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Biogeosciences

Effects of climate warming and declining species richness in grassland model ecosystems: acclimation of CO_2 fluxes

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Abstract. To study the effects of warming and declining species richness on the carbon balance of grassland communities, model ecosystems containing one, three or nine species were exposed to ambient and elevated (ambient +3°C) air temperature. In this paper, we analyze measured ecosystem CO_2 fluxes to test whether ecosystem photosynthesis and respiration had acclimated to warming after 28 months of continuous heating, and whether the degree of acclimation depended on species richness. In order to test whether acclimation occurred, short term temperature response curves were established for all communities in both treatments. At similar temperatures, lower flux rates in the heated communities as compared to the unheated communities would indicate thermal acclimation. Because plant cover was significantly higher in the heated treatment, we normalized the data for plant cover. Subsequently, down-regulation of both photosynthesis and respiration was observed. Although CO_2 fluxes were larger in communities with higher species richness, species richness did not affect the degree of acclimation to warming. These results imply that models need to take thermal acclimation into account to simulate photosynthesis and respiration in a warmer world.

1 Introduction

Both photosynthesis and respiration are known to increase with temperature, albeit not necessarily to the same degree, until an optimum temperature is reached (Saxe et al., 2001; Larcher, 2003). Because rising global temperatures could influence the global carbon balance, it is important to study to what extent future climate will affect the carbon balance of terrestrial ecosystems.

Although photosynthetic and respiratory rates increase with temperature (Larcher, 2003), both processes and their resulting CO_2 fluxes may also acclimate to warming conditions (Rook, 1969; Körner and Larcher, 1988; Bryla et al., 1997, 2001; Atkin et al., 2000a, 2006; Atkin and Tjoelker, 2003; King et al., 2006). Acclimation can be defined as the adjustment of processes such that plant performance is adapted to the new growth temperature (Lambers et al., 1998). Acclimation could mitigate, offset, or even enhance the predicted increases in photosynthesis and respiration. According to Atkin and Tjoelker (2003), acclimation of respiration can be associated with temperature-mediated changes in respiratory capacity, availability of substrates, and/or the demand for respiratory energy. Heterotrophic respiration (R_h) is reported to acclimate to higher temperatures as well (Luo et al., 2001), which is most likely due to depletion of labile soil organic carbon pools (Melillo et al., 2002; Kirschbaum, 2004; Eliasson et al., 2005). Acclimation of photosynthesis, on the other hand, might be due to inactivation of Rubisco and/or leakiness of photosynthetic membranes (Sharkey, 2005). Photosynthetic acclimation also can result from either a shift in optimum temperature or an adjustment of photosynthetic rates at all temperatures (Berry and Björkman, 1980; Saxe et al., 2001).

Global change comprises several factors beyond climate warming, among which the loss of biodiversity is key. The functional importance of biodiversity is reported in numerous studies, describing for example reductions in biomass resulting from declining species richness (e.g. Hector et al., 1999; Van Ruijven and Berendse, 2005). Three mechanisms might explain how diversity influences productivity in plant communities: (a) complementarity, (b) facilitation, and (c) the sampling effect (Fridley, 2001).

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1. Complementarity implies the variation in location, time and type of resource utilization by different species. This can result in reduced competition and more complete exploitation of resources in more species-rich communities (Fridley, 2001).

2. Vandermeer (1989) defined facilitation as the circumstance where a species modifies its environment in a way favorable to co-occuring species. Examples of facilitative mechanisms are: nitrogen enrichment by N-fixers, water sharing via hydraulic lift (Caldwell et al., 1998), and nutrient sharing via mycorrhizal networks (Read, 1997).

3. The sampling effect can be separated into the greater likelihood of selecting a species (i) better adapted to the particular site conditions, and (ii) of higher potential growth rate or larger mature size (Fridley, 2001).

In accordance with these mechanisms, declining species richness may result in reduced plant productivity (Tillman et al., 1996; Symstad et al., 1998), and subsequently in smaller CO_2 fluxes. In particular, functional group diversity is assumed to be an important factor (Sphen et al., 2000; Roy, 2001), because between-functional-group differences are larger than within-functional-group differences, and thus complementarity, facilitation and sampling effects will be more pronounced in communities with higher functional diversity (Sphen et al., 2000).

Because the effects of different global change factors are not simply additive, it is important to know to what extent factors such as climate warming and biodiversity loss may interact. However, to our knowledge, experiments investigating interactions between these two types of global change have not been performed before. In this paper we present results from a long-term warming experiment in which different species richness levels are incorporated. We hypothesize that both photosynthesis and total ecosystem respiration acclimate to elevated temperatures after long-term continuous heating. This hypothesis was tested by making short term temperature response curves for all communities in both treatments. At similar temperatures, lower flux rates in the heated communities as compared to the unheated communities would then indicate thermal acclimation. However, because photosynthesis and respiration are known to be interdependent (Hoefnagel et al., 1998), the ratio of respiration to photosynthesis is hypothesized to remain unaffected.

Last, we hypothesize that plant biomass and ecosystem CO_2 fluxes are negatively influenced by loss of biodiversity. However, because acclimation is a physiological process, we do not expect species richness to have any influence on acclimation. Nonetheless, this has never been investigated. Therefore, we also tested the hypothesis that declining species richness results in reduced acclimation.

2 Materials and methods

2.1 Study site

This study was conducted at the Drie Eiken Campus of the University of Antwerp (Belgium, 51°09′ N, 04°24′ E). The climate of northern Belgium is characterized by mild winters and cool summers, with average annual air temperatures (T_{air}) varying around 9.6°C. Annual precipitation averages 776 mm, and is more or less equally distributed throughout the year.

In July 2003, an experimental platform containing 288 artificially assembled grassland model ecosystems was established. The platform consisted of 12 sunlit, climate-controlled chambers, of which six were exposed to ambient T_{air} (unheated chambers). The other six (heated chambers) were continuously heated 3 °C above T_{air}, such that temperatures in these chambers varied to the same degree as T_{air}. Each of these 12 chambers contained 24 different grassland communities of varying plant species richness (S): nine S=1 communities, nine S=3 communities and six S=9 communities. We selected 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.). Species, representing the three functional groups, were used to create the S=3 communities, and each species combination occurred only once. The S=9 communities contained all nine species. For more details regarding community compositions, we refer to De Boeck et al. (2006). The position of the different communities varied between the chambers, but one unheated and one heated chamber always exhibited the same positioning. Each plant community was placed in a PVC tube with a height of 60 cm and an inner diameter of 24 cm. The PVC tubes were filled with sieved loamy soil from an arable field. Soil texture and carbon content are given in Table 1. The communities were packed closely together within the chambers, with a space of 1 cm between neighboring pots.

Further details regarding the experimental set-up are given by Lemmens et al. (2006).

2.2 Measurements of carbon dioxide fluxes

Carbon dioxide fluxes were measured in November and December 2005, 28 months after the start of the experiment, and all measurements were made between 9 h 30 and 17 h. First, a transparent polymethyl pentene cuvette (60 cm high; 25 cm in diameter) was placed on the permanent soil collars. This cuvette was shaped such that it fitted the collars perfectly and air tightness was ensured by a gas tight seal. Flux measurements were performed with an infrared gas analyzer (IRGA; EGM-4; PP Systems, Hitchin, UK), which was coupled to

Table 1. Texture and soil carbon content of the soil in the plant communities at the start of the experiment. Values in parentheses represent the standard errors.

Texture	
Sand (>53 μm):	8.7% (1.4)
Silt(2–53 μm):	76.3% (0.9)
Clay (<2 μm):	14.8% (0.4)
Soil carbon content	
g C/100 g soil:	1.6 (0.2)

the cuvette. During the measurements, a quantum sensor (JYP 1000, SDEC, France) inside the cuvette measured photosynthetic photon flux density (*PPFD*). Two aerators guaranteed well-mixed air.

After placing the cuvette, net ecosystem exchange of CO_2 (*NEE*) was measured. Subsequently, measurements of total ecosystem respiration (*TER*) were performed in the dark, immediately after we covered the cuvette with a black cloth, preventing photosynthesis. Gross primary productivity (*GPP*) could then be calculated as: *GPP=NEE–TER*. We are aware of the fact that this calculation can cause a slight (max 5–10%) overestimate of *GPP*, because leaf respiration is partially inhibited in the light (Atkin et al., 2000b). However, in this study our interest primarily lies in the relative differences in *GPP* among temperature treatments, not in absolute values of *GPP*. We therefore believe that the method used was suitable for our study.

Carbon dioxide fluxes were measured in the eight central plant communities (three of S=1, three of S=3 and two of S=9) inside each of six chambers (three unheated and three heated). Inside each of the chambers air temperature and relative humidity were measured continuously with a combined QFA66 humidity-temperature sensor (Siemens, type QFA66, Germany), and 30-min means were stored.

To assess acclimation, CO_2 fluxes from all plant communities were measured twice at three different air temperatures. Measurements were performed during three periods of approximately one week. One month earlier, from 24 October until 28 October, aboveground biomass was harvested at 3.5 cm height. As a result of this harvest, plants never spread out of the pots during our measurement periods. Two days before each measurement period, air temperatures inside the chambers were altered in order to enlarge the temperature range (T_{air} was still fluctuating in accordance with ambient temperatures). This was necessary for making regressions of *TER* versus temperature. Throughout this study, air temperatures ranged between 3 and 13°C in both temperature treatments. Furthermore, during our flux measurements, soil temperatures did not differ significantly between unheated and heated plant communities (data not shown). In Table 2 the experimental set-up of the measurements is presented.

Table 2. Experimental set-up of the measurements.

Period	Temperature Treatment	
	Unheated	Heated
1 (22–25/11/2005)	ambient	ambient +3°C
2 (28–30/11/2005)	ambient +8°C	ambient +8°C
3 (5–8/12/2005)	ambient +3°C	ambient

2.3 Water supply

One day before each of the three measurement periods, soil water content (*SWC*) was measured in all communities with a TRIME portable TDR soil moisture meter (MESA systems Co., USA). When *SWC* differed between temperature treatments, we adjusted the water supply, such that variation in *SWC* between heated and unheated communities was minimal. During the flux measurements, *SWC* never differed significantly between temperature treatments (p values being 0.46, 0.37 and 0.59 for periods 1, 2 and 3, respectively; paired t-test). Consequently, observed differences in CO_2 fluxes should not be due to variations in *SWC*.

2.4 Plant cover and specific leaf area

Ecosystem-scale CO_2 effluxes include not only physiological temperature responses, but also phenological and allometrical responses. Therefore, we determined total green plant cover and specific leaf area for each of the 48 plant communities immediately after the flux measurements. Green plant cover, a measure for plant biomass, was estimated using the pin-frame method. We recorded the plant species touched by a vertical needle at each point of a 60 point matrix. Specific leaf area (*SLA*) ($m^2\,kg^{-1}$) of each species was also determined for each community. For this purpose, we sampled three leaves of all plant species in each of the 48 plant communities. Surface areas were measured with a Li-3000A area meter (Li-Cor, Nebraska, USA), and after being dried for 48 h at 70°C, all samples were weighed. Subsequently, we calculated average *SLA* (average (area/mass)) for all plant species, such that differences between unheated and heated chambers could be computed.

2.5 Data analysis

The aim of our research was to determine whether acclimation had occurred. Because T_{air} was constantly fluctuating, we wanted to compare respiration rates at a standard temperature (7°C), which occurred in all plant communities. In order to estimate *TER* at 7°C (TER_7), we fitted regressions for *TER* in Origin7 (Origin 7, Originlab, Northampton, MA, USA), using the following function:

$$TER = TER_7 * Q_{10}^{(T_{air}-7)/10} \quad (1)$$

in which Q_{10} is the temperature sensitivity of *TER*, and T_{air} (in °C) is the recorded air temperature at the time of measurement. Regressions were fitted to the six measurements made for each plant community (two measurements during each of the three periods). All regressions were significant, and the uncertainty surrounding TER_7 was on average 15%. Furthermore, our raw data and fitted basal rates of respiration and Q_{10} values (data not shown) did not suggest any sign of acclimation within the three day periods of our measurements. Hence, we are confident that the predicted rates at 7°C are correct.

Photosynthesis correlated better with light intensity than with T_{air} (*GPP* vs. T_{air}: correlation coefficient ρ=0.15; p=0.013; *GPP* vs. *PPFD*: ρ=0.52; p<0.0001). Therefore, regressions for *GPP* were calculated as a function of *PPFD*. Since light intensities fluctuated continuously as well, GPP_{100} (*GPP* at a *PPFD* value of 100 μmol photons $m^{-2}\,s^{-1}$) was estimated for each community. For this purpose, we used the following function:

$$GPP = (QE * P_{max} * PPFD)/(QE * PPFD + P_{max}) \quad (2)$$

in which *QE* is the quantum efficiency and P_{max} the maximum photosynthesis. In order to obtain GPP_{100}, *PPFD* in Eq. (2) was set at 100 μmol photons $m^{-2}\,s^{-1}$. Regressions were fitted to the six measurements made in each plant community.

Since plant cover varied strongly among plant communities and between temperature treatments, it was also necessary to correct for the effect of plant cover in order to make CO_2 fluxes of different communities comparable. To this end, the following function was fitted to the data, for both TER_7 and GPP_{100}:

$$y = a * b^x \quad (3)$$

in which y is TER_7 or GPP_{100} and x is the plant cover. Values a and b are fitted constants, determined by Origin7. Subsequently, we used the fitted functions to calculate $TER_{7(pc1)}$ and $GPP_{100(pc1)}$ for the plant cover measured in each community. (To clarify: $TER_{7(pc1)}$ and $GPP_{100(pc1)}$ are predicted flux rates based on the measured plant cover and Eq. (3), whereas TER_7 and GPP_{100} are measured fluxes, albeit normalized for temperature or light). Corrections for effects of plant cover were accomplished by computing residuals as $TER_7 - TER_{7(pc1)}$ and $GPP_{100} - GPP_{100(pc1)}$ (residuals were termed $Resid_{TER}$ and $Resid_{GPP}$). Using these residuals, unheated and heated communities with equal species composition could be compared.

In order to calculate the degree of acclimation, regressions were again fitted to TER_7 and GPP_{100} (using Eq. 3), but now this was performed for both temperature treatments separately. Subsequently, these two functions were used to calculate $TER_{7(pc2)}$ and $GPP_{100(pc2)}$ for all communities, such that we could make a comparison between flux rates in the heated and unheated chambers and were able to calculate the degree of acclimation at a particular plant cover.

In summary, $TER_{7(pc1)}$ and $GPP_{100(pc1)}$ are predicted fluxes, based on one fit for both temperature treatments together, and were used to calculate residuals for individual communities. These residuals could then be compared statistically to test for differences between heated and unheated communities with equal species composition. The $TER_{7(pc2)}$ and $GPP_{100(pc2)}$, on the other hand, are predicted flux rates, based on separate fits for unheated and heated communities, and were computed to test for differences among temperature treatments, not communities.

2.6 Statistics

All statistical analyses were performed in SAS (SAS system 9.1, SAS Institute, Cary, NC, USA). To test whether TER_7 was correlated with plant cover, a spearman-rank correlation was used, since these data were not normally distributed. For GPP_{100}, a Pearson correlation test could be used. Comparisons of unheated and heated communities with equal species composition were performed with a paired t-test. Multiple analysis of variance (MANOVA) was used to test effects of species richness and to check whether the TER_7:GPP_{100} ratio differed among temperature treatments. Three outliers were deleted from all analyses because the plant communities were severely disturbed. In the pair wise comparison of unheated and heated communities with equal species composition, we were obliged to delete some other communities as well, because, after two years of treatment, different species dominated in these unheated and heated communities. These plant communities could thus not be compared. As a result, comparisons of communities with equal species composition were performed for 36 communities, 18 unheated and 18 heated communities.

3 Results

3.1 Phenological/allometrical responses

Plant cover differed significantly between temperature treatments (p=0.02; paired t-test), with an average cover of 66% in unheated communities and 81% in heated communities. This difference in cover was most pronounced in the monocultures, where plant cover was on average 33% higher in the heated communities (p=0.07; paired t-test). For both S=3 and S=9 plant cover was only 11% and 9% higher in the heated communities and this difference was statistically not significant (p=0.17 and p=0.99, respectively; paired t-test). Species richness affected plant cover as well, with lower average cover in monocultures than in S=3 and S=9 (p=0.05 and p=0.05, respectively; MANOVA). We observed no difference between S=3 and S=9 (p=0.98; MANOVA). Furthermore, no interactions were found between temperature treatment and species richness level (p=0.45; MANOVA).

Table 3. Comparison of specific leaf area (*SLA*) (m^2/kg) in unheated (U) and heated (H) plant communities. Values for n correspond to the number of plant communities in which leaves of the respective species were sampled. Values for *SLA* represent the average value for all plant communities in the temperature treatment and *p* values represent the probability for a statistically significant difference between *SLA* of species in unheated and heated communities. *Bellis perennis L.* is not shown, because it only occurred in two communities and therefore we could not carry out a statistical analysis.

Species	n (U)	*SLA* (U)	n (H)	*SLA* (H)	*p* value
Dactylis glomerata L.	7	27.81	10	27.80	0.86
Festuca arudinacea Schreb.	6	19.97	7	19.37	0.85
Lolium perenne L.	3	19.08	3	19.70	0.70
Lotus corniculatus L.	2	24.10	5	20.18	0.50
Medicago sativa L.	8	25.76	9	25.29	0.67
Plantago lanceolata L.	2	26.60	7	22.00	0.12
Rumex acetosa L.	9	23.70	7	24.86	0.47
Trifolium repens L.	2	24.43	2	22.97	0.80

Comparison of *SLA* of each species between both temperature treatments did not reveal a significant heating effect for any of the species (Table 3).

3.2 Temperature responses of CO_2 fluxes

Neither TER_7, nor GPP_{100} was affected by heating (Fig. 1), not even when comparing unheated and heated communities with equal species composition (p=0.57 and p=0.98, for TER_7 and GPP_{100}, respectively; paired t-test). Similar to plant cover, both CO_2 fluxes were significantly lower in monocultures than in S=3 and S=9 (TER_7: p=0.02 and p=0.01; GPP_{100}: p=0.004; p=0.01; each time comparing S=1 with S=3 and S=9, respectively; MANOVA). Again, we observed no difference between S=3 and S=9 (p=0.84 and p=0.99, for TER_7 and GPP_{100}, respectively; MANOVA). We also found no interaction between temperature treatment and S level, neither for TER_7, nor for GPP_{100} (p=0.81 and p=0.94, respectively; MANOVA).

The TER_7:GPP_{100} ratio did not differ between unheated and heated communities with equal species composition (p=0.09; paired t-test; Table 4), and also species richness did not have any influence on this ratio (p=0.89; MANOVA).

3.3 CO_2 fluxes normalized by plant cover

Because TER_7 and GPP_{100} are both strongly (positively) correlated with plant cover (ρ=0.79, p<0.0001 and ρ=0.80, p< 0.0001, respectively), it was necessary to take plant cover into account in order to verify whether physiological acclimation could have occurred. Therefore, Eq. (3) was fitted to the data to calculate $TER_{7(pc1)}$ and $GPP_{100(pc1)}$ (see Materials and Methods; Fig. 2 and Table 5). Subsequently, $Resid_{TER}$ and $Resid_{GPP}$ were computed. We observed a reduction of both $Resid_{TER}$ and $Resid_{GPP}$in the heated chambers as compared to the unheated chambers (p=0.01 and p=0.01, respectively; paired t-test; Fig. 3).

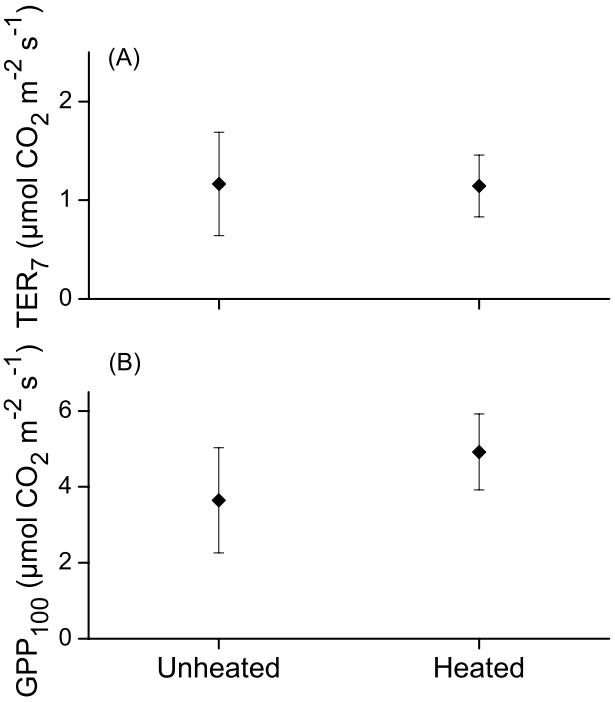

Fig. 1. (a) Total ecosystem respiration at 7°C (TER_7) and **(b)** gross primary productivity at 100 μmol photons m^{-2} s^{-1} (GPP_{100}) and their 95% confidence levels (error bars) for the two temperature treatments (unheated and heated).

This suggests that, across the entire plant cover spectrum, TER_7 and GPP_{100} were lower in the heated than in the unheated treatment. Species richness did not have any effect on $Resid_{TER}$ or $Resid_{GPP}$ (p=0.96 and p=0.92, respectively; MANOVA), and no interactions were observed between temperature treatment and species richness level (p=0.50 and p=0.90, for $Resid_{TER}$ and $Resid_{GPP}$, respectively; MANOVA). For this reason it was not necessary to

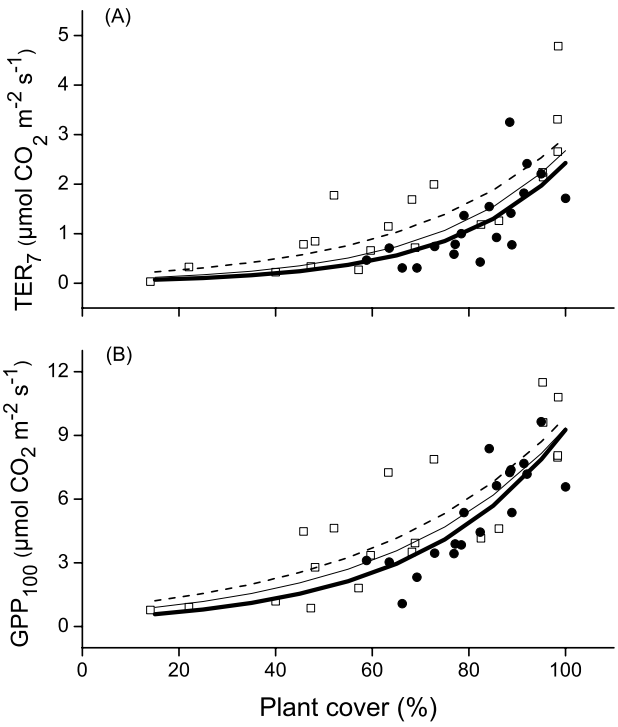

Fig. 2. (a) Total ecosystem respiration at 7°C (TER_7) and **(b)** gross primary productivity at 100 μmol photons $m^{-2}\,s^{-1}$ (GPP_{100}) in function of plant cover. □ = unheated communities; • = heated communities. Exponential regressions are given for the temperature treatments separately as well as combined. — = unheated and heated communities together; −− = unheated communities only; ▬ = heated communities only. Parameter values and statistics for all regressions are provided in Table 5.

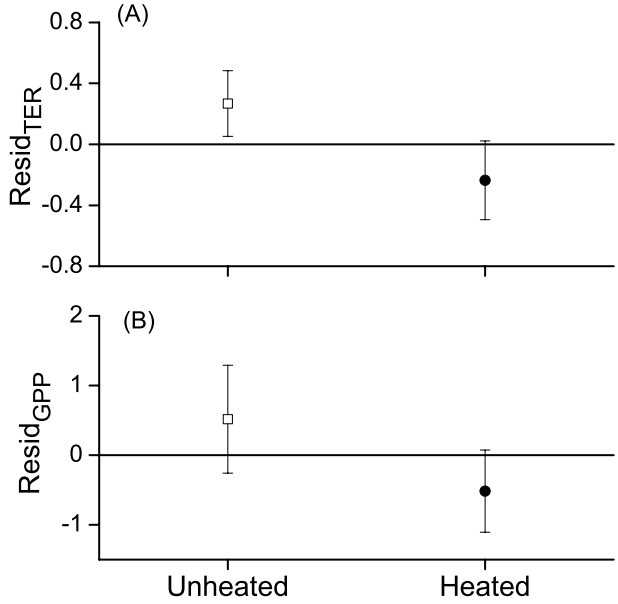

Fig. 3. Residuals between **(a)** measured total ecosystem respiration at 7°C (TER_7) and **(b)** gross primary productivity at 100 μmol photons $m^{-2}\,s^{-1}$ ($GPP100$) and the predicted values based on the observed plant cover. Error bars indicate the 95% confidence level. □ = unheated communities; • = heated communities.

determine the effects of heating for each S level separately, and thus, all S levels could be treated together.

In order to determine the degree of acclimation, $TER_{7(pc2)}$ and $GPP_{100(pc2)}$ were computed (see Materials and methods; Fig. 2 and Table 5). At a standard plant cover of 75%, we obtained a 39% lower $TER_{7(pc2)}$ and a 23% lower $GPP_{100(pc2)}$ in the heated chambers as compared to the unheated chambers. Error propagation indicated that the degrees of acclimation did not differ significantly (at p=0.05). Furthermore, when comparing the TER_7:GPP_{100} ratio of heated and unheated communities with equal species composition, we observed no statistical difference either (p=0.09; paired t-test). Hence, we conclude that there was no difference in the degree of acclimation between total ecosystem respiration and photosynthesis.

4 Discussion

4.1 Heating effect

The higher plant cover in the heated chambers points towards a phenological response to heating. We assume that the higher temperatures in the heated chambers enhanced plant and organ development, resulting in the higher plant cover observed in these communities. According to Pollock (1990), elevated temperature can indeed influence productivity.

In contrast to our results, Lemmens et al. (2006) reported a negative temperature effect on plant biomass for the same experiment at the November 2003 harvest. This reduction in biomass was, however, most likely due to enhanced soil drought in the heated chambers during summer 2003 (Lemmens et al., 2006). Furthermore, although *SWC* was observed to be significantly lower in the heated chambers during the post-harvest time preceding our measurements (data not shown), we believe that the communities were never exposed to drought stress. Moreover, prior to our measurements, water supply was adjusted such that differences in *SWC* were avoided during our measurements (see Materials and Methods). Therefore, because *SWC* was sufficiently high during this current study and air temperatures were suboptimal, heated chambers likely provided better growing conditions, resulting in the higher plant cover in the heated communities.

Table 4. Mean values and standard deviations (SD) for total ecosystem respiration at 7°C (TER_7), gross primary productivity at 100 μmol photons $m^{-2}\,s^{-1}$ (GPP_{100}) and the TER_7:GPP_{100} ratio for unheated and heated communities.

Temperature treatment	TER_7 (μmol CO_2 $m^{-2}\,s^{-1}$)		GPP_{100} (μmol CO_2 $m^{-2}\,s^{-1}$)		TER_7:GPP_{100}	
	Mean	SD	Mean	SD	Mean	SD
Unheated	1.41	1.02	5.17	3.39	0.27	0.11
Heated	1.20	0.80	5.26	2.33	0.22	0.08

Table 5. Parameter values and R^2 for the fitted exponential regressions of TER_7 (Total ecosystem respiration at 7°C) and GPP_{100} (gross primary productivity at 100 μmol photons $m^{-2}\,s^{-1}$) versus plant cover (displayed in Fig. 2). The R^2 (m vs. p) and p values (m vs. p) represent the R^2 and p values for the linear regressions fitted for measured versus predicted fluxes. Values in parentheses represent the standard errors.

	Unheated only		Heated only		Both temperature treatments combined	
	TER_7	GPP_{100}	TER_7	GPP_{100}	TER_7	GPP_{100}
a	0.146 (0.058)	0.838 (0.287)	0.037 (0.034)	0.355 (0.159)	0.068 (0.033)	0.592 (0.173)
b	1.030 (0.004)	1.025 (0.003)	1.043 (0.008)	1.033 (0.004)	1.037 (0.004)	1.028 (0.003)
R^2	0.771	0.729	0.481	0.680	0.592	0.680
R^2 (m vs. p)	0.212	0.287	0.564	0.383	0.339	0.303
p value (m vs. p)	0.041	0.015	0.0002	0.005	0.0001	0.0003

Despite the positive heating effect on plant cover, neither TER_7, nor GPP_{100} differed among temperature treatments. This result points towards the occurrence of thermal acclimation. When normalized for plant cover, heated communities did indeed exhibit lower values for both CO_2 fluxes than did unheated communities, which is evidence for acclimation to elevated temperatures. Thermal acclimation of CO_2 fluxes was found in several other studies (Rook, 1969; Larigauderie and Körner, 1995; Loveys et al., 2003; Tjoelker et al., 1998; Atkin et al., 2006), although it did not occur in some studies on root respiration (Sowel and Spomer, 1986; Weger and Guy, 1991; Zogg et al., 1996; Burton and Pregitzer, 2003). Unfortunately, we could not partition our measured ecosystem respiratory fluxes into their autotrophic and heterotrophic components and therefore, we cannot state whether thermal acclimation of *TER* was due to decreased plant respiration, decreased heterotrophic respiration, or both.

The degree of acclimation appeared to differ slightly between TER_7 and GPP_{100}. At 75% plant cover, we observed a higher degree of acclimation for TER_7 than for GPP_{100}, which was, however, not significant. The 75% plant cover was chosen because it occurred in both temperature treatments, and because at 75% plant cover we believe to have mitigated "the compression effect". By compression effect we refer to the fact that, whereas increases in *LAI* are theoretically unlimited, plant cover can never exceed 100%. At high values, plant cover will therefore saturate if *LAI* continues to increase. Since respiration and photosynthesis both depend on *LAI*, it was important to restrict the use of plant cover to a range where its changes are not uncoupled from those in *LAI*.

Although TER_7 tended to acclimate to a higher degree than GPP_{100}, the TER_7:GPP_{100} ratio did not differ among temperature treatments, indicating no difference in acclimation degrees. This latter result is considered more accurate, since the TER_7:GPP_{100} ratio was determined for each community separately, such that unheated and heated communities with equal species composition could be compared. Therefore, we believe that both respiration and photosynthesis exhibited equal degrees of acclimation. Moreover, degrees of acclimation are highly variable amongst species (Larigauderie and Körner, 1995; Turnbull et al., 2001; Atkin and Tjoelker, 2003, Loveys et al., 2003) and therefore comparisons should be made between communities of equal species composition. Thus, whereas the first method, computing the degree of acclimation over all plant communities, gives us an impression about the overall degree of acclimation, the second method can be considered as evidence for equal acclimation degrees for respiration and photosynthesis.

In accordance with our results, Gifford (1994, 1995) reported the ratio of respiration to photosynthesis in diverse species to be remarkably insensitive to growth temperature. Furthermore, he noted that the assumption that plant respiration is more temperature-dependent than photosynthesis (Ryan et al., 1996; Woodwell, 1990) is based on short-term

experiments (Gifford, 1994). The equal TER_7:GPP_{100} ratio in our unheated and heated communities points towards the interdependence of respiratory and photosynthetic rates (Hoefnagel et al., 1998). This interdependence is due to the fact that respiration relies on the substrate provided by photosynthesis (Bouma et al., 1995), whereas respiration itself is crucial for the maintenance of photosynthetic activity (Krömer et al., 1995; Hoefnagel et al., 1998). Such interdependence of respiration and photosynthesis was also reported in several other experiments (e.g. Ziska and Bunce, 1998; Loveys et al., 2003; Atkin et al., 2006), and this information could be essential for determining whether global warming could alter the carbon balance of terrestrial ecosystems.

In order to determine whether the lower normalized flux rates in the heated chambers could be caused by differences in leaf morphology, we verified whether the specific leaf area differed among temperature treatments. For all species, *SLA* appeared to be unaffected by heating. This result is in accordance with Lemmens et al. (2006), who observed no temperature effect on *SLA* either, in the same set-up in September and October 2003. By contrast, Loveys et al. (2002) reported increases in *SLA* with increasing growth temperature in 14 out of 16 species. Since we did not find a significant heating effect on *SLA*, we believe that in our study, acclimation was not caused by differences in specific leaf area.

4.2 Effects of declined species richness

The second global change factor in this experiment, declining species richness, significantly affected plant cover, with monocultures exhibiting the lowest plant cover. This is in accordance with our expectations, since complementarity and facilitation can only occur in communities with more than one species. The fact that plant cover did not differ between S=3 and S=9, which both contained three functional groups, is in agreement with the reported importance of functional group diversity (Sphen et al., 2000). In accordance, we found values for both TER_7 and GPP_{100} (not normalized for plant cover) to be lower in the monocultures than in S=3 and S=9 communities. Because the S level no longer affected respiration or photosynthesis when normalized for plant cover ($Resid_{TER}$ and $Resid_{GPP}$ were unaffected by S level), the effect of species richness can be ascribed to differences in plant biomass.

As a final point, the S level did not influence the TER_7:GPP_{100} ratio, and also $Resid_{TER}$ and $Resid_{GPP}$ remained unaffected. Furthermore, we observed no interactions between temperature treatment and species richness level. This implies that species richness had no effect on the normalized CO_2 fluxes and therefore, we assume that species richness will not have had any influence on the degree of thermal acclimation beyond its direct effect via altered plant cover.

5 Conclusions

In this study, we tested the hypotheses (1) that photosynthesis and total ecosystem respiration both acclimate to elevated temperatures after long-term, continuous heating; (2) that both CO_2 fluxes acclimate to the same degree; and (3) that declined species richness does not affect the degree of acclimation.

In accordance with our first hypothesis, we observed physiological acclimation of both photosynthesis and total ecosystem respiration after 28 months of continuous heating. This physiological acclimation was entirely compensated by the higher biomass in the heated chambers. Hence, at equal air temperatures, both temperature treatments exhibited similar ecosystem-scale CO_2 fluxes. Moreover, our second hypothesis could not be falsified either, since heating had no effect on the ratio of respiration to photosynthesis, and degrees of acclimation are thus considered equal for both CO_2 fluxes.

Declined species richness, on the other hand, resulted in reduced plant cover, and accordingly also in decreases in both TER_7 and GPP_{100}. Nonetheless, neither the TER_7:GPP_{100} ratio, nor $Resid_{TER}$ or $Resid_{GPP}$ was affected by species richness. Thus, declined species richness did not induce or affect the observed physiological response to long-term heating, which is in accordance with our last hypothesis.

With regard to global warming, we conclude that models need to take into account the opposing effects of increasing plant cover and physiological down-regulation of photosynthesis and respiration in a warmer world.

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