would be consistent with the biomass shift that we observed, *S* also promoted the grasses that were productive in monoculture, so resources did not simply shift from fast to slow growers, but more total resource became available in agreement with the complementarity hypothesis (Hooper & Vitousek 1998; Figure 2a). This is consistent with leaf net photosynthesis being greater at high *S* before the heat wave, as the latter suggests enhanced availability of nitrogen during the exponential growth phase shortly after cutting (leaf photosynthetic capacity being closely coupled with nitrogen content). Positive interactions between species could produce results similar to complementarity, and their role in diversity effects has been demonstrated in grassland (Hooper & Dukes 2004) as well as in bryophyte communities (Mulder *et al*. 2001). In vascular plants, taller species protecting shade-tolerant neighbours of smaller stature against photoinhibition could be such a facilitation pathway.

As well as the changes originating from species composition, *S* enhanced water use by more subtle (physiological or structural) means (cf. observed water use exceeding expected water use at higher *S* in Fig. 4). Increased stomatal conductance might be responsible for this, given that the more species-rich systems had access to more water (saturating curve in Fig. 3). Figure 1(b) suggests that this greater water availability might be ascribed, at least partly, to species capable of

© 2004 British Ecological Society,

extracting more water from the soil in monoculture, such as *Lolium perenne* (Fig. 4a), which tripled its biomass from $S = 1$ to $S = 8$ (before the stress, so not in response to the stress; but note this is based on $n = 1$ in monoculture). However, before the stress, biomass was also increased in species with poor water extraction potential in monoculture such as *Lolium multiflorum* (Fig. 4b), and no general relationship was found for all species between monoculture water use and biomass change from monoculture to mixture. Moreover, during the stress, mean water use in the most diverse mixtures (72·1%, Fig. 3) exceeded the consumption in seven of eight monocultures (Fig. 4), indicating that water use was enhanced by complementarity rather than by dominance of species consuming a lot of water. The data thus support the hypothesis that plant diversity also enhances resource acquisition under stress, as observed by Pfisterer & Schmid (2002) in grasslands during drought. Nevertheless, water supply in the more species-rich systems was enhanced less than demand, because leaves were more stressed at higher S, as shown by chlorophyll fluorescence; this imbalance probably led to the observed greater mortality. We conclude that complementarity in resource use for biomass production had a cost in terms of reduced survival under stress, despite the likelihood of complementarity in water acquisition during exposure. This may add to the already relatively high inherent sensitivity to climate warming observed in perennial grasses (Grime *et al*. 2000). Note that S effects on water use saturated earlier than on biomass, suggesting weaker complementarity for water uptake than for productivity.

The lower survival of our grasses at higher S does not support the 'insurance hypothesis' that more species leads to greater diversity in species' tolerances to different types of environmental fluctuations (Walker, Kinzig & Langridge 1999; Yachi & Loreau 1999). However, we used only one type of extreme, so a potentially wider diversity of tolerances may have remained unexpressed. If communities are exposed to a single type of extreme, it is important to know whether the changes in species relative abundance that occur with increases in S (for example through competition or facilitation) select for species with either low or high susceptibility to that extreme, or do not select at all. 'Selection' suggests that the biomass proportions of either class of species increase more than do those of the other. This could occur if morphogenetic or physiological traits important for competitive success in mixture are inversely related to stress susceptibility. Growth rate or ultimate stature could qualify as two such traits. However, before the heat wave there was no selection for inherent (monoculture) sensitivity to stress in the current experiment, so increased species richness did not make the model ecosystems more vulnerable by this means (although exposure to stress can itself shift community composition to favour more resistant species, e.g. from C_3 to C_4 species; White *et al*. 2000). Furthermore, compositional resistance in our stands was maximal, as none of the species was completely lost within the timeframe of the stress event. Influences of S on susceptibility to lose species (rather than individuals) therefore cannot be estimated directly. However, evenness of the survival proportions of the species within the communities decreased with S. As, at the same time, more individuals were lost, on average, the risk of local extinction of species had become greater. In contrast to this, Sankaran & McNaughton (1999) observed that compositional stability between years was greater in more diverse than in less diverse natural savanna grasslands exposed to experimental perturbations. The disparity with our experiment could be because we used survival as the resistance measure. Survival expresses short-term changes following a stress peak, whereas resistance to species turnover between years, as used by Sankaran & McNaughton (1999), combines resistance in the sense of immediate change with processes across longer timescales, for example slow local extinction of stress-sensitive species after they have been affected by a stress peak, and subsequent recolonization of vegetation gaps. Timescale and type of stability (cf. Pimm 1984) are clearly critical factors in comparisons between studies.

Despite the generally good relationship between ϕ and plant stress (Srinivasan, Takeda & Senboku 1996), ϕ was not an adequate predictor of species or of community survival. Apparently, basal meristems of grasses can often still regenerate when the adult leaves (in which ϕ was measured) are dead. Possibly cellular-morphological techniques applied to the meristematic zone are more useful to characterize the stress intensities and exposure times at which individual grass plants or tillers effectively die. Alternatively, statistical inference could be used to investigate which plant traits are coupled specifically with resistance to lethal stress (cf. Fernandez, Wang & Reynolds 2002; Olyslaegers *et al*. 2002).

In the current climate, the smaller population sizes of many infrequent or rare species already make diverse systems more vulnerable to extremes for stochastic reasons (lower buffering to environmental fluctuation). The question arises as to whether the mechanisms that led to higher susceptibility to extremes in our synthesized, more species-rich grasslands also operate within ecosystems in general, which would add to this vulnerability.

Acknowledgements

This research was supported by the Federal Office for Scientific, Technical and Cultural Affairs (Prime Minister's Office, Brussels, Belgium), under the programme Global Change, Ecosystems and Biodiversity.

References

Bengtsson, J. (1998) Which species? What kind of diversity? Which ecosystem function? Some problems in studies of relations between biodiversity and ecosystem function. *Applied Soil Ecology* **10**, 191–199.

© 2004 British Ecological Society,

Buckland, S.M., Grime, J.P., Hodgson, J.G. & Thompson, K. (1997) A comparison of plant responses to the extreme drought of 1995 in northern England. *Journal of Ecology* **85**, 875–882.

Chapin, F.S., III, Sala, O.E., Burke, I.C. *et al.* (1998) Ecosystem consequences of changing biodiversity. Experimental evidence and a research agenda for the future. *Bioscience* **48**, 45–52.

Díaz, S. (2001) Terrestrial communities: ecosystem measurement. *Encyclopedia of Biodiversity*, Vol. 2 (ed. S.A. Levin), pp. 321–344. Academic Press, London.

Fernandez, R.J., Wang, M. & Reynolds, J.F. (2002) Do morphological changes mediate plant responses to water stress? A steady-state experiment with two C_4 grasses. *New Phytologist* **155**, 79–88.

Frank, D.A. & McNaughton, S.J. (1991) Stability increases with diversity in plant communities: empirical evidence from the 1998 Yellowstone drought. *Oikos* **62**, 360–362.

Givnish, T.J. (1994) Does diversity beget stability? *Nature* **371**, 113–114.

Grime, J.P., Brown, V.K., Thompson, K. *et al.* (2000) The response of two contrasting limestone grasslands to simulated climate change. *Science* **289**, 762–765.

Hector, A., Schmid, B., Beierkuhnlein, C. *et al.* (1999) Plant diversity and productivity experiments in European grasslands. *Science* **286**, 1123–1127.

Hooper, D.U. (1998) The role of complementarity and competition in ecosystem responses to variation in plant diversity. *Ecology* **79**, 704–719.

Hooper, D.U. & Dukes, J.S. (2004) Overyielding among plant functional groups in a long-term experiment. *Ecology Letters* **7**, 95–105.

Hooper, D.U. & Vitousek, P.M. (1998) Effects of plant composition on nutrient cycling. *Ecological Monographs* **68**, 121–149.

Hooper, D.U., Solan, M., Symstad, A. *et al.* (2002) Species diversity, functional diversity and ecosystem functioning. *Biodiversity and Ecosystem Functioning: Synthesis and Perspectives* (eds M. Loreau, S. Naeem & P. Inchausti), pp. 195–208. Oxford University Press, Oxford, UK.

Howarth, C.J. (1991) Molecular responses of plants to an increased incidence of heat shock. *Plant, Cell & Environment* **14**, 831–841.

Huston, M.A. (1997) Hidden treatments in ecological experiments: re-evaluating the ecosystem function of biodiversity. *Oecologia* **110**, 449–460.

Koch, G.W., Bloom, A.J. & Chapin, F.S., III (1991) Ammonium and nitrate as nitrogen sources in two *Eriophorum* species. *Oecologia* **88**, 570–573.

Loreau, M., Naeem, S., Inchausti, P. *et al.* (2001) Biodiversity and ecosystem functioning: current knowledge and future challenges. *Science* **294**, 804–808.

MacGillivray, C.W., Grime, J.P. & the Integrated Screening Programme team (1995) Testing predictions of the resistance and resilience of vegetation subjected to extreme events. *Functional Ecology* **9**, 640–649.

McCarthy, J.J., Canziani, O.F., Leary, N.A., Dokken, D.J. & White, K.S. (2001) *Climate Change 2001: Impacts, Adaptation and Vulnerability*. Cambridge University Press, Cambridge, UK.

McGrady-Steed, J., Harris, P.M. & Morin, P.J. (1997) Biodiversity regulates ecosystem predictability. *Nature* **390**, 162–165.

Mulder, C.P.H., Uliassi, D.D. & Doak, D.F. (2001) Physical stess and diversity-productivity relationships: the role of positive interactions. *Proceedings of the National Academy of Sciences USA* **98**, 6704–6708.

Naeem, S. & Li, S. (1997) Biodiversity enhances ecosystem reliability. *Nature* **390**, 507–509.

Naeem, S., Hakansson, K., Lawton, J.H., Crawley, M.J. & Thompson, J. (1996) Biodiversity and plant productivity in a model assemblage of plant species. *Oikos* **76**, 259–264.

Nijs, I. & Impens, I. (2000a) Biological diversity and probability of local extinction of ecosystems. *Functional Ecology* **14**, 46–54.

Nijs, I. & Impens, I. (2000b) Underlying effects of resource use efficiency in diversity–productivity relationships. *Oikos* **91**, 204–208.

Nijs, I., Kockelbergh, F., Teughels, H., Blum, H., Hendrey, G. & Impens, I. (1996a) Free Air Temperature Increase (FATI): a new tool to study global warming effects on plants in the field. *Plant, Cell & Environment* **19**, 495–502.

Nijs, I., Teughels, H., Blum, H., Hendrey, G. & Impens, I. (1996b) Simulation of climate change with infrared heaters reduces the productivity of *Lolium perenne* in summer. *Environmental and Experimental Botany* **36**, 271–280.

Olyslaegers, G., Nijs, I., Roebben, J. *et al.* (2002) Morphological and physiological indicators of tolerance to atmospheric stress in two sensitive and two tolerant tea clones in South Africa. *Experimental Agriculture* **38**, 397–410.

Petchey, O.L., McPhearson, P.T., Casey, T.M. & Morin, P.J. (1999) Environmental warming alters food-web structure and ecosystem function. *Nature* **402**, 69–72.

Pfisterer, A.B. & Schmid, B. (2002) Diversity-dependent production can decrease the stability of ecosystem functioning. *Nature* **416**, 84–86.

Pimm, S.L. (1984) The complexity and stability of ecosystems. *Nature* **307**, 321–326.

Sankara, M. & McNaughton, S.J. (1999) Determinants of biodiversity regulate compositional stability of communities. *Nature* **401**, 691–693.

Soulé, M.E. (1987) *Viable Populations for Conservation*. Cambridge University Press, Cambridge, UK.

Srinivasan, A., Takeda, H. & Senboku, T. (1996) Heat tolerance in food legumes as evaluated by cell membrane thermostability and chlorophyll fluorescence techniques. *Euphytica* **88**, 35–45.

Tilman, D. (1996) Biodiversity: population vs. ecosystem stability. *Ecology* **72**, 350–363.

Tilman, D. & Downing, J.A. (1994) Biodiversity and stability in grasslands. *Nature* **367**, 363–365.

Tilman, D. & El Haddi, A. (1992) Drought and biodiversity in grasslands. *Oecologia* **89**, 257–264.

Tilman, D., Lehman, C.L. & Thomson, K.T. (1997) Plant diversity and ecosystem productivity: theoretical considerations. *Proceedings of the National Academy of Sciences, USA* **94**, 1857–1861.

Van Peer, L., Nijs, I., Bogaert, J., Verelst, I. & Reheul, D. (2001) Survival, gap formation, and recovery dynamics in grassland ecosystems exposed to heat extremes: the role of species richness. *Ecosystems* **4**, 797–806.

Virginia, R.A. & Wall, D.H. (2001) Principles of ecosystem function. *Encyclopedia of Biodiversity*, Vol. 2 (ed. S.A. Levin), pp. 345–352. Academic Press, London.

Walker, B., Kinzig, A. & Langridge, J. (1999) Plant attribute diversity, resilience, and ecosystem function: the nature and significance of dominant and minor species. *Ecosystems* **2**, 95–113.

Wardle, D.A., Bonner, K.I. & Barker, G.M. (2000) Stability of ecosystem properties in response to above-ground functional group richness and composition. *Oikos* **89**, 11–23.

Weiner, J. & Solbrig, O.T. (1984) The meaning and measurement of size hierarchies in plant populations. *Oecologia* **61**, 334–336.

White, T.A., Campbell, B.D., Kemp, P.D. & Hunt, C.L. (2000) Sensitivity of three grassland communities to simulated extreme temperature and rainfall events. *Global Change Biology* **6**, 671–684.

Yachi, S. & Loreau, M. (1999) Biodiversity and ecosystem productivity in a fluctuating environment: the insurance hypothesis. *Proceedings of the National Academy of Sciences, USA* **96**, 1463–1468.

Received 5 February 2004; revised 10 May 2004; accepted 17 May 2004

© 2004 British Ecological Society,