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Warming alters the metabolic balance of ecosystems

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The carbon cycle modulates climate change, via the regulation of atmospheric CO_2, and it represents one of the most important services provided by ecosystems. However, considerable uncertainties remain concerning potential feedback between the biota and the climate. In particular, it is unclear how global warming will affect the metabolic balance between the photosynthetic fixation and respiratory release of CO_2 at the ecosystem scale. Here, we present a combination of experimental field data from freshwater mesocosms, and theoretical predictions derived from the metabolic theory of ecology to investigate whether warming will alter the capacity of ecosystems to absorb CO_2. Our manipulative experiment simulated the temperature increases predicted for the end of the century and revealed that ecosystem respiration increased at a faster rate than primary production, reducing carbon sequestration by 13 per cent. These results confirmed our theoretical predictions based on the differential activation energies of these two processes. Using only the activation energies for whole ecosystem photosynthesis and respiration we provide a theoretical prediction that accurately quantified the precise magnitude of the reduction in carbon sequestration observed experimentally. We suggest the combination of whole-ecosystem manipulative experiments and ecological theory is one of the most promising and fruitful research areas to predict the impacts of climate change on key ecosystem services.

Keywords: global warming; carbon sequestration; carbon cycle; metabolic theory; gross primary production; ecosystem respiration

1. INTRODUCTION

The biosphere is in the midst of a pronounced warming trend. Global surface temperature has risen by approximately 0.74°C in the past century and is projected to increase by a further 3–5°C over the next 100 years (Houghton 2001; IPCC 2007). Evidence for the ecological impacts of global warming on individual taxa is now unequivocal as represented by range expansions and poleward migrations (Walther *et al.* 2002; Parmesan & Yohe 2003; Rosenzweig 2008) but the potential responses of whole ecosystems are uncertain (Walther *et al.* 2002). This may be at least partially due to the perceived difficulties of dealing with such seemingly complex systems (Walther *et al.* 2002; Montoya *et al.* 2006; Memmott *et al.* 2007).

Changes to the carbon cycle are regarded as one of the greatest impacts on ecosystem service supply associated with climate change (Schroter *et al.* 2005). These changes include direct effects—e.g. on productivity, CO_2 sequestration, resource quality—but also indirect effects—e.g. on precipitation patterns, water availability, crop production.

Of special interest are those changes in the biogeochemical cycling of carbon that could potentially alter the 'metabolic balance' of ecosystems. This balance is defined as the rate of carbon fixation by photosynthesis relative to remineralization by respiration, and it determines whether an ecosystem acts as a source or a sink for atmospheric CO_2 (Woodwell *et al.* 1998; del Giorgio & Duarte 2002; Woodward 2007).

Some recent evidence has highlighted the potential for feedback between warming and ecosystem CO_2 sequestration (Cox *et al.* 2000; Canadell *et al.* 2007; Piao *et al.* 2008). For instance, in terrestrial ecosystems there is a strong positive feedback between temperature and CO_2 emission due to elevated rates of soil respiration (Lloyd & Taylor 1994; Cox *et al.* 2000; Knorr *et al.* 2005; Davidson & Janssens 2006; Arnone *et al.* 2008), and it has also been suggested that as the oceans warm their ability to sequester CO_2 from the atmosphere may weaken (del Giorgio & Duarte 2002; López-Urrutia *et al.* 2006).

Recently, several attempts have been made with coupled climate–carbon models to incorporate some of the key biotic components of the carbon cycle

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(Cox *et al.* 2000; Friedlingstein *et al.* 2006). However, there is little agreement as to exactly how this should be done in a systematic and predictive manner. In relation to this point the two fundamental questions that we address here, are:

— How will the metabolic balance of ecosystems respond to warming?
— Can we predict the precise magnitude of such changes for any probable warming scenario?

To answer these questions we combine a whole-system experiment with predictive ecological theory, to enable us to simulate experimentally future warming scenarios and their probable consequences on ecosystem processes. In particular, the experimental component permits direct comparisons to be made between contemporary ecosystems with their 'future' warmed counterparts, and also gives us the opportunity to explore the underlying drivers behind the observed responses. Furthermore, by using materially closed systems (i.e. the only inputs of carbon are through gaseous exchange with the atmosphere) we are able to avoid the confounding effects of changes in the movements of allochthonous carbon into and out of the system and focus on the mechanisms affecting changes in the balance of autochthonous carbon.

Here, we first present and test the metabolic theory of ecology (MTE) (*sensu* Brown *et al.* 2004) by attempting to establish the temperature dependence of the fundamental components of the carbon cycle (net primary production (NPP), gross primary production (GPP) and ecosystem respiration (ER), respectively) and their dependence on individual metabolism. We then use the theoretical platform of the MTE to explore whether warming will alter carbon sequestration rates in ecosystems. Finally, through extension of the MTE, we attempt to predict quantitative changes in the metabolic balance of ecosystems in response to a probable warming scenario predicted for the end of the next century (IPCC 2007). We tested our predictions at the ecosystem scale using a whole system manipulative experiment in aquatic mesocosms that mimicked this degree of warming.

Lentic freshwater ecosystems are tractable as mesocosms because the unit of the ecosystem is easily delimited and replicable. Importantly, these systems enable the assembly of functioning ecosystems, which although simplifications of their natural counterparts, allow us to understand the mechanisms behind the ecosystem level changes that may occur as a result of warming. Furthermore, freshwater ecosystems (e.g. wetlands) are known to be fundamental components of the global carbon cycle with respect to carbon sequestration (Whiting & Chanton 2001). Therefore, understanding how carbon sequestration rates behave in response to warming in these systems is critical.

2. THEORETICAL FRAMEWORK

Metabolism is a fundamental process that regulates the flux of energy and matter through multiple levels of biological organization, from individuals to ecosystems (West *et al.* 1997; Brown *et al.* 2004). According to the MTE, individual metabolic rate (i.e. the power required to sustain an organism), can be explained by the general metabolic model (West *et al.* 1997; Gillooly *et al.* 2001; Brown *et al.* 2004)

$$B_i = b_0 \mathrm{e}^{-E/kT} M_i^{\alpha}, \tag{2.1}$$

where B_i is the basal metabolic rate of an individual i, b_0 is a normalization constant independent of body size and temperature, $\mathrm{e}^{-E/kT}$ is the Boltzmann factor that describes the temperature, T, dependence of metabolic rate, where k is Boltzmann's constant (8.62×10^{-5} eV K^{-1}) and E is the activation energy of metabolism. M_i corresponds to the body mass of an individual i, and α is the allometric scaling exponent (West *et al.* 1997; Brown *et al.* 2004). By summing the individual metabolic rates of all the organisms within an ecosystem it is possible to predict total ecosystem metabolic rates (Enquist *et al.* 2003; Allen *et al.* 2005; López-Urrutia *et al.* 2006). This general metabolic model has been extended to describe three ecosystem processes that underpin the carbon cycle: NPP, GPP and ER (Enquist *et al.* 2003; Allen *et al.* 2005; López-Urrutia *et al.* 2006).

The rate of GPP for a whole ecosystem can be estimated from the sum of the individual photosynthetic rates of all of its autotrophic organisms (Allen *et al.* 2005; López-Urrutia *et al.* 2006):

$$\mathrm{GPP} = \frac{1}{V}\sum_{i=1}^{na} P_i = \frac{1}{V} n_0 \mathrm{e}^{-E_\mathrm{p}/kT} \sum_{i=1}^{na} M_i^{\alpha}, \tag{2.2}$$

where na is the number of autotrophic organisms in volume V, n_0 is a normalization constant independent of body size M_i and temperature T, E_p is the effective activation energy governing the temperature dependence of photosynthetic reactions reported in the literature (approx. 0.32 eV; Allen *et al.* 2005; López-Urrutia *et al.* 2006), and α is the allometric scaling exponent. The parameter E_p, which is the 'effective' activation energy of photosynthesis, approximates the hyperbolic temperature dependence of photosynthesis with an exponential function over the temperature range (0–30°C) to permit direct comparison with the exponential temperature dependence of respiration. (Allen *et al.* 2005; López-Urrutia *et al.* 2006). The photosynthesis–temperature response is typically hyperbolic, declining at high temperatures due to deactivation of the component reactions (Bernacchi *et al.* 2001; Medlyn *et al.* 2002). However, photosynthetic temperature optima are generally correlated with the environmental temperature range experienced by plants and deactivation is uncommon within the annual environmental temperature range experienced by plants in their natural environment (Larcher 1995). We, therefore, approximate the hyperbolic photosynthesis–temperature relationship with E_p, following Allen *et al.* (2005) using a well-established model of leaf photosynthesis (Farquhar *et al.* 1980) and reasonable assumptions (internal CO_2 concentrations are about 70% of ambient, co-limitation of photosynthesis by Rubisco, similar kinetic properties for Rubisco across species) that are frequently used

in carbon cycling models. It is important to note here, that the derivation of E_p is based on the expected concentrations of CO_2 at the sites of photosynthesis in terrestrial plants (Allen *et al.* 2005). Therefore, potential differences between aquatic and terrestrial photosynthesis, for instance, changes in the concentration gradient of CO_2 at the site of photosynthesis, due to Henry's law or slight differences in Rubisco kinetics between aquatic and terrestrial plants, may result in a divergence from the expected E_p of approximately 0.32 eV in aquatic ecosystems, a point that has been previously neglected in tests of MTE in aquatic systems (López-Urrutia *et al.* 2006).

The GPP of an ecosystem is the gross absorption of CO_2. Therefore, GPP also accounts for the photosynthate that is respired by autotrophs. Because autotrophic respiration is ultimately limited by, and tightly coupled to, photosynthate production within individual autotrophs (i.e. by substrate availability; Dewar *et al.* 1999; Atkin & Tjoelker 2003) the temperature dependence of autotrophic respiration should be constrained by the photosynthetic activation energy over temporal scales as short as days to weeks. This process is called type-I respiratory acclimation, and has been observed empirically (Atkin & Tjoelker 2003) and experimentally (Dewar *et al.* 1999). In our model for GPP we therefore assume that autotrophic respiration (AR) has an activation energy equivalent to E_p over the comparatively long temporal scale of our experiment (Allen *et al.* 2005).

$$\begin{aligned} \mathrm{AR} &= \frac{1}{V}(1-\varepsilon)\mathrm{GPP} \\ &= \frac{1}{V}(1-\varepsilon)n_0 \mathrm{e}^{-E_p/kT}\sum_{i=1}^{na} M_i^{\alpha}, \end{aligned} \tag{2.3}$$

where $(1-\varepsilon)$ is the fraction of photosynthate that is respired by autotrophs.

The NPP of an ecosystem is defined as its GPP minus the carbon respired by autotrophs, AR (i.e. the net fixation of CO_2 into plant biomass; Allen *et al.* 2005; Woodward 2007):

$$\mathrm{NPP} = \frac{1}{V}\varepsilon\mathrm{GPP} = \frac{1}{V}\varepsilon n_0 \mathrm{e}^{-E_p/kT}\sum_{i=1}^{na} M_i^{\alpha}, \tag{2.4}$$

where ε is the fraction of photosynthate allocated to the net primary production of producer biomass.

In a similar way, the rate of ecosystem respiration (ER) can be estimated from the individual respiratory rates of all of its autotrophic (AR) and heterotrophic (HR) organisms (Enquist *et al.* 2003; Allen *et al.* 2005; López-Urrutia *et al.* 2006):

$$\begin{aligned} \mathrm{ER} &= \frac{1}{V}[\mathrm{AR}+\mathrm{HR}] \\ &= \frac{1}{V}\left[(1-\varepsilon)n_0 \mathrm{e}^{-E_p/kT}\sum_{i=1}^{na} M_i^{\alpha,a} + r_0 \mathrm{e}^{-E_r/kT}\sum_{i=1}^{nh} M_i^{\alpha,h}\right], \end{aligned} \tag{2.5}$$

where na is the total number of autotrophic organisms and nh is the number of heterotrophic organisms in a volume V, r_0 is a normalization constant which is independent of M_i and T. We assume the scaling exponent α is the same for autotrophs, a and heterotrophs, h, (West *et al.* 1997; Gillooly *et al.* 2001; Brown *et al.* 2004). The average activation energy governing the temperature dependence of respiratory reactions, E_r is approximately 0.65 eV (Gillooly *et al.* 2001; Enquist *et al.* 2003).

In equation (2.5) because ER is the sum of both HR and AR it does not have a simple exponential temperature dependence governed by a single activation energy, unlike NPP or GPP. At steady state, in a closed system, ER is limited for substrate and must equal GPP over the course of a year. Therefore, under conditions of substrate limitation the activation energy for heterotrophic metabolism, E_r, should approach the activation energy for photosynthetic reactions, E_p, resulting in equivalent temperature dependences between GPP and ER over the relevant temporal scale (Allen *et al.* 2005). However, when an ecosystem deviates from steady state (i.e. $\mathrm{ER} < \mathrm{GPP}$ or $\mathrm{ER} > \mathrm{GPP}$), ER is not constrained by GPP. During non-steady-state dynamics, providing there is sufficient stored carbon, heterotrophic respiration may exceed NPP (i.e. the potential contemporary carbon substrate) over temporal scales dependent on the turnover time of the carbon stores. Under such conditions heterotrophic metabolism can proceed at maximum capacity. Therefore, during non-steady-state dynamics, because $E_r > E_p$, ER should have a temperature dependence approaching that of heterotrophic metabolism, E_r, and therefore greater than the activation energy for GPP.

Equations (2.2), (2.4) and (2.5) yield general expressions for the temperature dependence of NPP, GPP and ER and highlight the importance of the activation energies for individual metabolism in controlling the temperature response of whole ecosystem metabolic rates. Importantly, the theory outlined above differs from previous work modelling the temperature dependence of the carbon cycle based on individual metabolism (Allen *et al.* 2005). Here, we do not make the assumption of steady state. Rather, because we are simulating the consequences of global warming on ecosystem metabolism (i.e. a perturbation) we attempt to understand what happens to the metabolic balance of ecosystems during the transitory phase between steady states. As such, GPP and ER have the potential to go out of balance. In a scenario where $\mathrm{ER} > \mathrm{GPP}$, ER may be fuelled by baseline respiration (i.e. respiration uncoupled from contemporary primary production) which is dependent on the carbon stored within the system (Trumbore 2000; del Giorgio & Williams 2005). On the other hand, when $\mathrm{ER} < \mathrm{GPP}$, ER is not substrate limited. In either case ER is not constrained by GPP and can exhibit non-steady-state dynamics in response to warming.

The theory above provides a platform from which a mechanistic understanding of the potential consequences of global warming on the metabolic balance of ecosystems can be drawn and leads to a number of important predictions, which we tested experimentally. First, the temperature dependence of NPP is governed by the effective activation energy that characterizes photosynthetic reactions, and the relationship between ln(NPP) and $1/kT$ should approximate a

slope of $E_p \approx 0.32$ eV. Second, the temperature dependence of GPP is constrained by the activation energy for photosynthetic reactions because of the acclimation of AR and the slope of the relationship between ln(GPP) and $1/kT$ should be indistinguishable from that of NPP. Third, assuming non-steady-state dynamics, the temperature dependence of ER should be greater than that of GPP and the slope of the relationship between ln(ER) and $1/kT$ should approach the activation energy of heterotrophic metabolism, $E_r \approx 0.65$ eV. Finally, and most importantly, because of the differential temperature dependences of the two processes, ecosystem respiration should increase more rapidly than primary production as ecosystems warm.

With an understanding of the mechanisms controlling the temperature dependence of NPP, GPP and ER it is possible to predict how the metabolic balance (ER/GPP)—which is the ability of an ecosystem to sequester carbon—will respond to warming. We define $R_{H:U}$ as the ratio of the metabolic balance (ER_H/GPP_H) in the heated, i.e. future, systems to the ratio of the metabolic balance (ER_U/GPP_U) in the unheated, i.e. present, ecosystems, which is given by

$$R_{H:U} = \frac{ER_H/GPP_H}{ER_U/GPP_U} = e^{[(E_r - E_p)(T_H - T_U)]/kT_H T_U}, \qquad (2.6)$$

where E_r and E_p are the activation energies for ecosystem respiration and photosynthesis, respectively, and T_H and T_U are the temperatures of heated and unheated ecosystems (see the electronic supplementary material, S1 for a full derivation of the theory). Equation (2.6) suggests that the response of the metabolic balance of an ecosystem to warming can be predicted and quantified from the knowledge of two parameters: the difference between the activation energies of respiration and photosynthesis ($E_r - E_p$) and the temperature increase affecting the system ($T_H - T_U$).

3. MATERIAL AND METHODS

(a) *Experimental set-up*

We tested these predictions by comparing ecosystem metabolism rates in freshwater mesocosms designed specifically for ecosystem scale manipulations (figure 1). The field-based study was carried out between December 2005 and April 2008 at the Freshwater Biological Association River Laboratory (2°10′ W, 50°13′ N), East Stoke, Dorset, UK. We used 20 artificial ponds, each holding 1 m^3 of water: this scale of mesocosm reproduces the key elements of community structure (e.g. diversity, trophic complexity) and functioning (e.g. nutrient cycling) of shallow lake ecosystems (Jones *et al.* 2002; McKee *et al.* 2003; Ventura *et al.* 2008). Half were warmed 3–5°C (mean 4°C) above ambient temperature (see the electronic supplementary material, figures S3 and S4, and table S6), in accordance with global warming projections for the next 100 years for temperate areas in the Northern Hemisphere (IPCC 2007). Experimental warming was achieved by an electronic heating element connected to a thermocouple which monitored the temperature in a given heated and unheated treatment pair of mesocosms. Treatments were arranged in a randomized block design (five blocks of four mesocosms) such that each block contained two replicates of each treatment. The mesocosms were seeded in December 2005 with organic substrates and a suite of organisms, representing an interconnected pelagic and benthic community that contained representative species from primary producers (phytoplankton, macrophytes) to top predators (Roach, *Rutilus rutilus*), and a suite of intermediate invertebrate consumers (Zooplankton, including *Daphnia* and *Bosmina*, and benthic macroinvertebrates, including Mollusca, Malacostraca, Trichoptera, Ephemeroptera and Odonata; see the electronic supplementary material, S7 for a full species list) to mimic the organismal composition, trophic complexity and physical structure of shallow lake ecosystems (Jones *et al.* 2002; McKee *et al.* 2003; Ventura *et al.* 2008). The biota was left to establish for 10 months prior to experimental warming, which commenced on 11 September 2006, thereby allowing time for further natural colonization before the onset of the study on 11 April 2007. NPP, GPP and ER were measured every two months for one year.

(b) *Calculation of metabolic parameters*

Whole ecosystem metabolic fluxes (NPP, GPP and ER) were measured over a 24 h diel cycle for each replicate of each treatment on alternate months over the course of one year (April 2007 to April 2008) using the dissolved oxygen (DO) change technique (Marzolf *et al.* 1994; Mulholland *et al.* 2001; see the electronic supplementary material, S2 for additional details). This technique assumes that changes in DO concentration over a diel cycle represent the metabolic activity (photosynthetic and respiratory) of an aquatic ecosystem.

The record of continuous DO measurements was used to calculate the NPP, GPP and ER for each pond on each sampling occasion. The DO change (ΔDO) for each 15 min time interval was calculated as the difference in O_2 concentration between t_1 and t_2 (i.e. $t_2 - t_1$; see the electronic supplementary material, figure S5). The daylight and night-time analysis periods were delimited as follows: the total analysis period was defined from the minimum O_2 concentration on the 1st night and extended for 24 h to include the minimum O_2 concentration on the 2nd night. Photosynthetic dawn was identified as the minimum O_2 concentration after which all subsequent values were greater than it. Photosynthetic dusk was defined as the maximum O_2 concentration after which all subsequent values were lower (see the electronic supplementary material, figure S5; Bales & Nardi 2007). Each O_2 change value was then assigned to a day- or night-time category. Subsequently the metabolic parameters were calculated by numerical integration. NPP was calculated as

$$NPP = \sum \Delta O_{2day}. \qquad (3.1)$$

GPP was calculated as

$$GPP = NPP + R_{day}, \qquad (3.2)$$

where R_{day} is the day-time respiration. Since it is impossible to directly measure R_{day}, it was estimated,

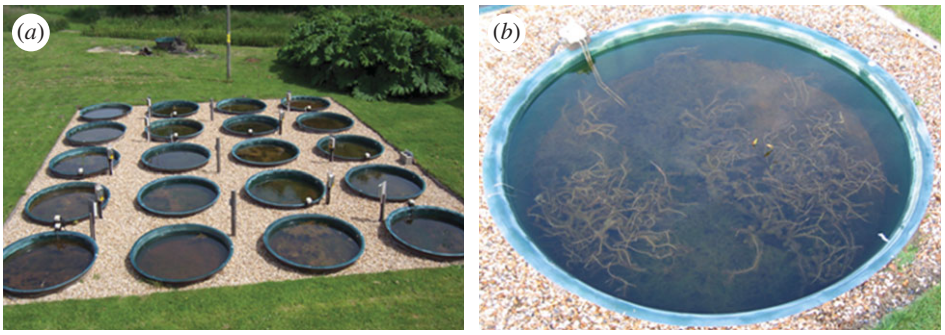

Figure 1. (*a*) Aerial view of the global warming mesocosm experiment in April 2008. The experimental plot consisted of 20 mesocosms: 10 heated and 10 unheated. (*b*) Close-up of mesocosm 1 (heated) in April 2008, highlighting the presence of diverse floral and faunal assemblages.

in keeping with the literature, by extrapolating the mean night-time respiration value across the hours of daylight (Marzolf *et al.* 1994; Mulholland *et al.* 2001; Bales & Nardi 2007). ER was calculated as

$$\mathrm{ER} = R_{\mathrm{day}} + \sum \Delta \mathrm{O}_{2\mathrm{night}}. \tag{3.3}$$

The metabolic balance of each replicate of each treatment was then determined as the ratio of ER : GPP. In the rare event of significant instrument drift or failure, the entire replicate was removed from the final analysis (nine measurements were removed from a total of 140; $n = 131$).

(c) *Statistical analyses*

Statistical analyses of the temperature dependence of ln(NPP), ln(GPP) and ln(ER) (treating temperature ($1/kT$) as a continuous variable) using analysis of covariance (ANCOVA) were computed in R statistical software (R Developmental Core Team 2006). To account for temporal pseudoreplication in the statistical model we included pond identity nested within sampling occasion to account for temporal random effects. Comparison of NPP, GPP, ER and the ratio of ER/GPP among treatments (treating temperature as a categorical factor) was conducted with restricted maximum likelihood methods (PROC MIXED) in SAS, using a blocked, factorial design with repeated measures. This procedure is comparable to repeated measures ANOVA, in that temporal pseudo-replication is accounted for, but has a covariance structure that enables measurements to be included where replicates are not present on all occasions, a prerequisite of other repeated measures tests (Wolfinger & Chang 1998).

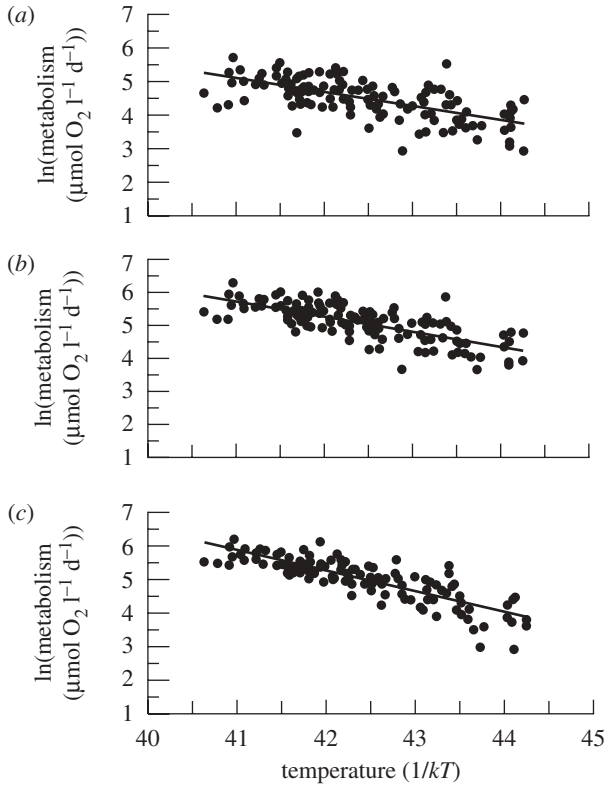

Figure 2. Temperature dependence of (*a*) net primary production, NPP, (*b*) gross primary production, GPP and (*c*) whole ecosystem respiration, ER. The slope of the temperature response equates to the activation energy of the respective process rate. Each data point corresponds to either the NPP, GPP or ER of a single mesocosm on each of the seven sampling occasions. The slope of the temperature dependence of ER was more sensitive to increases in temperature than NPP and GPP (see main text). (*a*) $y = -0.41x + 22.1$; $r^2 = 0.4$; (*b*) $y = -0.45x + 24.4$; $r^2 = 0.5$; (*c*) $y = -0.62x + 31.2$; $r^2 = 0.7$.

4. RESULTS

(a) *The temperature dependence of NPP, GPP and ER*

NPP, GPP and ER all increased with temperature (figure 2*a–c* and table 1). There were no significant differences in the slopes or intercepts of the temperature dependences of NPP, GPP or ER between heated and unheated mesocosms (table 1). Furthermore, we observed no significant interactions between temperature, treatment, pond identity or sampling occasion for NPP, GPP or ER, suggesting that temporal random effects did not influence our results and their temperature dependencies were equivalent across months and between sampling occasions (table 1). This facilitated the use of a single linear model to characterize each of the empirically determined temperature dependences of NPP, GPP and ER (figure 2*a–c*). Empirical measures of photosynthetic and respiratory activation energies were close to our theoretical expectations and those values reported in the literature. For NPP, E_{p} was

Table 1. Results from analysis of co-variance (ANCOVA). The first ANCOVA tests for relationships between ecosystem level metabolic rates (NPP, GPP or ER) and sampling occasions and temperature, parallelism between treatments and differences between intercepts. Metabolic rates are used as dependent variables, temperature ($1/kT$) as the covariate, and treatment (heated or control) as the independent variable. The second ANCOVA tests for differences in the slope of the temperature dependence between metabolic rates (i.e. ER × NPP and ER × GPP). Here metabolic rate is used as the dependent variable, temperature ($1/kT$) as the covariate and metabolic rate ID (e.g. NPP or ER) as the independent variable.

relationship	d.f.	*f*-ratio	*p*-value
ln(NPP) versus $1/kT$	1,123	85.9	**<0.0001**
ln(GPP) versus $1/kT$	1,123	146.6	**<0.0001**
ln(ER) versus $1/kT$	1,123	294.85	**<0.0001**
difference in slope of ln(NPP) versus $1/kT$ between treatments	1,123	0.51	0.47
difference in intercept of ln(NPP) versus $1/kT$ between treatments	1,123	2.05	0.15
difference in slope of ln(GPP) versus $1/kT$ between treatments	1,123	0.23	0.82
difference in intercept of ln(GPP) versus $1/kT$ between treatments	1,123	2.55	0.11
difference in slope of ln(ER) versus $1/kT$ between treatments	1,123	0.56	0.46
difference in intercept of ln(ER) versus $1/kT$ between treatments	1,123	2.27	0.13
difference in slope between ln(GPP) × ln(NPP) versus $1/kT$	1,254	0.45	0.5
difference in slope between ln(ER) × ln(NPP) versus $1/kT$	1,254	12.88	**<0.001**
difference in slope between ln(ER) × ln(GPP) versus $1/kT$	1,254	3.2	**0.0015**

0.41 eV (95% CI 0.32–0.5 eV, $n = 131$) which is slightly steeper than the predicted value ($E_p \approx 0.32$ eV; Allen *et al.* 2005; figure 1*a*). This small overestimate may be ascribed to the fact that NPP measures based on O_2 production are inevitably influenced to some extent by heterotrophic metabolism and may therefore more accurately be described as 'net ecosystem production' (Bales & Nardi 2007). Because it is currently impossible to completely disentangle autotrophic and heterotrophic processes in a systematic way at the ecosystem level (Baldocchi *et al.* 2001; see the electronic supplementary material, S2), heterotrophic metabolism could not be isolated from our measurements of O_2 production. Nevertheless, the effective activation energy of NPP reported in the literature ($E_p \approx 0.32$ eV; Allen *et al.* 2005) falls within the 95 per cent confidence limits of our empirically determined activation energy for NPP. For GPP, E_p was 0.45 eV (95% CI 0.38–0.53 eV, $n = 131$; figure 2*b*), which was statistically indistinguishable from the activation energy from NPP (table 1) though slightly steeper than predicted from the activation energy of photosynthesis. This discrepancy is likely to again be attributable to the inability to isolate autotrophic and heterotrophic processes when measuring whole system metabolism with measurements of O_2 change. Additionally, deviations between our predictions and experimental results may arise from assuming that the activation energies of aquatic and terrestrial photosynthesis are equivalent in the derivation of equations (2.2) and (2.4) after Allen *et al.* (2005). Nevertheless, these results provide substantial evidence for our assumption that the temperature dependence of GPP is governed by the activation energy for photosynthesis due to the type-I acclimation of AR to photosynthate production over periods of months to years (e.g. Dewar *et al.* 1999; Atkin & Tjoelker 2003).

For ER, the activation energy was 0.62 eV (95% CI 0.55 to 0.69 eV, $n = 131$), and approached the activation energy expected for heterotrophic metabolism ($E_r \approx 0.65$ eV; Gillooly *et al.* 2001; Enquist *et al.* 2003; Allen *et al.* 2005; figure 2*c*). Importantly, the activation energy for ER was greater than that of GPP, validating our assumption that ER and heterotrophic metabolism were not limited by GPP (i.e. the mesocosms exhibit non-steady-state dynamics). Further, the empirically determined temperature dependence of NPP and GPP differed from ER (table 1) and, as predicted, ER was more sensitive to temperature increases than NPP and GPP, further substantiating the non-steady-state dynamics exhibited by the experiment.

The conformity between our empirical data and theoretical predictions provides strong support for the suggestion that the rate of ecosystem metabolism is ultimately constrained by the activation energies of photosynthesis and respiration at the individual level (Enquist *et al.* 2003; Brown *et al.* 2004; Allen *et al.* 2005). Further, because ER responds more rapidly to rising temperatures than NPP and GPP, warming could alter the metabolic balance (i.e. the balance between GPP and ER) and carbon sequestration rates within ecosystems.

(b) *Whole-system metabolic balance: quantitative predictions*

Our experimental manipulation showed that GPP and ER were consistently elevated (both within and across seasons) in the warmed mesocosms (figure 3*a*,*b*). Correspondingly, mean annual GPP ($F_{1,113} = 9.58$, $p = 0.0025$; figure 3*a*) and mean annual ER ($F_{1,113} = 33.37$, $p < 0.0001$; figure 3*b*) were significantly higher in the warmed mesocosms, but the magnitude of their responses to warming differed markedly. In agreement with our qualitative theoretical predictions, ER increased at a faster rate under experimental warming than did GPP. As such, experimental warming increased ER considerably more than GPP which showed smaller differences between warmed and control mesocosms (figure 3*a*,*b*).

Given the differential responses of GPP and ER to warming, which were governed by their activation energies at the individual level, we sought to predict how the metabolic balance of our mesocosms would

respond to warming (i.e. equation (2.6) $R_{H:U}$). In figure 4, we show how the metabolic balance of a given ecosystem should change quantitatively with increasing temperatures. For a constant reference value of T_U (e.g. present-day temperatures), carbon sequestration is reduced ($R_{H:U}$ increases) as T_H increases. The magnitude of the increase (i.e. the slope) is governed by the difference in the activation energies of respiration and photosynthesis ($E_r - E_p$).

We tested this general prediction with data from our experiment. Here, E_r and E_p were our empirically observed values of 0.62 and 0.45 eV, respectively. We used T_H and T_U as the mean annual absolute temperatures in the heated and unheated mesocosms (290.9 and 286.1 K, respectively). After substituting the empirical values into equation (2.6) we would expect the ratio $R_{H:U}$ to be 1.12, i.e. carbon sequestration will be reduced by 12 per cent in the warming scenario. Our empirically measured $R_{H:U}$ (mean annual ratio) was 1.13 (95% CI 1.07–1.19), and was statistically indistinguishable from our theoretical prediction (figure 4). Accordingly, the metabolic balance (ER : GPP ratio) of the warmed mesocosms was significantly elevated over the course of the year ($F_{1,113} = 12.71$, $p < 0.005$, figure 3*c*). In fact, in four months during the study (June, August and October 2007 and April 2008) the ER : GPP ratio was greater than 1, suggesting that the warmed systems became net sources of CO_2 to the atmosphere over the growing season.

5. DISCUSSION

Our results suggest that the temperature dependences of whole ecosystem respiration and primary production are fundamentally different, as suggested by their activation energies at the individual level. This finding provides a simple mechanistic platform with strong predictive power for understanding how global warming may alter carbon sequestration rates within ecosystems. Because the activation energy for ecosystem respiration is higher than that of primary production, ecosystem respiration increased proportionately more than production under the experimentally induced global warming scenarios predicted for the end of the century. The shift in the metabolic balance of the warmed ecosystems in our experiment suggests that a larger fraction of the carbon fixed by photosynthesis was remineralized and released as CO_2, thus compromising the capacity of these systems to sequester carbon as they warm.

In our experiment both warmed and control mesocosms were net sinks for CO_2. However, the carbon sequestration capacity of the warmed systems relative to the control systems was severely compromised. In both warmed and control mesocosms the carbon balance deviated from steady state because ER/GPP was less than 1 averaged over the year, validating the assumptions of our theoretical models. Importantly, in the control mesocosms, at ambient temperature, ER/GPP averaged over the year was considerably lower than 1, indicating that these systems were strong sinks for CO_2. In the warmed mesocosms ER/GPP was less than 1 when averaged over the year; however, during the summer and autumn months these systems were net CO_2 sources (i.e. ER/GPP > 1) indicating that a portion of heterotrophic metabolism was fuelled by stored organic carbon. Because the mesocosms were not at steady state (i.e. ER/GPP < 1 or ER/GPP > 1), ER was not substrate limited by contemporary NPP. As such, heterotrophic metabolism increased in response to warming, and was unconstrained by the weaker temperature dependence of GPP. This corroborates our assumption that the activation energy for ER closely reflected the activation energy for heterotrophic metabolism in response to warming. In our mesocosm experiment the temperature response of ER was not constrained by GPP and warming increased the fraction of absorbed carbon (GPP) that was respired (ER), thereby reducing carbon sequestration.

In general, caution must be exercised when extrapolating from mesocosm experiments to natural ecosystems. Having a general theoretical framework that is supported by experimental observations may assist this extrapolation. In particular, the effects of temperature on the metabolic balance observed in our whole-ecosystem manipulations should be treated somewhat cautiously when extrapolating to other systems where other limiting resources (e.g. light, nutrients, organic carbon) might alter the temperature response of primary production and, to a lesser extent, ecosystem respiration (Woodwell *et al.* 1998). For instance, in both marine (López-Urrutia & Moran 2007) and terrestrial (Woodwell *et al.* 1998) systems it has been suggested that resource limitation may override the effects of temperature on primary production at the ecosystem level. Nevertheless, if at higher temperatures resource limitation were to curtail the temperature response of primary production to a greater extent than respiration, as seen in oceanic carbon cycling (López-Urrutia & Moran 2007), we might expect the shift in the metabolic balance to be further amplified over temporal scales relevant to the turnover times of stored organic carbon pools. This is because the large stores of organic carbon in these systems will be available to fuel ER even if contemporary primary production is reduced.

Acclimation is of fundamental importance to any discussion of the potential effects of warming on the metabolic balance of ecosystems (Dewar *et al.* 1999; Melillo *et al.* 2002; Atkin & Tjoelker 2003; Allen *et al.* 2005). It has often been suggested that over temporal scales relevant to the study of the effects of global warming ER must balance GPP (i.e. the ecosystems reach steady state; Gifford 2003; Allen *et al.* 2005). The acclimation of ER to GPP arises from the assumption that oxidative metabolism is ultimately limited by carbon from GPP (Gifford 2003; Allen *et al.* 2005). If this is correct, the consequences of warming revealed in our study may be viewed as transient non-steady-state effects which, in natural ecosystems, would eventually reach metabolic equilibrium. However, the consequences of warming for the carbon balance of natural ecosystems depend fundamentally on the turnover times of the organic carbon pools. For instance, studies of soil organic carbon (SOC) pools suggest that the majority of contemporary respiration is

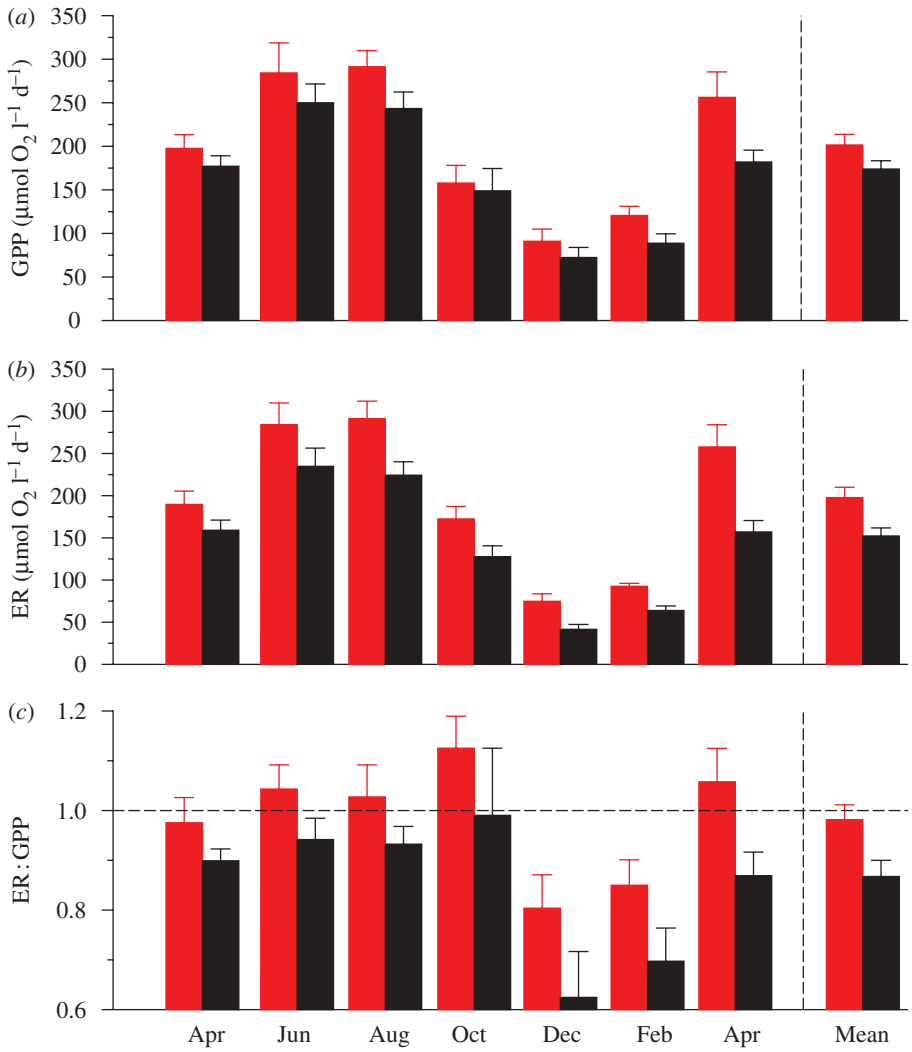

Figure 3. Differences in ecosystem metabolism (±s.e.) between heated (red bars) and unheated (black bars) experimental treatments. Both (*a*) gross primary production, GPP, and (*b*) ecosystem respiration, ER, were consistently elevated in warmed treatments. The magnitude of the increase in ER between warmed and unheated systems was markedly greater than the increase in GPP, reflecting its stronger temperature dependence. Correspondingly, there was a highly significant treatment effect on the (*c*) ER : GPP ratio, such that the metabolic balance of the warmed mesocosms shifted towards heterotrophy, both seasonally and over the whole year (represented by mean annual values). The dotted line represents the metabolic balance (ER = GPP). Warmed ecosystems were net sources of CO_2 to the atmosphere in June, August, October and April (i.e. ER : GPP > 1).

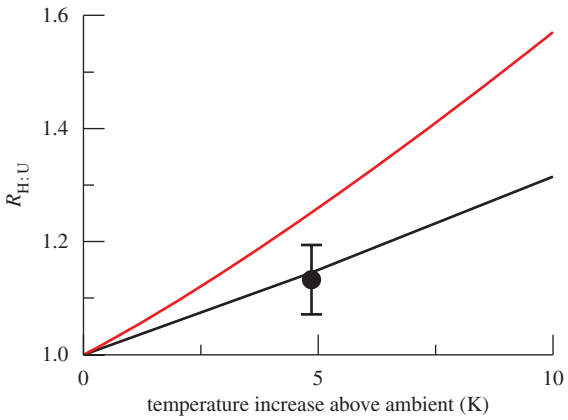

Figure 4. Quantitative changes in the ratio of the metabolic balance between warmed and ambient ecosystems ($R_{H:U}$ in equation (2.6) as temperature, T_H, increases. The black line corresponds to the prediction for the experimentally observed activation energies for respiration, E_r, and photosynthesis, E_p (0.62 and 0.42 eV, respectively). The red line is the prediction for the mean value reported in the literature, based on E_r of 0.65 eV and E_p of 0.32 eV. The dot corresponds to the mean annual value (and 95% CIs) measured empirically in our mesocosms, which is undistinguishable from our theoretical prediction.

driven by organic matter fixed more than 2 years but less than 30 years ago (Trumbore 2000). Furthermore, the effects of warming on soil respiration are most pronounced on the non-labile SOC pools that have large turnover times (decades to centuries), which increases the potential for strong long-term positive feedback to warming (Knorr *et al.* 2005). Given the considerable reserves of 'stored' organic carbon in natural ecosystems (Trumbore 2000; del Giorgio & Williams 2005), particularly in soils and aquatic sediments, any increase in baseline respiration (i.e. respiration uncoupled from contemporary primary production) relative to primary production driven by the

differential activation energies of heterotrophic and autotrophic processes could shift the carbon balance of many ecosystems from being net sinks for atmospheric CO_2 to becoming net sources.

Importantly, and as we have shown in our experiments, ecosystems are likely to exhibit non-steady-state dynamics with respect to carbon sequestration in response to warming. Over geological time-scales these 'transient' dynamics must reach steady state because ultimately ER requires fixed carbon as a substrate. However, understanding the effects of global warming on the carbon sequestration of ecosystems is crucial over much shorter temporal scales, and those which are relevant to the manifestations of positive feedback which may hasten global warming (i.e. decades). In this context, the use of manipulative experiments to inform short-term consequences of warming can be very useful (Benton *et al*. 2007).

6. CONCLUSION

The biotic regulation of atmospheric CO_2 constitutes one of the most important 'ecosystem services' of value to humans (Schroter *et al*. 2005). It is surprising then, that there is still no general consensus as to how the metabolic balance of ecosystems will respond to projected global warming (del Giorgio & Duarte 2002; Knorr *et al*. 2005; López-Urrutia *et al*. 2006; López-Urrutia & Moran 2007). In addressing these problems we have used a combination of ecological theory, tested explicitly in experimental ecosystems. Our approach revealed a fundamental mechanism, ultimately driven by the metabolic rates of individuals, which dictated the effects of temperature on the metabolic balance of ecosystems. Furthermore, our results demonstrate that predicting how the metabolic balance of ecosystems may respond to environmental warming may not require a bespoke model plagued with detail and numerous parameters specific to the system under study. A significant portion of the biological complexity of an ecosystem (Montoya *et al*. 2006; e.g. community composition, trophic architecture) can be reduced to two fundamental parameters: the activation energies for the metabolic processes and temperature. However, given the inherent complexity and diversity of biotic and abiotic factors influencing the dynamics of carbon cycling in natural ecosystems, caution should be exercised in extrapolating our findings in mesocosms to natural systems. The generality of the quantitative predictions developed here to other systems may be achieved after verification in other natural ecosystem types (e.g. terrestrial and marine). Nevertheless, our models and their experimental verification provide an important baseline and foundation for understanding the mechanisms dictating the effects of temperature on the metabolic balance of ecosystems, and for predicting future change.

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