The Temperature Dependence of the Carbon Cycle in Aquatic Ecosystems

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SUMMARY

The carbon cycle modulates climate change via the regulation of atmospheric CO$_2$, and represents one of the most important ecosystem services of value to humans. However, considerable uncertainties remain concerning potential feedbacks between the biota and the climate. We developed theoretical models derived from the metabolic theory of ecology (MTE), and tested them in an ecosystem-level manipulative experiment in freshwater mesocosms. The year-long experiment simulated a warming scenario (A1B; [IPCC, 2007]) expected by the end of the century. The key components of the carbon cycle – that is gross primary production (GPP), ecosystem respiration (ER) and CH$_4$ efflux (ME) – measured in our experiment were all strongly related to temperature. Their temperature dependence was typically constrained by the average activation energy of their particular metabolic pathway, and as predicted by our models, this increased progressively for GPP, ER and ME. Warming of 4 °C decreased the sequestration of CO$_2$ by 13%, increased the fraction of primary production effluxing as methane by 20% and the fraction of ER as methane by 9%, in line with the offset in their respective activation energies. Because methane has 21 times the greenhouse gas radiative potential of CO$_2$, these results suggest aquatic ecosystems could drive a previously unknown positive feedback between warming and the carbon cycle.

We then used a series of global data compilations of measurements of rates of primary production and respiration to better understand the temperature dependence of the carbon cycle in other aquatic ecosystems and to compare them with data from terrestrial systems. Our experimental results were mirrored by our global data compilations, with the effective activation energy for marine and freshwater primary production identical to GPP measured in our experiment. Similarly, the temperature dependences of respiration in estuaries, lakes and the ocean were indistinguishable from that of ER in our experiment. Finally, our study suggests that the temperature dependence of primary production and respiration in aquatic ecosystems might differ from those in terrestrial ecosystems, and this could be crucial in predicting the future response of the carbon cycle in these different systems to global warming.

I. INTRODUCTION

A. The Ecological Consequences of Global Warming

The biosphere is in the midst of a pronounced warming trend: global surface temperatures have already risen by an average of ~0.74 °C over the last century and are projected to increase by a further 3–5 °C over the next
century (IPCC, 2007). Future warming has important consequences for all levels of ecological organisation, from individual organisms to entire ecosystems (Montoya and Raffaelli, 2010; Walther et al., 2002). At the population and species levels, ecological responses to global warming have been demonstrated for a large and growing list of taxa; these responses include extirpation of local populations, latitudinal and altitudinal shifts in the distribution of species populations, and altered phenology (Parmesan and Yohe, 2003; Woodward et al., 2010a,b). At the ecosystem level, however, there is still no firm consensus regarding the magnitudes, types and directions of biotic responses to warming. This uncertainty is often ascribed to the complexity of ecological networks (e.g. food webs) that are embedded within ecosystems, and which is often perceived as confounding in attempts to derive predictions at these higher levels of organisation (Montoya et al., 2006; Perkins et al., 2010; Ptacnik et al., 2010; Purdy et al., 2010; Reiss et al., 2010a,b; Woodward et al., 2010a,b). The composition of the interaction networks in the new communities that will emerge as temperatures rise will be determined by differential rates of range shifts, with certain species moving faster and further than others; however, since the direction and magnitude of a particular species’ response to climate change are often unexpected, such community-level responses are often difficult to predict (Montoya and Raffaelli, 2010; Walther et al., 2002). In contrast, predicting the effects of environmental change on ecosystem-level processes (i.e. primary production, ecosystem respiration, nutrient cycling) might be substantially easier because comparable rates of ecosystem processes can result from vastly different community compositions (Manning et al., 2006; Perkins et al., 2010; Reiss et al., 2010a).

In this chapter we focus on the effects of warming – a key component of future climate change – on ecosystem processes in aquatic systems. Global warming has the potential to affect ecosystem processes and the provisioning of their associated services to humanity (e.g. carbon sequestration, crop production) profoundly, via a variety of direct and indirect mechanisms (Montoya and Raffaelli, 2010; Schroter et al., 2005). Temperature may, for example, directly alter the carbon sequestration capacity and the greenhouse gas efflux potential of ecosystems through its effects on the balance between plant growth and decomposition (Allen et al., 2005; Yvon-Durocher et al., 2010b). Both of these rates exhibit predictable temperature dependencies at the organismal level, which will ultimately determine responses at the whole ecosystem level (Allen et al., 2005; Gillooly et al., 2001; Lopez-Urrutia et al., 2006; Yvon-Durocher et al., 2010a). In terrestrial ecosystems, the potential for strong positive feedbacks between warming and carbon sequestration through temperature-induced enhancement of microbial respiration is well established (Davidson and Janssens, 2006; Knorr et al., 2005). However, its magnitude remains unclear because the
fraction of soil carbon susceptible to enhanced decomposition in response to warming is highly variable between ecosystems (Bellamy et al., 2005; Luo et al., 2001). Given that turnover rates of some soil carbon fractions span many years (Trumbore, 2000), and that a 10% change in total soil organic carbon would be equivalent to all the anthropogenic CO₂ emitted over a 30-year period (Kirschbaum, 2000), temperature-induced enhancement of soil respiration has the potential to affect atmospheric carbon chemistry for decades (Bellamy et al., 2005). Continued warming may also help facilitate decomposition of large quantities of carbon currently stored in permafrost, thereby substantially increasing efflux of carbon dioxide and methane to the atmosphere (Mastepanov et al., 2008).

Recent work in aquatic ecosystems has also highlighted the potential for significant enhancement of respiration rates (Lopez-Urrutia et al., 2006) and associated declines in carbon sequestration (Gudasz et al., 2010), again due to the strong temperature dependence of metabolism. Furthermore, concerns have recently been raised regarding the colossal potential source of methane which lies beneath the sea bed in the form of clathrate methane hydrates which, if they were to become unstable due to warming, could have a catastrophic impact on our climate (Westbrook et al., 2009).

Overall, the potential importance of positive feedbacks between biogeochemical cycles and global warming highlights the need to link biotic responses explicitly to the components of the carbon cycle that are likely to be affected by climate change (Cox et al., 2000; Friedlingstein et al., 2006). This paper outlines several approaches, via the use of bioenergetic models, whole-ecosystem experiments and global meta-analyses, to address the potential for feedbacks between warming and the biogeochemical cycling of carbon in aquatic ecosystems. The models and their experimental verification are primarily derived from, and represent extensions of, the metabolic theory of ecology (MTE) (Allen et al., 2005; Brown et al., 2004; Enquist et al., 2003; Lopez-Urrutia et al., 2006; Yvon-Durocher et al., 2010a,b), which uses fundamental physical and chemical principles to understand and predict fluxes of energy and matter through individuals and ecosystems. We focus on the biotic controls that determine the dynamics of carbon dioxide and methane – the dominant end products of the remineralisation of organic carbon in aquatic ecosystems and the two most important greenhouse gases with potential to cause positive feedbacks with global warming. Our paper is therefore structured around two central questions. First: how and by how much will CO₂ sequestration in aquatic ecosystems be affected by the 4 °C rise in temperature expected by the end of the century (IPCC, 2007)? Second: how and by how much will CH₄ emissions change relative carbon sequestration under the warming scenario expected by the end of the century? We begin by presenting the predictions
of our theoretical models, which attempt to delineate the temperature dependence of the key components of the carbon cycle in aquatic ecosystems – that is, gross primary production (GPP), ecosystem respiration (ER) and methane efflux. We then discuss the degree of concordance between our theoretical predictions and two direct tests of their assumptions. The first of these uses a large-scale freshwater mesocosm experiment as a model system, and the second uses global compilations of data of measurements of primary production and respiration in natural marine and freshwater ecosystems. We conclude each section, which is delineated by a particular metabolic flux, by emphasising the similarities and differences between its temperature dependence in aquatic and terrestrial ecosystems. We then attempt to predict how the balance between GPP, ER, and methane efflux will respond to future warming and test our predictions in our mesocosm experiment. Finally, we conclude by discussing the prospects and challenges for extending these approaches in the future and in a broader context.

B. Carbon Cycling Within an Ecosystem: A Tractable Model

Given that carbon is the universal currency used by biota to store and expend energy (Baird and Ulanowicz, 1989), delineating the factors governing its transformations and fluxes is key to understanding biotic interactions within ecosystems and how they might be affected by environmental warming. We therefore begin by considering the cycling of carbon between the organic (i.e. biotic) and inorganic (i.e. abiotic) pools with a generic and hypothetical standing freshwater ecosystem (Figure 1). In this simplified view, biomass is synthesised either by capturing photons (light energy) via photosynthesis, or by harnessing exergonic redox reactions of inorganic chemical compounds via chemosynthesis. This biomass, which is predominantly composed of organic carbon compounds, is transferred through the food web from autotrophs (i.e. the photo- and chemo-synthesisers) to heterotrophic primary consumers, secondary consumers, and so on via consumption, and also partially to the microbial consortium via senescence and biomass excretion. This detrital biomass is then remineralised by microbes via aerobic and anaerobic metabolism, yielding inorganic constituents. At each step in the transfer of energy (≈biomass), a substantial proportion is lost due to metabolism and heat production (Lindeman, 1942), such that energy availability declines towards the top of trophic pyramids, and this is one of the key reasons why large predators are typically rare (Hutchinson, 1959).
The net carbon balance of an ecosystem is defined as the difference between the gross fixation of CO₂ from the atmosphere through photosynthesis (GPP) and the total metabolism of fixed carbon, which is released back to the atmosphere primarily as CO₂ (ER) and/or methane (CH₄ efflux; ME) (e.g. Yvon-Durocher et al., 2010a,b). In an idealised closed system, where the sizes of the autotrophic and heterotrophic carbon pools and fluxes between them remain constant, the net carbon balance is zero at steady state. Because CO₂ and methane are both greenhouse gases, and therefore have radiative forcing potential, the net carbon balance of ecosystems is crucial to the regulation of global temperature as it determines whether ecosystems act primarily as either sources or sinks of atmospheric CO₂ (Lovelock, 1972;
Whiting and Chanton, 2001; Woodwell et al., 1998). A related concept, the greenhouse gas efflux potential of an ecosystem, concerns the overall effect of a non-zero net carbon balance on potential radiative forcing in the atmosphere (Whiting and Chanton, 2001). Consequently, in the context of biotic feedbacks with climate change, the two most important gaseous end products of the remineralisation of organic carbon are CO₂ and CH₄. Understanding how the carbon balance and greenhouse gas efflux potential of aquatic ecosystems are related to environmental temperature is thus crucial for generating robust predictions from coupled climate–carbon models (Cox et al., 2000; Friedlingstein et al., 2006). Achieving this goal will require a detailed understanding of the mechanisms that control the temperature dependence of the key metabolic process in the carbon cycle at the ecosystem level (i.e. GPP, ER, and CH₄ efflux [ME]). Although recent progress has been made in this area within the last decade, particularly in terrestrial systems (Allen et al., 2005; Davidson and Janssens, 2006; Enquist et al., 2003, 2007; Gudasz et al., 2010; Knorr et al., 2005; Laws et al., 2000; Lopez-Urrutia et al., 2006; Luo et al., 2001; Melillo et al., 2002; Yvon-Durocher et al., 2010a,b), there remains much uncertainty, especially in aquatic ecosystems.

In the terrestrial carbon cycle, the temperature dependence of GPP has been demonstrated to be constrained by the rate of Rubisco carboxylation, which is determined primarily by the concentration of CO₂ at the active site of the enzyme. Therefore, potential differences between aquatic and terrestrial plants (e.g. temperature-dependent changes in aqueous concentration gradient of CO₂ at the site of photosynthesis due to Henry’s Law, or differences in Rubisco kinetics between aquatic and terrestrial plants) may result in a divergence in the temperature dependence of photosynthesis between these systems. In terrestrial ecosystems the temperature dependence of ER is constrained to be equal to that of GPP at steady state because fixed carbon from GPP provides the substrate that fuels ER in these systems, which receive little if any carbon subsidies from adjacent ecosystems. This acclimation of the temperature dependence of ER to GPP over temporal scales relevant to the potential feedbacks between warming and the carbon cycle has led some authors to suggest that the potential positive feedbacks between warming and terrestrial respiration may be weaker than expected from short-term experimental studies (Gifford, 2003; Luo et al., 2001). The long-term temperature sensitivity of respiration in aquatic ecosystems may be complicated by the fact that many aquatic ecosystems (e.g. lakes, streams, rivers, estuaries and coastal seas) receive considerable subsidies of carbon from adjacent ecosystems – for example coastal seas receive carbon from estuaries, estuaries receive carbon from rivers, while lakes, streams and rivers receive carbon from the terrestrial catchments they drain (Cole and
Caraco, 2001; Cole et al., 2000, 2002; del Giorgio and Peters, 1994; del Giorgio et al., 1997; Pace et al., 2004; Ram et al., 2007). Therefore, in many aquatic ecosystems, ER is not necessarily at steady state with respect to GPP and might not be constrained by autochthonous production (Cole et al., 2000; del Giorgio and Peters, 1994; del Giorgio et al., 1997). This simple, yet fundamental, difference may have far reaching consequences for the propagation of positive feedbacks between warming and the carbon cycle in aquatic ecosystems.

C. Predicting the Effects of Warming: Combining Theory, Experiments and Empirical Data

Metabolic rate is the speed at which an organism uptakes energetic and material resources from its environment, transforms them into useable forms, and provisions them to the biochemical processes necessary for growth, survival, and reproduction (Brown et al., 2004). Thus, it is a fundamental determinant of the contribution an individual organism makes to the overall flux of energy and materials in an ecosystem (Brown et al., 2004). The MTE describes how two key variables, body size and temperature, influence the metabolic rates of cells, individual organisms, communities and ecosystems (e.g. Allen et al., 2005). Therefore, despite concerns raised by its critics (Clarke, 2004; Kozlowski and Konarzewski, 2005; Makarieva et al., 2005) the MTE provides a potentially useful framework to build a predictive understanding of the feedbacks between warming, and the biogeochemical cycling of carbon within ecosystems (Allen et al., 2005; Lopez-Urrutia et al., 2006; Yvon-Durocher et al., 2010a,b), and forms the basis of much of the theoretical component of this chapter.

The experimental component of this study involved simulating the potential effects of future warming on aquatic ecosystems using a replicated, ecosystem-level manipulation in freshwater mesocosms (Figure 2). The experimental design is described in detail in Section III (Yvon-Durocher et al., 2010a,b). Briefly, the experiment was conducted using 20 freshwater mesocosms, 10 of which were maintained at 3–5 °C above ambient temperature (mean 4 °C), in line with the A1B warming scenario predicted for temperate latitudes by the end of the twenty-first century (IPCC, 2007). The remaining 10 were maintained at ambient temperature, but were otherwise identical, and served as unheated controls (Yvon-Durocher et al., 2010a,b).

Mesocosm experiments represent an inevitable compromise between the control and replication of laboratory studies and the realism of
descriptive field surveys. Despite their potential limitations, because they allow cause-and-effect relationships to be identified in relatively realistic settings they represent a useful tool for predicting how global change scenarios might affect ecosystem-level processes that cannot be achieved using laboratory studies or field surveys (Benton et al., 2007; Woodward et al., 2010a,b). In particular, they afford the opportunity to isolate the effects of temperature from other potentially confounding variables (e.g. latitude, altitude and nutrient availability) and permit direct comparisons to be made between ecosystems under ambient and warmed conditions from the perspective of both applied and basic science. Such results could provide much-needed insight into the potential future consequences of warming on aquatic ecosystems and from a basic science perspective, when interpreted in light of theoretical predictions, they can aid in developing more mechanistic, predictive capabilities for addressing future climate change and for understanding fundamental relationships between community structure and ecosystem functioning (Woodward et al., 2010a,b).

Because mesocosm experiments are limited in their spatial and temporal extent, caution must be exercised when extrapolating their findings to natural systems and other ecosystem types (e.g. lakes, rivers and the coastal and open ocean). To address these potential caveats related to generality and ecological scales of investigation, we also compiled a broad database that combined many previously published global data compilations of measurements of respiration and primary production across a range of aquatic ecosystems (e.g. lakes, streams, estuaries and the open ocean) to further scrutinise our theoretical models and the application of our experimental findings to natural ecosystems.

Figure 2  (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. Figure from Yvon-Durocher et al. (2010a)
D. Aims of the Study

The overarching goal of this study was to develop a more predictive understanding of how temperature regulates carbon cycling in aquatic ecosystems and to provide at least an initial glimpse of potential responses to future warming. More specifically, our objectives were:

1. To understand the mechanisms controlling the temperature dependence of GPP, ER and the efflux of CH₄ to the atmosphere (i.e. the three key metabolic fluxes in the aquatic carbon cycle).
2. To understand the mechanisms that control the temperature dependence of balance between GPP and ER, which determines the carbon sequestration capacity of aquatic ecosystems and to gain predictive capabilities to assess how this balance might respond to future global warming scenarios.
3. To develop a mechanistic understanding of how the rate of methane efflux in relation to GPP (i.e. CO₂ fixation) and ER (CO₂ efflux) will be affected by warming. Together, this balance determines the greenhouse gas efflux potential of aquatic ecosystems.
4. To compare potential differences between these processes in aquatic and terrestrial ecosystems.

II. THEORETICAL FRAMEWORK

A. Carbon Fluxes at the Individual-Level

At the level of an individual organism, carbon fluxes occur during photosynthesis, respiration and methanogenesis, all of which have predictable mass $M_i$ and temperature $T$ (K) dependencies, which can, in theory, ultimately be scaled up to the entire ecosystem (Brown et al., 2004; Gillooly et al., 2001):

$$B_i = b_0 e^{-E_i/kT} M_i^\alpha$$

In this expression, $B_i$ is the basal metabolic rate of individual $i$ (g carbon s⁻¹), $\alpha$ is a scaling exponent that typically takes a value near 0.75 (Savage et al., 2004), and $b_0$ is a normalization constant independent of mass and temperature (g carbon g⁻α s⁻¹). For what follows, we will replace $b_0$ with metabolic pathway-specific normalization constants of $r_0$, $p_0$, and $m_0$, which correspond to respiratory, photosynthetic, and methanogenic fluxes of heterotrophs, autotrophs, and methanogens, respectively. The Boltzmann–Arrhenius factor, $e^{-E_i/kT}$, describes the exponential effect of temperature on metabolic rate, where $k$ is Boltzmann’s constant (8.62 × 10⁻⁵ eV K⁻¹) and $E_i$ is the average activation energy of the $i$th metabolic pathway. The Boltzmann–Arrhenius
factor quantifies how the speed of a reaction (e.g. ATP turnover in respiration) is governed by the average kinetic energy (i.e. temperature) of molecules, which must collide with a force exceeding the activation energy barrier, $E$, required for a given reaction to proceed.

The magnitude of the activation energy, $E$, varies depending on the metabolic pathway. Therefore, we will use $E_r$, $E_m$ and $E_p$ to characterize to the temperature dependence of aerobic respiration, methanogenesis and photosynthesis, respectively. The average activation energy of heterotrophic respiratory metabolism is $E_r \approx 0.65$ eV (Gillooly et al., 2001), which corresponds to a 15-fold increase in rates from 0 to 30 °C, whereas methanogenesis increases by about 35-fold (activation energy $E_m \approx 0.85$ eV, Yvon-Durocher et al., 2010b). In contrast, photosynthesis increases only about four-fold over this temperature range (effective activation energy of $E_p \approx 0.32$ eV, Allen et al., 2005). We refer to $E_p$ as an “effective” activation energy because the temperature dependence of C3 photosynthesis is hyperbolic (Medlyn et al., 2002), rather than exponential, due primarily to the process of photorespiration leading to the presence of temperature optima (Badger and Collatz, 1977). Using $E_p$ as an approximation allows for direct comparison between the temperature dependences of photosynthesis with those of respiration and methanogenesis (Allen et al., 2005; Lopez-Urrutia et al., 2006). This approximation was derived by Allen et al. (2005) using a well-established model of C3 photosynthesis (Farquhar et al., 1980) based on assumptions that are reasonable for terrestrial plants (internal CO$_2$ concentrations are about 70% of ambient, co-limitation of photosynthesis by Rubisco, similar kinetic properties for Rubisco across species).

### B. Relating Individual-Level Fluxes to Ecosystem Processes: The Temperature Dependence of the Aquatic Carbon Cycle

Metabolic flux at the ecosystem level is equal to the sum of the metabolic fluxes of all its constituent individual organisms, and this knowledge may help in understanding important aspects of the structure and functioning of ecosystems (Allen et al., 2005; Brown et al., 2004; Enquist et al., 2003; Lopez-Urrutia et al., 2006; Yvon-Durocher et al., 2010a). Here we consider how each of the three fundamental components of the carbon cycle (GPP, ER and ME) could potentially respond to warming.

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; Lopez-Urrutia et al., 2006; Yvon-Durocher et al., 2010a), following Eq. 1:
In this expression, \( N_p \) is the number of photosynthetic organisms in an ecosystem of volume \( V \), \( M_{TOT}^p = \frac{1}{V} \sum_{i=1}^{N_p} M_i \) is the total biomass of photosynthetic organisms per unit volume, and \( \langle M^{1-\omega} \rangle_p = \sum_{i=1}^{N_p} M_i^{3/4} / \sum_{i=1}^{N_p} M_i \) is an average for body size (Allen et al., 2005; Lopez-Urrutia et al., 2006). By taking logs of both sides of Eq. 2 we can derive a general expression for the temperature dependence of GPP:

\[
\ln(GPP) = -E_p \left( \frac{1}{kT} \right) + \ln \left[ p_0 M_{TOT}^p \langle M^{1-\omega} \rangle_p \right]
\] (3)

GPP is the gross fixation of CO\(_2\), and thus is equal to the sum of net primary production

\[
NPP = \varepsilon GPP = \varepsilon p_0 e^{-E_p/kT} M_{TOT}^p \langle M^{1-\omega} \rangle_p
\] (4)

and autotroph respiration

\[
AR = (1 - \varepsilon)GPP = (1 - \varepsilon)p_0 e^{-E_p/kT} M_{TOT}^p \langle M^{1-\omega} \rangle_p
\] (5)

where GPP = NPP + AR, and \( \varepsilon \) is the fraction of photosynthate allocated to net primary production. Equation 5 is consistent with the observation that autotrophic respiration is ultimately limited by, and therefore tightly coupled to, photosynthesis (Atkin and Tjoelker, 2003; Dewar et al., 1999). Consequently, whilst the temperature dependence of autotrophic respiration has an activation energy of \( E_r \) over the short term, it is ultimately constrained to equal to that of photosynthesis, \( E_p \), over longer time scales (Allen et al., 2005). This process, which is referred to as type I respiratory acclimation, has been observed empirically (Atkin and Tjoelker, 2003) and experimentally (Dewar et al., 1999). We therefore assume that autotroph respiration has an activation energy of \( E_p \) for the derivation that follows.

In a similar way, we can readily obtain ecosystem-level expressions for heterotroph respiration

\[
HR = r_0 e^{-E_r/kT} M_{TOT}^r \langle M^{1-\omega} \rangle_r
\] (6)

by summing the respiratory contributions of the heterotrophs (Allen et al., 2005; Enquist et al., 2003; Lopez-Urrutia et al., 2006; Yvon-Durocher et al., 2010a) where \( M_{TOT}^r \) is the total biomass of heterotrophs and \( \langle M^{1-\omega} \rangle_r \) is calculated based on the size distribution of heterotrophs. The total rate of ER is readily obtained by combining Eqs. 5 and 6:

\[
ER = AR + HR = (1 - \varepsilon)p_0 e^{-E_p/kT} M_{TOT}^p \langle M^{1-\omega} \rangle_p + r_0 e^{-E_r/kT} M_{TOT}^r \langle M^{1-\omega} \rangle_r
\] (7)
Note that in contrast to the previous equations, the temperature dependence of ER is not governed by a single activation energy because it includes contributions from both heterotrophic and autotrophic organisms. At steady state, ER is limited by substrate availability, and must therefore equal GPP. Here, we define steady state as the tendency for ER to equal GPP over time periods of 1 year or greater. During the short term - that is over diurnal or seasonal cycles - the carbon balance frequently deviates from steady state (e.g. during the spring bloom in aquatic ecosystems) and GPP can exceed ER (or vice versa), though over the year at steady state this is no net gain or loss of carbon. However, when an ecosystem deviates from steady state (i.e. ER < GPP or ER > GPP), ER is no longer constrained by GPP. During long term (i.e. a year or greater) non-steady state dynamics, such as those that may potentially occur in our experiment (i.e. because warming is expected to act as a perturbation), heterotrophic respiration may exceed NPP (i.e. the potential contemporary carbon substrate) over temporal scales dependent on the turnover time of the carbon stores in the ecosystem. Further, this situation of non-steady state dynamics might exist more generally in aquatic ecosystems provided that they hold sufficient stored carbon or receive significant allochthonous subsidies to fuel heterotrophic metabolism (Cole et al., 2000, 2002; del Giorgio and Peters, 1994; del Giorgio et al., 1997; Pace et al., 2004; Ram et al., 2007). Under such conditions heterotrophic metabolism tends towards maximum capacity. Therefore, during non-steady state dynamics, because $E_r > E_p$, ER is expected to have a temperature dependence approaching that of heterotrophic metabolism, $E_r$ – that is greater than the activation energy for GPP. Thus, assuming that ER and GPP are not at steady state, we can make the simplifying assumption that the term for AR in Eq. 7 is insignificant relative to HR in ER and remove it. We then have:

$$\ln(ER) = -E_r \left(\frac{1}{kT}\right) + \ln[r_0 M^m_{TOT} \langle M^{1-z}\rangle_m]$$

which is a general expression for the temperature dependence of ER at non-steady state.

An ecosystem-level expression for methane production can be obtained from summing the respiratory contributions of the methanogens in the ecosystem

$$MP = m_0 e^{-E_m/kT} M^m_{TOT} \langle M^{1-z}\rangle_m.$$  

where $M^m_{TOT}$ is the total biomass of methanogens, and $\langle M^{1-z}\rangle_m$ is calculated based on the size distribution of the methanogens. Here, we are attempting to understand how biogeochemical feedbacks will respond to global warming and to predict how the net efflux of CH$_4$ to the atmosphere behaves in response to temperature. There is, however, an important distinction
between CH$_4$ efflux and production (MP). CH$_4$ efflux (ME) to the atmosphere is the net result of the difference between CH$_4$ production in the anaerobic zone of the sediment and the oxidation of CH$_4$ by methanotrophs in the oxic layers of the sediment and the overlying water column (Segers, 1998). CH$_4$ oxidation has the potential to oxidize \( \sim 90\% \) of CH$_4$ produced in the sediments of lakes and wetlands (Schutz et al., 1989) and substantially reduce net CH$_4$ efflux. In our model, we make the simplifying assumption that although CH$_4$ oxidation can considerably reduce net CH$_4$ efflux, it has little effect on its temperature dependence (after Yvon-Durocher et al., 2010b). As such, we expect CH$_4$ efflux to be a constant proportion of CH$_4$ production with temperature, therefore the temperature dependence of CH$_4$ efflux should be equivalent to the activation energy for methanogenesis. Given the assumption that CH$_4$ oxidation has little effect on the temperature dependence of CH$_4$ efflux (ME), we can derive a general expression for the temperature dependence of ME by taking logs of both sides of Eq. 9, after Yvon-Durocher et al. (2010b)

\[
\ln(\text{ME}) = -E_m \left( \frac{1}{kT} \right) + \ln \left[ m_0 M^m_{\text{TOT}} \langle M^{1-z} \rangle_m \right] \quad (10)
\]

Equations 2–10 predict the temperature dependence of GPP, ER and MP (and therefore CH$_4$ efflux [ME]) and highlight the importance of the activation energies of sub-cellular metabolism in controlling the temperature response of ecosystem-level flux rates. The theory above provides a platform from which a mechanistic understanding of the potential consequences of global warming on the carbon balance of ecosystems can be drawn and leads to a number of important predictions, which we tested experimentally. First, the temperature dependence of GPP, ER and ME will be governed by their respective activation energies at the cellular level. Therefore, the relationship between \( \ln(\text{GPP}) \) and \( 1/kT \) should approximate a slope of \( E_p \sim 0.32 \text{ eV} \), \( \ln(\text{ER}) \) (after Allen et al., 2005). Assuming non-steady state dynamics, the temperature dependence of ER should be greater than that of GPP and the slope of the relationship between \( \ln(\text{ER}) \) and \( 1/kT \) should approach the activation energy of heterotrophic metabolism, \( E_r \sim 0.65 \text{ eV} \) (after Gillooly et al., 2001). The temperature dependence of CH$_4$ efflux should be governed by the activation energy of methanogenesis. Thus, the slope of the relationship between \( \ln(\text{ME}) \) and the reciprocal of absolute temperature \( 1/kT \) should approximate a slope equal to \( E_m \sim 0.88 \text{ eV} \) (after Yvon-Durocher et al., 2010b). Finally, and most importantly, because of the differential temperature dependences of these three processes, warming is expected to shift the overall carbon balance of ecosystems, thereby altering rates of carbon sequestration, greenhouse gas emission and thus potential biotic–abiotic feedbacks. We test these theoretical predictions using data
from our mesocosm experiment and global data compilations of primary production and respiration in natural ecosystems.

C. Relating Individual-Level Fluxes to Ecosystem Processes: The Carbon Balance

With an understanding of the mechanisms controlling the temperature dependence of GPP, ER and ME, using the above theory it is possible to predict the temperature dependence of the carbon balance of aquatic ecosystems. Here we will focus on the metabolic balance (ER/GPP), which is the ability of an ecosystem to sequester carbon, the balance between ME and GPP, and that of ME and ER, which determines the greenhouse gas efflux potential of aquatic ecosystems. The metabolic balance is defined as ER/GPP and combining Eqs. 3 and 8, we have:

\[
\frac{\text{ER}}{\text{GPP}} = \frac{r_0 e^{-E_r/kT} M_{\text{TOT}}^{r} (M^{1-z})_r}{p_0 e^{-E_p/kT} M_{\text{TOT}}^{p} (M^{1-z})_p}
\]

This can be simplified to

\[
\frac{\text{ER}}{\text{GPP}} = \frac{r_0}{p_0} (E_r - E_p) \frac{M_{\text{TOT}}^{r} (M^{1-z})_r}{M_{\text{TOT}}^{p} (M^{1-z})_p}
\]

which yields a general expression for the temperature dependence of the ER/GPP ratio during non-steady state dynamics where ER is not constrained by GPP. Similarly we can derive non-steady state solutions for the temperature dependence of the ME/GPP ratio by combining Eqs. 3 and 10:

\[
\ln(\text{ME}/\text{GPP}) = E_m - E_p \left( \frac{1}{kT} \right) + \ln \left[ \frac{r_0}{p_0} \times \frac{M_{\text{TOT}}^{m} (M^{1-z})_m}{M_{\text{TOT}}^{p} (M^{1-z})_p} \right]
\]

and the ME/ER ratio by combining Eqs. 8 and 10:

\[
\ln(\text{ME}/\text{ER}) = E_m - E_r \left( \frac{1}{kT} \right) + \ln \left[ \frac{m_0}{r_0} \times \frac{M_{\text{TOT}}^{m} (M^{1-z})_m}{M_{\text{TOT}}^{r} (M^{1-z})_r} \right]
\]

Equations 13–15 predict that in ecosystems where autochthonous carbon production (i.e. GPP) and consumption (i.e. ER and ME) are uncoupled, either through a perturbation (e.g. long-term changes in temperature) or by
allochthonous carbon subsidies, as is frequently prevalent in freshwater, estuarine and coastal ecosystems (Cole et al., 2000, 2002; del Giorgio and Peters, 1994; del Giorgio et al., 1997; Pace et al., 2004; Ram et al., 2007), the temperature dependence of the carbon balance of aquatic ecosystems will be governed by the differences in the activation energies of the key carbon fluxes. Therefore, our models predict that ln(ER/GPP) should approximate a linear function of 1/kT with a slope of $E_r - E_p$. While ln(ME/GPP) versus 1/kT should have a slope equal to $E_m - E_p$ and ln(ME/ER) versus 1/kT should have a slope of approximately $E_m - E_r$. We test these theoretical predictions using our mesocosm experiment.

### III. MATERIALS AND METHODS

#### A. Study Site and Experimental Design

The outdoor mesocosm experiment was based at the Freshwater Biological Association River Laboratory (2°10′W, 50°13′N) in East Stoke, Dorset, UK. Twenty artificial ponds, each holding 1 m$^3$ of water were set up to mimic shallow lake ecosystems (Figure 2): this scale of mesocosm is designed to reproduce many of 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). Ten of the 20 ponds were warmed 3–5°C above ambient temperature, in accordance with the IPCC A1B 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. Temperatures were logged every 5 min over the entire year using HOBO temperature-irradiance data loggers and regular adjustments were made to ensure that temperature differences between treatments were $±4°C$. The mean annual temperature difference between treatments was 4.1°C ± SE 0.01 (Yvon-Durocher et al., 2010a).

The mesocosms were seeded in December 2005 with organic substrates and a suite of organisms that included representative species from primary producers (phytoplankton, macrophytes) to top predators (Roach, Rutilus rutilus), and a suite of intermediate invertebrate consumers (zooplankton, including cladocerans, copepods and rotifers, and benthic macroinvertebrates, including Mollusca, Malacostraca, Trichoptera, Ephemeroptera and Odonata) to mimic, as far as possible, the general organismal composition, trophic complexity and physical structure of shallow lake ecosystems. The biota was left to establish for 10 months prior to the onset of experimental warming, which commenced in September 2006, thereby allowing time for
further natural colonisation before the start of the annual sampling period in April 2007. Populations of the introduced top predator, *R. rutilus* were maintained at constant densities [two individuals (age 1+) per mesocosm (~12 g carbon m$^{-3}$)] in all mesocosoms and monitored via regular electrofishing surveys. Because the fish were maintained at predetermined biomass-densities they merely served to ‘complete’ the food webs to mimic natural shallow lakes and are not considered further here.

**B. Measuring Primary Production and Respiration**

GPP and ER were measured over a 24 h diel cycle for each replicate of each treatment on alternate months over the course of 1 year (April 2007 to April 2008) using the dissolved oxygen (DO) change technique (Marzolf *et al.*, 1994; Mulholland *et al.*, 2001), resulting in a total of 140 measurements of each. This technique assumes that changes in DO concentration over a diel cycle represent the metabolic activity (photosynthetic and respiratory) of an aquatic ecosystem. To measure the concentration of DO in the mesocosms, YSI 600XLM multiparameter Sondes equipped with 6562 rapid pulse DO sensors were deployed in each heated and unheated treatment pair on each of the seven sampling occasions over the year. Measurements of DO, temperature and pH were taken every 15 min for 24 h at mid depth (0.25 m) in the water column of each pond. At the beginning of each sampling occasion the calibration of each Sonde was tested by deploying both Sondes in the same pond for 1 h to ensure equivalence in DO readings, and re-calibration was carried out when necessary. Subsequently, prior to deployment in each treatment pair, the Sondes were calibrated in water-saturated air with a correction for barometric pressure. Calibration accuracy was verified by monitoring the DO concentration of water-saturated air for 10 min and checking against 100% O$_2$ saturation for the measured temperature and pressure (Yvon-Durocher *et al.*, 2010a).

The record of continuous DO measurements was used to calculate the GPP and ER for each mesocosm on each sampling occasion, after Yvon-Durocher *et al.* (2010a). The dissolved oxygen 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. $t_2 - t_1$). 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 first night and extended for 24 h to include the minimum O$_2$ concentration on the second 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 (Bales and Nardi, 2007; Yvon-Durocher *et al.*, 2010a). Each O$_2$ change value was then assigned
to a day or night-time category. Subsequently the metabolic parameters were calculated by numerical integration. GPP was calculated as:

$$GPP = \sum \Delta O_{2\text{day}} + R_{\text{day}}$$

(16)

where $R_{\text{day}}$ is day-time respiration. Since it is impossible to directly measure $R_{\text{day}}$, it was estimated, in keeping with the literature, by extrapolating the mean night time respiration value across the hours of daylight (Bales, 2007; Marzolf et al., 1994; Mulholland et al., 2001). ER was calculated as (see appendix details):

$$ER = R_{\text{day}} + \sum \Delta O_{2\text{night}}$$

(17)

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 (9 measurements were removed from a total of 140; $n = 131$). Current biogeochemical techniques presently preclude the disentanglement of autotrophic and heterotrophic respiration at the ecosystem level (Mulholland et al., 2001) and rule out the estimation of photorespiration (Marzolf et al., 1994). Consequently, measures of GPP using the DO change technique may be slightly overestimated given the inclusion of heterotrophic respiration in calculation of $R_{\text{day}}$.

Benthic respiration (oxygen uptake) was measured using dark in situ benthic chambers which enclosed a sample of 500 mL at the sediment–water interface. A magnetic stirrer in the chamber ensured that the sample was evenly mixed. Benthic respiration was measured by the removal of 25 mL samples at the beginning and the end of the 6 h incubations. The samples were gently discharged into gas-tight vials (12 mL, Exetainers, Labco Ltd, High Wycombe, UK) and allowed to overflow twice (to minimize atmospheric gas exchange), and fixed for later Winkler analysis. The samples were immediately fixed and stored in a fridge at 5°C to minimize light and temperature fluctuations until they could be titrated in the laboratory (<5 days). To ensure linearity of oxygen uptake an initial timed series of samples was taken, subsequently only $T=0$ and $T=\text{final}$ samples were taken to limit sample extraction from the chambers. Oxygen uptake was then calculated as the difference between $T=\text{final}$ and $T=0$ samples and the duration of the incubation.

C. Measuring Methane Efflux

Measurements of the efflux of CH$_4$ were made simultaneously to those GPP and ER, after Yvon-Durocher et al. (2010b). A single gas chamber was positioned at the water surface of each mesocosm on each sampling occasion. The
chambers were made of polycarbonate and enclosed a headspace (300 mL) of ambient air at the air–water interface of the mesocosm [see Yvon-Durocher et al. (2010b) for details]. The lid of the gas chamber was equipped with a Teflon septum port, through which samples of gas (1 mL) were removed using a gas-tight syringe (2 mL VICI gas tight syringe) every 15 min for the first hour of the incubation, then hourly for up to 10 h thereafter. The samples were then transferred to water-filled gas-tight vials (3 mL, Exetainers; Labco, High Wycombe, UK) through a two-way valve venting through a narrow bore needle. The gas-tight vials were then stored upside down prior to analysis.

The concentration of CH$_4$ in the headspace of the sample was determined by gas chromatography as follows. Samples (50 µL) were withdrawn from the headspace of the sample vials and injected into a gas-chromatograph fitted with a flame ionising detector (GC/FID; Agilent Technologies, UK). Headspace concentrations of CH$_4$ were calculated from peak areas calibrated against known standards (Scientific and Technical gases, Staffs, UK) and the total amount of CH$_4$ in the gas tight vial (water plus headspace) was calculated using the appropriate solubility coefficients (Yamamoto et al., 1976). The efflux of CH$_4$ across the water–air interface was calculated by ordinary least squares regression analysis of the change in concentration of CH$_4$ in the chamber headspace over time. Subsequently, 1 h was used as an appropriate duration for accurately estimating the flux of CH$_4$ (Lambert and Frechette, 2005). Regression slopes with a significance of $P > 0.05$ and/or an $R$-squared of below 0.9 were considered non-significant and were excluded from further analyses (9 from the 140 individual flux measurements).

D. Dissolved Methane

The concentration of dissolved CH$_4$ in the water column was measured by removing a water sample (30 mL in a gas-tight syringe) and gently transferring it to a gas tight vial (12.5 mL Exetainers, Labco, High Wycombe, UK), allowing it to overflow, fixing it with a bactericide (100 µL 50%, w/v, ZnCl$_2$) and sealing it. Samples were collected at hourly time intervals (in total 6–10 h depending on the time of year) over a day for each replicate on alternate months for 1 year (April 2007 to April 2008, $n = 1416$ individual measurements). Upon return to the laboratory, a headspace (2 mL analytical grade helium) was introduced to the gas-tight vial and the sample was shaken vigorously for 0.5 min and then allowed to stand for a further 30 min to allow for headspace equilibration, before analysis of the headspace concentration of CH$_4$ using a gas chromatograph as described above, see also (Sanders et al., 2007; Yvon-Durocher et al., 2010b). Finally, the 1416 measurements were pooled in each case to give an average daily pool of dissolved CH$_4$ for each pond (140 measures over the year for 70 heated and 70 ambient).
E. Statistical Analyses

All experimental data were checked for normality using the Shapiro Wilks test for normality and were natural log or log_{10} transformed prior to statistical analysis, where necessary. The activation energy of any metabolism is given by the slope of the relationship of an Arrhenius plot between ln(x flux) and 1/kT, where k is Boltzmann’s constant and T is absolute temperature (K). Here we replace the 1/kT with 1/k(1/T – 1/T_c): this transformation centres the inverse temperature data at zero to make the intercept of the linear model equal to the flux rate at T_c (here we have chosen 15 °C). This greatly reduces the correlation between the activation energy and the intercept and makes the intercept more biologically meaningful. The activation energy of ln(CH_4 efflux), ln(GPP) and ln(ER) was determined by linear mixed effects models using the lme function in R (R Development Core Team, 2006). In the models, treatment and sampling occasions were treated as fixed effects, while a random effects term in which pond identity was nested within sampling occasion, accounted for temporal pseudo-replication due to repeated sampling of the mesocosms over the year. The most parsimonious model to describe the temperature dependence of metabolism was obtained by first fitting the most complex model (i.e. different slopes for each treatment and sampling occasion) to test for statistical differences in the slopes of each of these relationships between treatments and sampling occasions; subsequently, non-significant terms were deleted to give the best model to describe the data. Model comparison was carried out using the Akaike Information Criterion (AIC). The activation energies of the literature compilation data for primary production and respiration were determined by ordinary least squares regression.

Between-treatment differences in the overall mean annual values of GPP, ER, CH_4 efflux, inorganic nutrients, gas transfer velocities, ER/GPP, CH_4 efflux /GPP and CH_4 efflux /ER were also analysed using the lme (linear mixed-effects model) function in R (R Development Core Team, 2006). In the models, treatment (heated or unheated) was treated as the fixed effect, and temporal pseudo-replication from repeated sampling of the mesocosms seasonally over the year was accounted for by including mesocosm identity nested with sampling occasion as random effects. The repeated measures model was used to test for overall statistical differences between treatments in mean annual values of the above parameters.

F. Literature Data Compilation and Meta-Analysis

Data for lake and stream primary production were extracted from Morin et al. (1999), and data for oceanic primary productivity were taken from the ocean primary productivity working group (OPPWG http://marine.rutgers.
Within this extensive data repository we used only data from the most comprehensive survey of oceanic primary production currently available, the MARMAP (1978–1982) survey, to minimise potential between-study methodological biases. Data for lake respiration were taken from Gudasz et al. (2010), and data for pelagic estuarine respiration data were from Hopkinson and Smith (2005). Marine pelagic bacterial respiration data were taken from a large database on bacterial metabolism (http://www.uea.ac.uk/env/people/facstaff/robinsonc) in Robinson (2008) and from Rivkin and Legendre (2001). To investigate the effects of resource acclimation on the temperature dependence of ER further we divided the data on marine bacterial respiration into those from coastal seas (i.e. within the continental shelf; e.g. the North Sea) which are likely to receive allochthonous carbon subsidies, and those from the open ocean (i.e. beyond the continental shelf; e.g. the North East Atlantic ocean) which are unlikely to receive allochthonous carbon subsidies from estuaries.

IV. RESULTS

A. Ecosystem-Level Carbon Fluxes: Experimental Tests

Data from our mesocosm experiment broadly supported our theoretical predictions. Rates of GPP were strongly related to temperature (Table 1) and a plot of ln(GPP) versus 1/k(1/T – 1/Tc) revealed that its activation energy was 0.45 eV (Figure 3A; 95% CI 0.38–0.53 eV). This value was steeper than predicted [0.32 eV; Allen et al. (2005)], based on assumptions made for C3 photosynthesis, and might reveal fundamental differences between aquatic and terrestrial photosynthesis. ER was also strongly related to temperature (Table 1) and its activation energy in a plot of ln(ER) versus 1/k(1/T – 1/Tc) was 0.62 eV (Figure 3B; 95% CI 0.55–0.69 eV). The activation energy for ER was statistically indistinguishable from the predicted value of 0.65 eV (Gillooly et al., 2001) and revealed that our assumption of long-term non-steady-state dynamics in response to experimental warming was validated because the temperature dependence of ER was not constrained by GPP over the year (Table 2). Finally, ME was similarly strongly related to temperature (Table 1), and a plot of ln(ME) versus 1/k(1/T – 1/Tc) revealed that its activation energy (Figure 3C; 95% CI 0.64–1.02 eV) was also indistinguishable from the predicted value (≈0.88 eV) based on the activation energy of methanogenesis (Yvon-Durocher et al., 2010b). This finding also provides strong support for our model assumption that the temperature dependence of ME and CH4 production (MP) are equivalent and that CH4 oxidation has little effect on the temperature dependence of ME.
The three fundamental metabolic fluxes of the carbon cycle exhibited strong seasonal trends in the mesocosm experiment (Figure 4). For example, flux rates of GPP, ER, and ME peaked in early summer and were lowest in winter, presumably reflecting the temperature dependence of metabolism. Rates of benthic respiration, however, exhibited slightly different seasonal dynamics with rates peaking in late summer and autumn (Figure 4D). For all metabolic fluxes, the mean annual values were significantly elevated in the warmed mesocosms (Table 3).

As predicted from our models, the key metabolic fluxes of the carbon cycle had sequentially greater activation energies (i.e. $GPP = 0.45 \text{ eV} < ER = 0.62 \text{ eV} < ME = 0.85 \text{ eV}$) in the mesocosm experiment, that were predictable from the sub-cellular kinetics of their particular metabolic pathways.

### Table 1  Results from Linear Mixed Effects (lme) models

<table>
<thead>
<tr>
<th>Relationship</th>
<th>d.f.</th>
<th>$F$ ratio</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\ln(\text{CH}_4 \text{ efflux}) \text{ vs. } 1/kT$</td>
<td>1,123</td>
<td>97.98</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>$\ln(\text{GPP}) \text{ vs. } 1/kT$</td>
<td>1,123</td>
<td>146.6</td>
<td>&lt;0.0001</td>
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<tr>
<td>$\ln(\text{ER}) \text{ vs. } 1/kT$</td>
<td>1,123</td>
<td>294.85</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Difference in slope of $\ln(\text{CH}_4 \text{ efflux})$ vs. $1/kT$ between treatments</td>
<td>1,123</td>
<td>1.23</td>
<td>0.23</td>
</tr>
<tr>
<td>Difference in intercept of $\ln(\text{CH}_4 \text{ efflux})$ vs. $1/kT$ between treatments</td>
<td>1,123</td>
<td>2.29</td>
<td>0.132</td>
</tr>
<tr>
<td>Difference in slope of $\ln(\text{GPP})$ vs. $1/kT$ between treatments</td>
<td>1,123</td>
<td>0.23</td>
<td>0.82</td>
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<tr>
<td>Difference in intercept of $\ln(\text{GPP})$ vs. $1/kT$ between treatments</td>
<td>1,123</td>
<td>2.55</td>
<td>0.11</td>
</tr>
<tr>
<td>Difference in slope of $\ln(\text{ER})$ vs. $1/kT$ between treatments</td>
<td>1,123</td>
<td>0.56</td>
<td>0.46</td>
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<tr>
<td>Difference in intercept of $\ln(\text{ER})$ vs. $1/kT$ between treatments</td>
<td>1,123</td>
<td>2.27</td>
<td>0.13</td>
</tr>
<tr>
<td>Difference in slope between $\ln(\text{CH}_4 \text{ efflux})$ vs. $1/kT$ and $\ln(\text{GPP})$ vs. $1/kT$</td>
<td>1,254</td>
<td>21.61</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Difference in slope between $\ln(\text{CH}_4 \text{ efflux})$ vs. $1/kT$ and $\ln(\text{ER})$ vs. $1/kT$</td>
<td>1,254</td>
<td>8.36</td>
<td>0.0042</td>
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<tr>
<td>Difference in slope between $\ln(\text{ER}) \times \ln(\text{GPP})$ vs. $1/kT$</td>
<td>1,254</td>
<td>3.2</td>
<td>0.0015</td>
</tr>
</tbody>
</table>

The first set of models test for relationships between ecosystem-level metabolic rates (GPP, ER or ME) and temperature [$1/k(1/T_1 - 1/T_2)$], parallelism between treatments, and differences between intercepts in the linear models. Metabolic rates are used as dependent variables, temperature [$1/k(1/T - 1/T_c)$] as the independent variable, while treatment (warmed or ambient) was a fixed factor and mesocosm nested within sampling occasion were treated as random effects to account for temporal pseudoreplication. The second set of models tested for differences in the slope of the temperature dependence between metabolic rates (i.e. $ER \times GPP$ and $ER \times ME$, $ME \times GPP$). Here metabolic rate is used as the dependent variable, temperature ($1/kT$) as the independent variable and metabolic rate ID (e.g. NPP or ER) as the fixed factor. Significant P values are given in italics.
Figure 3  Temperature dependence of the three main ecosystem-level carbon fluxes in the mesocosm experiment. (A) Gross primary production (GPP), (B) Ecosystem respiration (ER), and (C) CH\textsubscript{4} efflux (ME). The slope of the temperature–metabolism relationship is equivalent to the activation energy of the metabolic process. Each data point corresponds to either the GPP, ER or CH\textsubscript{4} efflux from a single mesocosm on each of the seven sampling occasions (n = 131). Ambient treatments are denoted by circles and warmed treatments by plus signs. Note that the three fundamental metabolic fluxes in the carbon cycle have sequentially greater activation energies. Dashed lines are predicted from theory (a = 0.32 eV, b = 0.62 eV, c = 0.88 eV). Figure redrawn from Yvon-Durocher et al. (2010a,b).
This divergence among the temperature dependences of these metabolic pathways suggests that under future global warming these processes have the potential to go out of balance.

**B. Ecosystem-Level Carbon Fluxes: Meta-Analysis of Field Survey Data**

An extensive global compilation of data on rates of primary production in the ocean revealed that its temperature dependence in a plot of $\ln(PP)$ versus $1/k (1/T - 1/T_c)$ was 0.44 eV (95% CI 0.39–0.49 eV) (Figure 5A) which was again steeper than the expected value of 0.32 eV derived for terrestrial C3 plants (Allen et al., 2005), but almost identical to the value of 0.45 eV measured in our mesocosm experiment (Figure 5A). Similarly, a global database of rates of primary production compiled for freshwater ecosystems (e.g. lakes and streams) demonstrated a temperature dependence identical to that observed in the ocean with a plot of $\ln(PP)$ versus $1/k(1/T - 1/T_c)$ revealing an activation energy of 0.48 eV (95% CI 0.35–0.62 eV) (Figure 5B). Again this value is

<table>
<thead>
<tr>
<th>Pond</th>
<th>Annual activation energy GPP</th>
<th>Annual activation energy ER</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-0.497</td>
<td>-0.642</td>
</tr>
<tr>
<td>2</td>
<td>-0.467</td>
<td>-0.608</td>
</tr>
<tr>
<td>3</td>
<td>-0.502</td>
<td>-0.673</td>
</tr>
<tr>
<td>4</td>
<td>-0.473</td>
<td>-0.639</td>
</tr>
<tr>
<td>5</td>
<td>-0.461</td>
<td>-0.623</td>
</tr>
<tr>
<td>6</td>
<td>-0.374</td>
<td>-0.535</td>
</tr>
<tr>
<td>7</td>
<td>-0.459</td>
<td>-0.633</td>
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<td>8</td>
<td>-0.504</td>
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<tr>
<td>9</td>
<td>-0.541</td>
<td>-0.645</td>
</tr>
<tr>
<td>10</td>
<td>-0.477</td>
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</tr>
<tr>
<td>11</td>
<td>-0.367</td>
<td>-0.553</td>
</tr>
<tr>
<td>12</td>
<td>-0.454</td>
<td>-0.634</td>
</tr>
<tr>
<td>13</td>
<td>-0.431</td>
<td>-0.580</td>
</tr>
<tr>
<td>14</td>
<td>-0.477</td>
<td>-0.632</td>
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<tr>
<td>15</td>
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<tr>
<td>16</td>
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<td>17</td>
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<td>18</td>
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<td>-0.676</td>
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<td>19</td>
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</tr>
<tr>
<td>20</td>
<td>-0.522</td>
<td>-0.645</td>
</tr>
</tbody>
</table>

Data reveal that the activation energy of ER is consistently higher than that of GPP suggesting that in all mesocosms ER was not at steady with GPP over the year, validating our theoretical assumptions.

This divergence among the temperature dependences of these metabolic pathways suggests that under future global warming these processes have the potential to go out of balance.
steeper than the 0.32 eV expected for terrestrial photosynthesis, but identical to that observed in our experiment and also in the ocean.

Data compiled on rates of community respiration, from a range of aquatic ecosystem types from across the globe revealed a similar coherence with our theoretical predictions (Figure 6). For example, in estuarine ecosystems, the activation energy of short-term pelagic community respiration was 0.58 eV (95% CI 0.46–0.7; Figure 6A), close to both the predicted value of 0.65 eV, and also the 0.62 eV observed in our experiments. Similarly, for the same estuaries, mean annual (i.e. long term) respiration had an activation energy of 0.54 eV (Figure 6B; 95% CI 0.41–0.8) which was statistically indistinguishable from the short-term flux data and the predicted theoretical value. Data compiled on rates of short-term and long-term sediment respiration in lakes across

![Figure 4](image_url)

**Figure 4** Seasonality of gross primary production (GPP; A), Ecosystem respiration (ER; B), CH₄ efflux (ME; C) and Benthic respiration (BR; D). Ambient treatments are denoted by solid lines and circles, while heated treatments are given by dashed lines and squares. Data points are the mean values (±SE) of each treatment on each month. Data reveal strong seasonal trends with rates peaking during early summer. Warmed treatments (dashed lines) are typically greater than the ambient treatments (solid line), as expected from the differences in their activation energies. Mean annual metabolic fluxes of each process were significantly elevated in the warmed mesocosms (Table 3). Note that data for ME, GPP and ER were standardised to μmol m⁻² day⁻¹ in the derivation of the carbon balance ratios in Figures 7 and 8. Figure redrawn from Yvon-Durocher et al. (2010a,b).
the arctic tundra was consistent with both the predicted values of 0.65 and 0.62 eV revealed by our experiment and exhibited an activation energies of 0.63 eV (Figure 6C; 95% CI 0.57–0.69) and 0.66 eV (Figure 6D; 95% CI 0.44–0.9), respectively. The activation energy of short-term bacterial respiration measured in coastal seas was 0.68 eV (Figure 6D; 95% CI 0.54–0.98 eV), again almost identical to the expected value of 0.65 eV. However, the activation energy of short-term bacterial respiration measured in the open ocean was 0.27 eV (Figure 6E; 95% CI 0.12–0.45) and was much shallower than that of heterotrophic metabolism, but closer to the activation energy of photosynthesis.

C. The Carbon Balance

The metabolic balance (i.e. ER/GPP) of the mesocosm experiment exhibited strong seasonal trends (Figure 7A). In 4 months of the annual study (June, August, October and April 2008) the metabolic balance was >1, indicating that ER > GPP and that the warmed mesocosms were therefore net sources of CO₂ to the atmosphere. As expected from the differential activation energies of photosynthesis and respiration, the metabolic balance was significantly elevated in the warmed mesocosms (Table 3; Figure 7A). Moreover, the mean annual ratio of ER/GPP was elevated by 13% in the warmed mesocosms (Table 3). Similarly, the ratio of ME/GPP (Figure 7B) and ME/ER (Figure 7C) revealed strong seasonal trends with both carbon balances peaking in early summer and reaching seasonal lows in February. As predicted by the differential activation energies of these three metabolic

<table>
<thead>
<tr>
<th>Variable</th>
<th>d.f.</th>
<th>F ratio</th>
<th>P value</th>
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<tr>
<td>ER</td>
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<tr>
<td>GPP</td>
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<td>42.9</td>
<td>&lt;0.0001</td>
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<tr>
<td>ln(CH₄ efflux)</td>
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<td>Benthic respiration</td>
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<tr>
<td>ER/GPP</td>
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<tr>
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<td>6.23</td>
<td>0.0139</td>
</tr>
</tbody>
</table>

Analysing differences between heated and unheated treatments in the annual means of gas transfer velocity [ln(k transfer)], ER, GPP, methane efflux [ln(CH₄ efflux)], the metabolic balance (ER/GPP), the ratio of methane efflux to GPP [ln(CH₄ efflux)/ln(GPP)], and the ratio of methane efflux to ER [ln(CH₄ efflux)/ln(ER)]. A linear mixed effects model was conducted with restricted maximum likelihood methods using the lme (linear mixed-effects model) function in R, treatment (heated or unheated) was the fixed effect, and temporal pseudo-replication from repeated sampling of the mesocosms over the year was accounted for by including mesocosm identity nested with sampling occasion as random effects. Significant P values are given in italic.
processes, both the ratio of ME/GPP and ME/ER were on average over the year, significantly elevated by 20% and 10%, respectively, in the warmed mesocosms (Table 3).

In line with their seasonal dynamics, the balance of the three key processes in the carbon cycle of the mesocosm experiment was related to temperature. As predicted by Eq. 13 a plot of ln(ER/GPP) and $1/k(1/T - 1/T_c)$ revealed that the temperature dependence of the metabolic balance in our experiment was 0.14 eV (95% CI 0.10 to 0.18 eV) and was statistically indistinguishable from the predicted value of 0.17 eV (Figure 8A) based on the empirically measured

Figure 5  Literature data for the temperature dependence of ecosystem-level primary production in (A) the ocean (Data are from the OPPWG http://marine.rutgers.edu/opp/Database/DB.html), and (B) freshwater ecosystems [i.e. lakes and streams; Data from Morin et al. (1999)]. The slope of the temperature–metabolism relationships is equivalent to the activation energy of the metabolic process. These data reveal that the activation energies of ecosystem-level primary production in the ocean and in freshwater ecosystems are identical to the slope of GPP in the mesocosm experiment (Figure 3A), though steeper than that predicted by Allen et al. (2005) for terrestrial plants (i.e. 0.32 eV).
difference between the activation energies for GPP and ER – that is $E_r - E_p$. This finding suggests that the temperature dependence of the carbon sequestration capacity of aquatic ecosystems can be predicted from the temperature dependences of photosynthesis and respiration at the cellular level. Similarly, a plot of $\ln(ME/GPP)$ and $1/k(1/T - 1/T_c)$ revealed that its activation

Figure 6  Literature data for the temperature dependence of community respiration. (A) Short-term pelagic estuarine ecosystems [data from Hopkinson and Smith (2005)]. (B) Long-term (i.e. mean annual) pelagic estuarine ecosystems [data from Hopkinson and Smith (2005)]. (C) Short-term sediment respiration in lakes [data from Gudasz et al. (2010)]. (D) Long-term (i.e. mean annual) sediment respiration in lakes [data from Gudasz et al. (2010)]. (E) Short-term bacterial respiration in the coastal ocean [data from Robinson (2008) and Rivkin and Legendre (2001)]. (F) Short-term bacterial respiration in the open ocean [data from Robinson (2008) and Rivkin and Legendre (2001)]. The slope of the temperature–metabolism relationships is equivalent to the activation energy of the metabolic process. These data reveal that the activation energies of ecosystem-level respiration in aquatic ecosystem are all close to the expected 0.62 eV predicted from our model and revealed in our mesocosm experiment, except for bacterial respiration in the open ocean which is likely constrained by GPP.
Figure 7  Seasonality of the carbon balance of the mesocosm experiment: (A) the metabolic balance (ER/GPP), (B) the balance between CH₄ efflux and gross primary production (ME/GPP), (C) the balance between CH₄ efflux and ecosystem respiration (ME/ER). Ambient treatments are denoted by solid lines and circles, while heated treatments are given by dashed lines and squares. Data points are the mean values (±SE) of each treatment on each month. Data reveal that the balance of the fundamental components of the carbon cycle are typically elevated in the warmed mesocosms as expected from the relative difference in their activation energies. The mean annual carbon balances were all significantly elevated in the warmed mesocosms (Table 3). Figure redrawn from Yvon-Durocher et al. (2010a,b).
Figure 8  Temperature dependence of the carbon balance of the mesocosm experiment. (A) The metabolic balance (ER/GPP), (B) the balance between CH₄ efflux and gross primary production (ME/GPP), (C) the balance between CH₄ efflux and ecosystem respiration (ME/ER). Ambient treatments are denoted by circles and warmed treatments by plus signs. The slopes of each of these relationships are indistinguishable from the differences in their empirically derived activation energies and are predicted by Eqs. 13–15 (e.g. a, $E_r - E_p = 0.62 - 0.45 = 0.17$. b, $E_m - E_p = 0.85 - 0.45 = 0.4$. c, $E_m - E_r = 0.85 - 0.62 = 0.23$). Figure redrawn from Yvon-Durocher et al. (2010a,b).
energy was 0.44 eV (95% CI 0.26 to 0.63 eV) (Figure 8B) and supported our prediction (Eq. 14) that the temperature dependence of ME/GPP should be equivalent to the difference in the activation energies of ME and GPP – that is $E_m - E_r = 0.4$. Moreover, our prediction of the importance of the activation energies of metabolism for the temperature dependence of the carbon balance of aquatic ecosystems were further supported by a plot of ln(ME/ER) and $1/k(1/T - 1/T_c)$ which revealed that its temperature dependence was 0.23 eV (95% CI 0.09 to 0.45 eV) (Figure 8C) and was indistinguishable from the predicted value of 0.27 eV, based on the difference in the activation energies of ME and ER – that is $E_m - E_p$.

V. DISCUSSION

A. The Temperature Dependence of the Key Components of the Carbon Cycle

The three key components of the carbon cycle in aquatic ecosystems that we measured in our experiment and compiled from global empirical databases were all strongly related to temperature. Moreover, their temperature dependence was typically constrained by the average activation energy of their particular metabolic pathway and, as predicted by our models, GPP, ER and ME had sequentially greater temperature dependences (i.e. GPP < ER < ME). The temperature dependence of the carbon cycle also differed between aquatic and terrestrial ecosystems which could have important implications for the propagation of feedbacks between future warming and the carbon cycle in these systems. In the following sections, we start by discussing each of the key metabolic fluxes in the carbon cycle, and subsequently assess the level of agreement between our experiments, meta-analyses and theory. We then focus on the apparent similarities and differences of the temperature dependence of the metabolic flux of interest between aquatic and terrestrial ecosystems. Finally, we shift our focus to the balance between the three key metabolic fluxes in the carbon cycle and consider the coherence between our theoretical predictions and experimental findings.

1. Experiments, Data and Theory

In general, there was strong agreement between our models, experiment and our empirically determined temperature dependence for each of the three metabolic processes. For instance, the ‘effective’ activation energy for GPP in our experiment was 0.45 eV and was identical to that observed in our global data compilations of primary production in the ocean (0.44 eV) and in freshwater ecosystems (0.48 eV). Similarly, the temperature dependence of ER in our experiment had an activation energy of 0.62 eV, which was
statistically indistinguishable from the average activation energy of heterotrophic metabolism [0.65 eV; Gillooly et al. (2001)] and supported our theoretical assumption of non-steady state between ER and GPP. The close coherence between the temperature dependence of ER observed in our experiment with the temperature dependence of lake (0.63 eV), estuarine (0.58 eV) and coastal bacterial respiration (0.68 eV) from the global data compilations again provide further support for the generality of our experimental findings in aquatic ecosystems. Rates of CH$_4$ efflux (ME) from the mesocosms were strongly dependent on temperature and, as predicted, had an activation energy (0.85 eV) which was almost identical to that of methanogenesis in pure cultures of methanogens (0.88 eV) (Yvon-Durocher et al., 2010b). These data corroborate the assumption in our theoretical model, that although CH$_4$ oxidation can considerably influence the absolute amounts of CH$_4$ production emitted to the atmosphere it has a negligible effect on the overall temperature dependence of ME.

The agreement between our experimentally and empirically derived activation energies provides strong support to suggest that the findings of our mesocosm experiment may be applicable more widely to aquatic ecosystems in general. These findings also support the notion that the ecosystem-level fluxes in the carbon cycle might be relatively independent of species composition (Allen et al., 2005; Enquist et al., 2003; Manning et al., 2006) because the temperature response of each of the metabolic fluxes in our experiment was identical to that observed in the ocean, estuaries and in freshwater ecosystems, each of which contrast markedly in their community structure and species composition. This point emphasises the potentially broad predictive power of our theoretical models based on biochemical kinetics (Allen et al., 2005; Brown et al., 2004) for understanding the temperature dependence of the aquatic carbon cycle and that these ecosystem-level processes might largely transcend seemingly contingent community-level differences between systems.

2. Aquatic-Terrestrial Comparisons

a. Primary production. The ‘effective’ activation energies of primary production in aquatic ecosystems documented in this study (experiment = 0.45 eV; Ocean = 0.44 eV; Freshwater = 0.48 eV) are steeper than the predicted value of 0.32 eV derived by Allen et al. (2005) based on assumptions for C3 photosynthesis in terrestrial plants [i.e. 1. internal CO$_2$ concentrations are about 70% of ambient; 2. co-limitation of photosynthesis by Rubisco; and 3. similar kinetic properties for Rubisco across species (Farquhar et al., 1980)]. This ‘effective’ activation energy of $\sim$0.32 eV has been verified for terrestrial net primary production in a global data compilation by Allen et al. (2005), and for individual level phytoplankton net photosynthesis in the ocean by Lopez-Urrutia et al.
(2006). However, the precision of the coefficient in the data reported in the latter study was low ($r^2 = 0.06$), so confidence in its absolute value is questionable. Our results suggest that the effective activation energy for aquatic photosynthesis might be greater than that of terrestrial photosynthesis, and this could have important implications for how these different ecosystem types may respond to future warming, i.e. an elevated capacity for CO$_2$ fixation at higher temperature in aquatic ecosystems.

The potential divergence in the temperature dependence of photosynthesis between aquatic and terrestrial plants might reflect fundamental differences in the physiology and biochemistry of photosynthesis in their respective autotrophic groups. For example, in the derivation of $E_p = 0.32$ eV, Allen et al. (2005) assume, following Farquhar et al.’s (1980) well-established model of leaf photosynthesis, that the rate of C3 photosynthesis in terrestrial plants is co-limited by the initial step in carbon fixation – that is the Rubisco carboxylation reaction and the light-dependent RuBP (Ribulose-1,5-bisphosphate) regeneration step. They demonstrated that the maximum rate of Rubisco carboxylation has an activation energy of $E_c = 0.68$ eV. However, the overall weaker effect of temperature on terrestrial photosynthesis is due to a decrease in the ratio of this process to photorespiration (i.e. oxygen fixation) at high temperatures (Allen et al., 2005). Oxygen and CO$_2$ compete at the binding site of Rubisco, which under conditions of low intracellular CO$_2$ concentrations, fixes O$_2$ rather than CO$_2$, thereby reducing the efficiency of photosynthesis (Falkowski and Raven, 1997). Moreover, intracellular CO$_2$ concentrations are strongly and negatively temperature dependent, increasing the prevalence of photorespiration and slowing photosynthesis at high temperatures in terrestrial plants (Berry and Bjorkman, 1980).

In aquatic systems, however, the rate-limiting step in photosynthesis tends to be governed by the diffusivity of CO$_2$ and/or HCO$_3^-$ across cell membranes, and the delivery of CO$_2$ to Rubisco (Smith and Walker, 1980). This raises the possibility that rates of photosynthesis in aquatic autotrophs may, in part, be limited by diffusion and therefore governed by fundamental physical laws. Another, more biologically based mechanism, relates to the fact that many aquatic plants have carbon concentrating mechanisms, including the inducible activation of the protein carbonic anhydrase that enables many unicellular green algae and cyanobacteria to utilise HCO$_3^-$ (Raven et al., 2008). Carbonic anhydrase is an efficient scavenger of DIC and is not inhibited by O$_2$, unlike Rubisco. The conversion of DIC to CO$_2$ by carbonic anhydrase provides elevated CO$_2$ to Rubisco, thus enabling more efficient CO$_2$ fixation at low concentrations, whilst simultaneously decreasing the oxygenase activity of Rubisco (i.e. photorespiration) (Raven et al., 2008). It seems possible then that carbon concentrating mechanisms in aquatic autotrophs might mitigate the constraints of elevated photorespiration to carboxylation ratios experienced
by terrestrial plants at high temperatures. This putative mechanism might explain why our data for aquatic photosynthesis has a considerably greater effective activation energy than terrestrial C3 photosynthesis.

Another possibility is that there are fundamental differences in the kinetic properties of the Rubisco enzyme between plants adapted to aquatic and terrestrial realms. Recent evidence suggests that the subcellular ratio of CO2:O2 is an important determinant of Rubisco kinetics that can differ markedly between autotroph species (Tcherkez et al., 2006). Furthermore, they find that the CO2:O2 specificity is inversely related to the maximal rate of Rubisco carboxylation. Thus, Rubiscos in the presence of high intracellular CO2 concentrations (e.g. organisms with carbon concentrating mechanisms) tend to have low CO2/O2 specificity but high maximal carboxylation rates, which may reflect a biochemical evolutionary adaptation to the sub-cellular environment. Therefore, the greater prevalence of carbon concentrating mechanisms in aquatic autotrophs (Raven et al., 2008) and the potential for elevated maximum Rubisco carboxylation rates might explain the stronger temperature dependence and higher effective activation energy of aquatic primary production reported in our experiment and global data compilations relative to those associated with terrestrial primary production.

b. Ecosystem respiration. For ER, our experimental findings, and those for aquatic ecosystems in general, appear to contrast with the model and global data compilation presented by Allen et al. (2005) for terrestrial ecosystems. In their model of the terrestrial carbon balance, Allen et al. (2005) make the assumption, for terrestrial ecosystems, of a steady state between GPP and ER over time periods of a year or greater. At steady state ER is constrained to equal GPP in the long term (i.e. 1 year or more), because GPP provides the substrate to fuel ER. Therefore, at steady state in a terrestrial ecosystem, Allen et al. (2005) predict that the temperature dependence of ER should be constrained to equal the effective activation energy of photosynthesis (i.e. \( \sim 0.32 \text{ eV} \)). They also predict that because \( E_p < E_r \), if growing season temperatures increase to a new long-term average, respiration at a given temperature (i.e. the normalisation constant, \( M_{T_{\text{TOT}}}^r (M_{T_{\text{TOT}}}^{-a})_r \), in Eq. 8) will decline. This is because \( M_{T_{\text{TOT}}}^r \) – that is the total biomass of heterotrophs – is predicted to decline with increases in temperature, because warming-induced increases in metabolism mean that fewer individuals can be supported per unit of GPP. These assumptions are intuitive for terrestrial ecosystems in which autochthonous production, and detritus derived from autochthonous production, is typically the dominant energy source that fuels heterotrophic food chains (Cebrian, 1999).

The results from our model, experiment and global data compilations suggest that the temperature dependence of ER in aquatic ecosystems might
be constrained by a somewhat different set of mechanisms from those operating in terrestrial ecosystems. In our experiment, the temperature dependence of ER (0.62 eV) was significantly greater than that of GPP (0.45 eV), indicating that over the temporal scale of the experiment (i.e., 1 year) ER was not at steady state with respect to GPP (Yvon-Durocher et al., 2010a). In both warmed and control mesocosms the carbon balance deviated from steady state because ER/GPP was $<1$ averaged over the year, suggesting that the mesocosms were accumulating carbon. Because the mesocosms were not at steady state (i.e., ER/GPP $<1$), ER was not substrate limited by contemporary NPP, and heterotrophic metabolism was unconstrained by the weaker temperature dependence of GPP. Moreover, our experimental data revealed that the intercept of $\ln(ER)$ versus $1/k(1/T - 1/T_c)$ – that is $M_{TOT}^{r} (M^{1-a})_{r}$ – was not significantly affected by long-term increases in temperature, contrary to the prediction of the Allen et al. (2005) steady-state model. Thus, because heterotrophic metabolism was not limited by organic carbon compounds from NPP, total heterotrophic biomass ($M_{TOT}^{r}$) did not decline in response to warming.

In our global data compilations, lake (0.63 eV), estuarine (0.58 eV) and coastal bacterial respiration (0.68 eV) were indistinguishable from one another and from the average activation energy of heterotrophic respiration [0.65 eV; Gillooly et al. (2001)], but greater than reported for terrestrial ecosystems [0.32 eV; Allen et al. (2005)]. This marked discrepancy between the temperature dependence of respiration in aquatic and terrestrial ecosystems could reflect fundamental differences in the equilibrium between autochthonous production, heterotrophic consumption and allochthonous carbon subsidies between these ecosystem types. As previously explained, the weaker temperature dependence of terrestrial ER arises from the fact that it is typically constrained by GPP over time periods greater than 1 year. However, in contrast, many aquatic ecosystems can receive considerable carbon subsidies from adjacent ecosystems that support heterotrophic metabolism beyond that which could be sustained solely by autochthonous primary production (Battin et al., 2008; Cole and Caraco, 2001; Cole et al., 2000, 2002; del Giorgio and Peters, 1994; del Giorgio et al., 1997; Pace et al., 2004; Ram et al., 2007). For example, lakes and rivers often drain sizeable terrestrial catchments and in doing so can receive substantial inputs of dissolved organic carbon, originally synthesised in the terrestrial biosphere (Battin et al., 2008; Cole and Caraco, 2001; Cole et al., 2000, 2002; del Giorgio and Peters, 1994; Pace et al., 2004). Similarly, estuaries are transitional ecosystems at the interface between riverine, terrestrial and marine ecosystems and can receive significant allochthonous subsidies of organic carbon (Ram et al., 2007). Many marine ecosystems, particularly coastal seas, are often influenced by nutrient upwellings along continental shelves and can also receive substantial carbon inputs from the estuaries (del Giorgio et al., 1997). The influence of carbon subsidies to aquatic ecosystems
complicates the concept of steady state between ER and GPP because a significant proportion of heterotrophic metabolism in aquatic ecosystems can be supported by carbon synthesised externally (Cole et al., 2000; del Giorgio and Peters, 1994; Pace et al., 2004). Thus, the stronger temperature dependence of respiration in the aquatic ecosystems highlighted here might arise if heterotrophic metabolism is not limited by GPP for organic carbon compounds. There is, however, one important exception to this pattern that is borne out in our data compilation: the open ocean – the region beyond the continental shelf – is typically oligotrophic or hyper-oligotrophic and receives few, if any, external carbon subsidies. In line with the argument outlined above, the temperature dependence of bacterial respiration from these regions was considerably lower (0.27 eV) than in coastal seas (0.68 eV; e.g. in the North Sea or the Mediterranean) and far closer to that reported in terrestrial systems.

B. The Carbon Balance of Aquatic Ecosystems

The balance of the three carbon fluxes investigated here and their response to future warming may affect the strength of biotic-atmospheric feedbacks on a potentially global scale (Woodwell et al., 1998). The strong correlation between temperature and each flux meant that overall rates of metabolism were significantly elevated in the warmed mesocosms over the course of the year (Yvon-Durocher et al., 2010a,b). However, more importantly, the different temperature dependences of these three processes resulted in substantial shifts in the carbon balance of the warmed mesocosms (Yvon-Durocher et al., 2010a,b). For example, the balance between ER and GPP was elevated by 13% on average over the year in the warmed mesocosms, resulting in a marked reduction in the capacity of the warmed mesocosms to sequester carbon. Similarly, the ratio between ME and GPP increased by 20%, while that of ME to ER increased by 9% on average over the year in the warmed mesocosms. These ratios determine the carbon balance (Whiting and Chanton, 2001; Yvon-Durocher et al., 2010b) and also the relative greenhouse gas efflux potential of ecosystems. Because CH$_4$ has up to 21 times the radiative forcing potential of CO$_2$ over periods of up to 20 years (Rodhe, 1990), the increase in ME relative to GPP and ER in response to a 4 °C rise in temperature could represent a substantial, but until now unknown, positive feedback between global warming and the carbon cycle if true of freshwater ecosystems on a global scale. Clearly, considerable caution must be exercised when extrapolating results from mesocosm experiments to global scale phenomena (Benton et al., 2007), but the close coherence between our theoretical models, experiments, and global data compilations suggest that our findings may be applicable to a wide range of natural ecosystems.
Our models predicted not only the direction but also the magnitude of the shift in the carbon balance of our mesocosms in response to warming. For example, our model, based on non-equilibrium dynamics, predicted that the ER/GPP ratio would be related to $1/k(1/T - 1/T_c)$ with a temperature dependence equal to the difference between the activation energy for respiration and that of Rubisco carboxylation ($E_r - E_p$). Data from our experiment strongly supported this prediction. Further, our model predictions for the temperature dependence of ME/GPP and ER/GPP were similarly corroborated by our experimental data and demonstrated that the temperature dependence of carbon balance could be predicted by the differences in the activation energies of these metabolic pathways (i.e. $E_m - E_p$ and $E_m - E_r$, respectively).

C. Conclusions, Caveats and Further Study

The divergence between the temperature dependence of the three key metabolic fluxes of the carbon cycle in our experiment resulted in significant imbalances between these biogeochemical processes at the ecosystem level and an overall reduction in the carbon sequestration capacity of the warmed mesocosms. Our models revealed that the constraints imposed by warming on individual metabolism were able to predict both the direction and magnitude of these experimentally observed shifts. Our data also suggest that the temperature dependence of primary production and respiration in aquatic ecosystems might be governed by a different set of mechanisms from those that constrain these processes in terrestrial ecosystems. For example, the temperature dependence of primary production in aquatic ecosystems was considerably stronger than that observed in terrestrial ecosystems, suggesting that aquatic photosynthesis might be more efficient at higher temperatures, relative to C3 photosynthesis in terrestrial plants. The prevalence of carbon-concentrating mechanisms in aquatic plants (Raven et al., 2008), combined with differences in the kinetic properties of the enzyme Rubisco (Tcherkez et al., 2006) might in part explain this phenomenon.

There are a number of important caveats that must also be considered when interpreting our results on the temperature dependence of primary production in both our experiment and in our global data compilations. For example, in Eqs. 2 and 3 and in our determination of the activation energy of primary production (Figures 3A and 5A) we assume that the body mass term (i.e. total biomass) is independent of environmental temperature. Co-variability of biomass and temperature may influence the observed activation energy of primary production in our experimental data and meta-analyses. In fact, in a recent study, we have demonstrated that on two of the seven sampling occasions in the mesocosm experiment, total phytoplankton biomass actually declined in response to warming (Yvon-Durocher et al.,
Similarly, a study in the open ocean has recently demonstrated that in the mesocosm experiment, total phytoplankton biomass tends to be lower in warmer, lower latitude regions (Moran et al., 2010). In both cases declines in autotroph biomass as a function of temperature should elevate the observed activation energy of aquatic primary production and further emphasise the differences between aquatic and terrestrial photosynthesis. In a similar manner, light and temperature are also typically correlated in temperate latitudes that experience large seasonal fluctuations (Hessen et al., 2005) and light is fundamental in controlling rates of photosynthesis in many aquatic ecosystems (Falkowski and Raven, 1997). Therefore, we cannot rule out that elevated light levels during the warmer months may at least in part explain the elevated temperature dependence of primary production in both our experiment and the global data compilations. If data were consistently available, rates of primary production should ideally have been corrected for latitudinal and seasonal differences in light levels before analysis. An additional caveat that relates to the MARMAP data set is that in the ocean, temperature and nutrient concentrations in the photic zone are often inversely correlated because the former determines the degree of stratification and hence the replenishment of nutrients from the deeper, more enriched waters (Finkel et al., 2005, 2010). However, greater nutrient limitation at higher temperatures would (assuming all else is equal) be expected to increase the strength of the temperature dependence of primary production in the ocean and accentuate the difference between aquatic and terrestrial ecosystems if accounted for in the analysis. Further, even the theoretical value of 0.32 eV derived by Allen et al. (2005) is based on experimental parameterisation, and is therefore not strictly mechanistic, and open to methodological error. Clearly more research is needed in this area to rigorously test the potential divergences between aquatic and terrestrial photosynthesis we have highlighted in this study.

As with GPP, the temperature dependence of ER in aquatic ecosystems was also much stronger than in terrestrial ecosystems, and might be explained by key differences in autochthonous production, consumption and external carbon subsidies in aquatic and terrestrial ecosystems. Many aquatic ecosystems receive significant subsidies of organic carbon that fuel heterotrophic metabolism over and above that which could be sustained on autochthonous production (Cole and Caraco, 2001; Cole et al., 2000, 2002; del Giorgio and Peters, 1994; del Giorgio et al., 1997; Pace et al., 2004; Ram et al., 2007; Tcherkez et al., 2006). Therefore, unlike terrestrial ecosystems, aquatic respiration is often not at steady state with autochthonous production (del Giorgio and Peters, 1994; del Giorgio et al., 1997), even over time periods of a year or more, and is thus not constrained by the weaker temperature dependence of photosynthesis. These potentially crucial differences between aquatic terrestrial ecosystems could be important in predicting their respective responses to future global warming. For example, autochthonous production as a
constraint on ER in terrestrial ecosystems is often cited as a negative feedback mechanism, causing acclimation over time periods (i.e. years) relevant to the feedbacks between warming and the carbon cycle (Dewar et al., 1999; Gifford, 2003; Luo et al., 2001). However, the potential lack of this ecosystem-level feedback in aquatic ecosystems might fuel the capacity of aquatic systems to remain net heterotrophic (i.e. net sources of CO₂ to the atmosphere) in a warmer climate long enough to seriously alter atmospheric chemistry.

ACKNOWLEDGEMENTS

We thank Brian Godfrey, Dan Perkins and the Freshwater Biological Association for their help with the experiment. Ricard Solé discussed ideas and provided comments on early drafts. We also thank Angel Lopez-Urrutia, Jon Cole and Jon Benstead for comments and suggestions that significantly improved the manuscript. G. Yvon-Durocher was supported by a Natural Environment Research Council studentship (NER/S/A2006/14029). J. Montoya was funded by the NERC Fellowship Scheme (NE/C002105/1), and a Ramon y Cajal Fellowship (RYC-2008-03664).

APPENDIX I. POTENTIAL CONFOUNDING VARIABLES AND SUPPLEMENTARY INFORMATION

A. Inorganic Nutrient Regime (Figure S1)

The overarching goal of this monograph was to build an understanding the effects of warming on the carbon cycle in aquatic ecosystems. Therefore, it was crucial to isolate the effects of warming per se from any other potentially confounding variables that might influence rates of carbon cycling. One such potentially confounding variable is the extent of inorganic nutrient limitation, because this can strongly influence rates of primary production (Woodward, 2007) and also the size structure of phytoplankton communities (Finkel et al., 2005; Winder et al., 2009). To determine whether the experimental treatment (i.e. warming) affected the extent of nutrient limitation in the mesocosms, we made detailed seasonal measurements of the major inorganic nutrients which might be expected to regulate primary production: water samples for measuring dissolved inorganic nutrient concentrations were collected from mid-depth in each mesocosm at 9 a.m. on each sampling occasion. Samples were filtered (Whatmann GF/F) and stored frozen (−20 °C) for subsequent determination of NO₃⁻, NO₂⁻, NH₄⁺, PO₄³⁻ and Si (Si(OH)₄) using a segmented flow auto-analyser (Skalar, San++, Breda, Netherlands), according to Kirkwood (1996).
Inorganic nutrients (NO$_3^-$, NO$_2^-$, NH$_4^+$, PO$_4^{3-}$ and Si) exhibited strong seasonal trends (Figure 3). For example, NO$_3^-$ concentrations peaked in spring and declined progressively throughout the summer, and were depleted to $\sim 0.005$ $\mu$mol l$^{-1}$ by October, before remineralisation in the winter. Concentrations of NO$_3^-$, NO$_2^-$, NH$_4^+$ and PO$_4^{3-}$ showed identical seasonal patterns in the warmed and ambient treatments, with no significant differences in the overall mean annual concentrations of these nutrients (Table S1). Furthermore, the stoichiometry of the inorganic nutrient pool exhibited remarkable similarity between treatments, with a mean annual ratio of total inorganic N to P of $\approx$11:1 in both heated and ambient mesocosms. The only inorganic nutrient which differed markedly between treatments was Si. Consistency of the dissolved inorganic nutrient concentrations between

Figure S1  Seasonality of inorganic nutrients in the mesocosm experiment: (a) Nitrite, (b) Nitrate, (c) Ammonium, (d) Silicate, (e) Phosphate, (f) the stoichiometry of the inorganic nutrient pool. Warmed treatments are denoted by the dashed lines and squares, while ambient treatments are denoted by the solid lines and circles. Data highlight that inorganic nutrient regimes were identical in warmed and ambient treatments (see Table S1).
experimental treatments meant that this potentially confounding variable, because it represents the potential resource supply rate for primary production, could be discounted from further analyses: that is, any changes in carbon cycling in the mesocosms could be ascribed to the effects of warming per se.

**B. Air–Water Gas Exchange Due to Advection and Diffusion** *(Figure S2)*

Apart from biological metabolic activity, gas exchange with the atmosphere due to diffusion and advection is an additional factor that might affect the concentration of dissolved gases *(Cole and Caraco, 1998)* and may also affect the interpretation of my results. Gas flux across the air–water interface is dependent on the concentration gradient between the water and the overlying air, and the gas transfer velocity, $k$ (otherwise known as the piston velocity). Gas flux across the air–water interface can be described by the following equation *(Cole and Caraco, 1998)*:

$$f = k \left( C_{\text{water}} - C_{\text{eq}} \right)$$

where $k$ is the gas transfer velocity (cm h$^{-1}$), and $C_{\text{water}} - C_{\text{eq}}$ is the concentration gradient of the gas between the water and the concentration that would be at equilibrium with the atmosphere. In running waters characterised by turbulent flow, reaeration due to physical processes is typically a crucial determinant of the concentration of dissolved gases, and must therefore be accounted for in calculation of metabolism from changes in the concentration of dissolved gases *(Marzolf et al., 1994; Mulholland et al., 2001)*. In still waters, however, $k$ is typically determined by wind velocity *(Cole and

<table>
<thead>
<tr>
<th>Inorganic nutrient</th>
<th>d.f.</th>
<th>$F$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>NO$_2^-$</td>
<td>1,120</td>
<td>0.06</td>
<td>0.812 (NS)</td>
</tr>
<tr>
<td>NO$_3^-$</td>
<td>1,120</td>
<td>0.65</td>
<td>0.420 (NS)</td>
</tr>
<tr>
<td>NH$_4^+$</td>
<td>1,120</td>
<td>0.23</td>
<td>0.632 (NS)</td>
</tr>
<tr>
<td>Si</td>
<td>1,120</td>
<td>6.08</td>
<td>0.015</td>
</tr>
<tr>
<td>PO$_4^{3-}$</td>
<td>1,120</td>
<td>0.68</td>
<td>0.412 (NS)</td>
</tr>
<tr>
<td>Total inorganic N to P</td>
<td>1,120</td>
<td>0.009</td>
<td>0.922 (NS)</td>
</tr>
</tbody>
</table>

A linear mixed effects model was conducted with restricted maximum likelihood methods using the *lme* (linear mixed-effects model) function in R, treatment (heated or unheated) was the fixed effect, and temporal pseudo-replication from repeated sampling of the mesocosms over the year was accounted for by including mesocosm identity nested with sampling occasion as random effects. $P$ values are given in italic.
Caraco, 1998) which determines surface water turbulence. In the present study measured wind velocities were typically very low (average 0.53 m s\(^{-1}\)), with only 2.22% of measurements above 3 m s\(^{-1}\). Importantly, \(k\) is largely independent of wind velocity at wind speeds less than \(\sim 3\) m s\(^{-1}\) (Cole and Caraco, 1998) therefore, enhanced gas exchange due to the turbulence created by wind was not considered in the calculations of ecosystem metabolism or CH4 efflux. However, advective processes which also determine \(k\) at low winds might still have been influenced by the heating of the mesocosms (i.e. through convection). To determine whether experimental warming systematically altered the gas transfer velocity we estimated \(k\) from simultaneous measurements of the efflux of CH4 and dissolved CH4 (detailed above) from:

\[
    k = \frac{f}{(C_{\text{water}} - C_{\text{eq}})}
\]

(2)

where \(f\) is the measured efflux of CH4 across the air–water interface, \(C_{\text{water}} - C_{\text{eq}}\) is the concentration gradient of the gas in the water and the concentration in the water at equilibrium with the atmosphere (\(C_{\text{eq}}\)). \(C_{\text{eq}}\) was calculated using the equations of Yamamoto et al. (1976) and the measured mixing ratio for CH4 in the air and temperature of the water on each occasion.

The gas transfer velocity, \(k\), exhibited no clear seasonal variability (i.e. it was independent of seasonal changes in temperature; Figure S2) and was not significantly different between treatments on average over the course of the experiment (\(F_{1, 123} = 3.46, \ P = 0.068\)). This evidence suggests that the
physical influence of heating the mesocosms by ~4 °C had little discernable effect on advective processes. Consequently, this potentially confounding variable was also discounted from further analyses of the biogeochemical processes in the experiment, and changes in the concentration of oxygen and methane in the water column and the efflux of methane across the air–water interface can be ascribed to biological metabolism.

REFERENCES


Lovelock, J.E. (1972). Gaia as seen through the atmosphere. Atmos. Environ. 6, 579.


