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Temperature and the metabolic balance of streams

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SUMMARY

1. It is becoming increasingly clear that fresh waters play a major role in the global C cycle. Stream ecosystem respiration (ER) and gross primary productivity (GPP) exert a significant control on organic carbon fluxes in fluvial networks. However, little is known about how climate change will influence these fluxes.

2. Here, we used a 'natural experiment' to demonstrate the role of temperature and nutrient cycling in whole-system metabolism (ER, GPP and net ecosystem production – NEP), in naturally heated geothermal (5-25 °C) Icelandic streams.

3. We calculated ER and GPP with a new, more accurate method, which enabled us to take into account the additional uncertainties owing to stream spatial heterogeneity in oxygen concentrations within a reach. ER ranged 1–25 g C m⁻² day⁻¹ and GPP 1–10 g C m⁻²day⁻¹. The median uncertainties (based on 1 SD) in ER and GPP were 50% and 20%, respectively. 4. Despite extremely low water nutrient concentrations, high metabolic rates in the warm streams were supported by fast cycling rates of nutrients, as revealed from inorganic nutrient (N, P) addition experiments.

5. ER exceeded GPP in all streams (with average GPP/ER = 0.6) and was more strongly related to temperature than GPP, resulting in elevated negative NEP with warming. We show that, as a first approximation based on summer investigations, global stream carbon emission to the atmosphere would nearly double from 0.12 Pg C year⁻¹ at 13 °C to 0.21 (0.15–0.33) Pg C year⁻¹ with a 5 °C warming.

6. Compared to previous studies from natural systems (including terrestrial ecosystems), the temperature dependence of stream metabolism was not confounded by latitude or altitude, seasonality, light and nutrient availability, water chemistry, space availability (water transient storage), and water availability.

7. Consequently, stream nutrient processing is likely to increase with warming, protecting downstream ecosystems (rivers, estuaries, coastal marine systems) during the summer low flows from nutrient enrichment, but at the cost of increased CO_2 flux back to the atmosphere.

Keywords: fluvial ecosystem, groundwater-fed stream, metabolic theory of ecology, nutrient spiralling, photosynthesis

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Introduction

Fresh waters play a major role in the global C cycle (Battin *et al.,* 2009a): stream ecosystem respiration (ER) and gross primary productivity (GPP) exert a significant control on organic carbon fluxes in fluvial networks and sum up to a global net ecosystem production (NEP = GPP–ER) of -0.12 Pg C year⁻¹ (Battin *et al.,* 2008, 2009b), with the negative sign indicating a source of CO₂ to the atmosphere. However, little is known about how climate change and especially temperature will influence these fluxes (Sand-Jensen, Pedersen & Sondergaard, 2007; Acuña *et al.,* 2008; Woodward *et al.,* 2010a).

In streams, ER is controlled not only by GPP but also by the allochthonous organic matter inputs, so ER generally exceeds GPP even during the summer period of maximum light availability for photosynthesis in groundwater-fed streams (Logue et al., 2004; Battin et al., 2008). Moreover, dissolved organic carbon (DOC) and nutrients are retained within surface biofilms, in matrices of extracellular polymeric substances (EPS) produced primarily by algae and bacteria (Battin et al., 2003; Romaní et al., 2004), so stream water nutrient supply rate might not control metabolic rates in mature biofilms (Freeman & Lock, 1995). Hence, in streams, ER should increase faster than GPP with increasing stream temperature because of the higher temperature dependence of respiration relative to photosynthesis, according to the metabolic theory of ecology (MTE; Allen, Gillooly & Brown, 2005; Lopez-Urrutia et al., 2006; Yvon-Durocher et al., 2010, in press). Hence, the temperature dependence of NEP will result from the differential in absolute metabolic flux (ER > GPP for a given temperature) and the differential in temperature dependence between ER and GPP (Lopez-Urrutia et al., 2006). This implies that NEP will become more negative with warming, so more respired carbon (CO₂) will efflux from streams to the atmosphere, thereby leading to a potential positive feedback in the greenhouse effect.

Here, we used a 'natural experiment' to demonstrate the role of temperature in whole-system metabolism (ER, GPP and NEP), in naturally heated geothermal (5–25 °C) Icelandic streams (Friberg *et al.*, 2009; Woodward *et al.*, 2010b). Compared to previous studies from natural systems (including terrestrial ecosystems), the temperature dependence of stream metabolism was not confounded by latitude or altitude, seasonality, light and nutrient availability, water chemistry, space availability (water transient storage), and water availability. Furthermore, we present a novel modification to the frequently used two-station dissolved oxygen change technique that enabled us to more accurately measure rates of wholestream metabolism.

Methods

Study area

The study area is situated in south-west Iceland (64°05' N, 21°30' W) on the mid-Atlantic ridge between the North American and Eurasian tectonic plates and is characterised by intense volcanic and geothermal activity (Arnason et al., 1969; Franzson et al., 2005). Heating of the stream water is by steam from boiling geothermal water reservoirs, which heats up the upper cold ground water that feeds the streams (Arnason et al., 1969). This explains why the water chemistry is very similar between streams despite large temperature differences. Precipitation, which exceeds 3000 mm per year, infiltrates the porous volcanic bedrock (Einarsson, 1984) and numerous small permanent streams, mostly groundwater-fed, that emerge from the valley side and discharge into the River Hengladalsá.

Before settlement in Iceland (900 AD), birch woodland (300–400 m a.s.l) and scrub covered the area with unbroken heathland vegetation up to 500–600 m a.s.l. Now, apart from the moss cover and sparse grassland of the plateau and plain, there are extensive areas stripped of vegetation and soil where rocks of volcanic origin protrude. Allochthonous organic matter input to the streams is therefore considered minimal beyond the dissolved organic carbon coming from the ground water. These long-term landscape changes (Simpson *et al.*, 2001) are the only known anthropogenic pressures on the streams investigated.

Study sites and general approach

We studied 13 groundwater-fed streams (discharge 1– 50 L s⁻¹) feeding a 2-km reach of the Hengladalsá River, with varying degrees of natural geothermal warming (5–25 °C) (Fig. S1, Table S1). We quantified the net metabolism of each stream in August 2008, when the streams were under steady-state conditions,

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i.e. when maximum standing biomass was assumed to be reached in all streams independently of temperature (see Enquist et al., 2003). Whole-stream metabolism estimates (ER and GPP) were based on a modified open-system O₂ change method using two stations (Odum, 1956; Marzolf, Mulholland & Steinman, 1994) corrected for lateral inflows (McCutchan et al., 2003; Hall & Tank, 2005). Essentially, this is an in-stream mass balance of oxygen requiring measurements of oxygen inflows and outflows along a river reach. Stream metabolism was measured in wholestream reaches (17–51 m long) during \sim 48 h within an 11-day period (6–16 August 2008). The necessary measurements on which the calculations are based are detailed below. A stream metabolism Excel workbook should always be kept for quality control including raw data, any corrections applied and all the calculations (Lighton, 2008; example available upon request to the corresponding author).

Oxygen measurements

Dissolved O₂ concentration and temperature were monitored at 1-min intervals with oxygen optic sensors fitted to multiparameter sondes TROLL9500 Professional (In-Situ Inc., Ft Collins, CO, USA) and Universal Controller SC100 (Hach Lange GMBF) and at 2-min intervals at one site with oxygen rapid pulse system sensors fitted on multiparameter sondes YSI600xlm (YSI Inc., Yellow Springs, OH, USA). Oxygen sensors were calibrated and checked in air-equilibrated water, in the laboratory, prior to and after field deployment. This method of calibration was cross-checked with independent measurements by the Winkler method (100 \pm 2% dissolved O₂ saturation in air-equilibrated water). No drift ($\pm 1\%$ of dissolved O₂ saturation) was observed, and the only correction applied was for minor discrepancies (<2%) between oxygen sensors during calibration.

Tracer studies

Conservative tracer studies (NaCl and propane) were run during the same period of fieldwork to quantify discharge (Q) at the top (TOP) and bottom (BOT) stations, groundwater lateral inflows (Q_g), mean travel time (τ), oxygen exchange coefficient (k_2), and hydraulic parameters (Table S1). Pre-weighted NaCl was dissolved in stream water immediately before slug injection. The mixing zone was generally sufficiently long (~11 m) for uniform dispersal to take place at the top station. In the shortest streams, additional deflectors and pools were created upstream the top station to increase mixing. Commercial propane (97.8%) was bubbled continuously across the width of the stream with microbubble gas diffusers (Point Four System Inc., Coquitlam, BC, Canada). Several 20mL glass vials for headspace analysis were crimped on site with chlorobutyl seals (Fisher Scientific, Loughborough, UK), and 5 mL of air was withdrawn from each vial using gas tight 10-mL Hamilton syringe and needles (non-coring point style 2). When the stream water conductivity reading returned to its ambient concentration at the bottom station, 5-mL water samples were taken from the top and bottom stations (three replicates) and flushed in the prepared vials. On return to the laboratory (within 1 month), the vials were shaken for one minute and 1 mL of the headspace was analysed by gas chromatography (Philips PU 4500 chromatograph equipped with Chrompack Poropak Q column, Pye Unicam flame photometric detector and Dyson Shimazu C-R3A chromatopac integrator). Gas samples were found to be stable for 1 month after collection in preliminary tests. No ambient propane was detected in the streams prior to addition.

Stream reaeration

The oxygen exchange coefficient k_2 (min⁻¹ with τ in min) was calculated as follows:

$$k_2 = 1.39 \frac{1}{\tau} \ln \left[\frac{G_{\text{TOP}} Q_{\text{BOT}}}{G_{\text{BOT}} Q_{\text{TOP}}} \right]$$

where 1.39 is a conversion factor (Rathburn *et al.*, 1978) and *G* steady-state concentration of propane (Marzolf *et al.*, 1994). This coefficient k_2 is commonly temperature-corrected as follows (Elmore & West, 1961):

$$k_T = k_2 \theta^{(T-T_{\text{tracer}})}$$

with T_{tracer} , stream temperature at the time of the tracer study and θ temperature coefficient (a constant derived from laboratory and channel experiments; and $\theta = 1.0241$ in Elmore & West, 1961). However, it has long been known that θ changes with stream turbulence. The film penetration model of Metzger & Dobbins (1967) and Metzger (1968) was used to quantify θ as a function of the absorption coefficient

 $K_{\rm L} = k_2 h$, with $K_{\rm L}$ in cm min⁻¹ and h, average stream depth, in cm (Demars, unpublished). In the present study, $K_{\rm L} \ge 1$ at all sites except site 12, where $K_{\rm L} = 0.3$ and T = 13.5 °C. Hence, $\theta = 1.005$ at all sites and the temperature correction had negligible effects on ER and GPP estimates (maximum 2.5% at site 1) as previously reported elsewhere (e.g. Genereux & Hemond, 1992): consequently, no temperature correction for k_2 was applied in the present study.

The expected oxygen solubility (C_x in mg L⁻¹) was calculated as previously described (Standing Committee of Analysts, 1980):

$$C_{\rm x} = \frac{C_{\rm atm}(P-V)}{(101.325 - V)}$$

where *P* is the observed atmospheric pressure (kPa), *V* saturation vapour pressure of water (kPa) and C_{atm} oxygen solubility (mg L⁻¹) under normal atmospheric pressure. The values of *V* and C_{atm} were expressed as functions of the averaged (top and bottom) observed stream water temperature:

$$V = 0.00005T^3 + 0.001T^2 + 0.0473T + 0.6089$$

using the saturated water pressure in a published table (Nave, 2006), within the range 0 < T < 30 °C,

$$C_{\rm atm} = -0.00008T^3 + 0.008T^2 - 0.404T + 14.609$$

using a published table of oxygen solubility (Standing Committee of Analysts, 1989). Continuous atmospheric pressure and temperature were taken from hourly records of Reykjavík and Hellisheidi meteorological stations and used to generate continuous atmospheric pressure data at the field sites, after correcting for differences in altitude (Demars, unpublished). These atmospheric pressure estimates agreed within 2 hPa with daily field measurements (Diplex precision barometer). Oxygen reaeration (K_A in mg L⁻¹) was calculated as in Young & Huryn (1998):

$$K_{\rm A} = (C_{\rm x} - C_{\rm AV})k_2\tau$$

where C_{AV} is the averaged observed oxygen concentration (mg L⁻¹).

Stream metabolism method: a reappraisal

Odum (1956) derived a theory to assess whole-stream metabolism based on the diel variability of dissolved oxygen at one station or two stations. Essentially, this

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is a mass balance of oxygen of *a thin parcel of water* moving downstream along the river channel (see McCutchan & Lewis, 2006). The problem is that the parameters for the calculations of stream metabolism are averages for the entire stream reach determined by tracer studies and that dissolved oxygen is only measured at one or two points. This leads to a discrepancy between the empirical data and the underlying assumptions (see Fig. 1). Important assumptions, implicit in both open-channel methods (single and two stations), are that the processes affecting the mass balance of oxygen for the parcel are spatially homogeneous not only within the studied reach but also, even for the two-station method, in an upstream zone of influence often defined as L = $3u/k_2$ (in the context of the single-station approach, see Chapra & Di Toro, 1991), with L distance (m), u water velocity (m s⁻¹) and k_2 coefficient of reaeration (s⁻¹). This assumption was not recognised in previous studies (Odum, 1956; Marzolf et al., 1994; McCutchan



Fig. 1 Schematic illustrating stream spatial heterogeneity. Lifeless stream where changes in dissolved oxygen (C_s) is simply attributable to reaeration (KA) and lateral groundwater inflows, with groundwater oxygen concentration (C_g) assumed much lower than C_s . Without lateral inflows, C_s equals the saturated oxygen concentration C_x . The direction and approximate size of the oxygen fluxes are represented with arrows. In this example, the mass of in-stream oxygen transported increases with lateral inflows and reaeration. Oxygen measurements are carried out at four stations (1-4). Current stream metabolism calculation with correction for groundwater inflows would perform well in the stream section 1-2 because lateral inflows are homogeneously distributed along the stream reach. More realistically, lateral inflows follow preferential flow paths as in section 2-3, and some reaches have no groundwater input as in section 3-4. Apparent 'loss' and 'gain' of oxygen appear in section 2-3 and 3-4, respectively. The former is attributable to within-reach spatial heterogeneity, while the latter is attributable to heterogeneity within a zone of influence from upstream station 3. Current metabolism calculations incorrectly convert these into 'consumption' and 'production' of oxygen (i.e. respiration and photosynthesis), creating biased results.

et al., 2002; Hall & Tank, 2005; McCutchan & Lewis, 2006), and as a result, the present two-station methods cannot be applied reliably in heterogeneous streams (see also Reichert, Uehlinger & Acuna, 2009). Here is a demonstration, i.e. falsification of the Odum (1956) method.

Let us assume that we have constant discharge, no lateral inflows and night-time to simplify the demonstration. Net stream metabolism (NEP) is calculated as follows (e.g. Marzolf *et al.*, 1994):

$$NEP_t = \left(C_{BOT_{t+\tau}} - C_{TOP_t} - K_{At}\right) \frac{Q}{wL}$$

with NEP_t net ecosystem production at time t (mg $O_2 \text{ m}^{-2} \text{ s}^{-1}$); $C_{BOT_{t+\tau}}$, concentration of oxygen at the bottom station at time $t + \tau$ (mg $O_2 \text{ L}^{-1}$); C_{TOP_t} , concentration of oxygen at the top station at time t (mg $O_2 \text{ L}^{-1}$); K_{At} , reaeration between the two stations at time t (mg $O_2 \text{ L}^{-1}$); Q, discharge (L s⁻¹); w, width (m); L, distance (m). Typically, oxygen concentrations are measured every minute.

At night, we expect an oxygen deficit at both stations ($C_{BOT} < C_x$, $C_{TOP} < C_x$ with C_x , the expected oxygen solubility defined earlier), an incoming flux of atmospheric oxygen ($K_A > 0$) and NEP < 0 (respiration consumes O₂). Let us also assume that the concentrations of oxygen at night are stable $(C_{BOT_t} = C_{BOT_{t+\tau}}, C_{TOP_t} = C_{TOP_{t+\tau}})$. Now, when the difference in oxygen concentration exceed reaeration, $(C_{\text{BOT}_{t+\tau}} - C_{\text{TOP}_t}) > K_A$ (Fig. 2), we have NEP > 0 which is not physically possible, as it would imply photosynthesis at night (Fig. 3). The more complex equation of McCutchan & Lewis (2006) gave the same results as Marzolf et al. (1994) and Hall & Tank (2005). If the stream is perfectly homogeneous, then we expect $C_{BOT_t} - C_{TOP_t} = 0$. In reality, it is highly improbable that there are two identical places in a stream and so $C_{BOT_t} - C_{TOP_t} \neq 0$ reflects stream heterogeneity. This spatial heterogeneity is not simply attributable to calibration errors of the sondes but to natural processes affecting oxygen concentrations, such as changes in benthic biomass and activity, reaeration rate and groundwater inflows.

Stream metabolism: new calculation method

To meet the necessary assumption of homogeneity, it suffices to take the average of the two oxygen records (top and bottom stations, C_{AV}) at time *t* and compute



Fig. 2 Data to illustrate the falsification of stream metabolism calculation. Top panel, dissolved oxygen saturation at two stations. The expected oxygen solubility $C_x = 100\%$. Bottom panel, reaeration K_A (with $k_2 = 0.167 \text{ min}^{-1}$) and resulting differences in oxygen concentrations (*C*) between the bottom and top stations according to the Odum (1956) method ($C_{BOT}_{t+\tau} - C_{TOP}_t$, see text).

the NEP for every time step (1 min) over a day (24 h) as follows:

$$\mathrm{NEP}_t = \left(C_{\mathrm{AV}_{t+\tau}} - C_{\mathrm{AV}_t} - K_{\mathrm{At}}\right) \frac{Q}{wL}$$

with C_{AV} averaged observed oxygen concentration at time *t* and *t* + τ , and with discharge (*Q*), width (*w*) and length (*L*) constant in this study.

This solution (see Fig. 3) allows also taking explicitly into account the spatial heterogeneity in the calculation of the uncertainties (using the standard deviation of the mean of the oxygen records), which is then propagated in the calculations of stream



Fig. 3 Falsification of published stream metabolism calculations. Net metabolism with data from Fig. 2. Note that all published methods (coloured lines, Marzolf *et al.*, 1994; Hall & Tank, 2005; McCutchan & Lewis, 2006) based on Odum (1956) are failing to indicate respiration at night, despite undersaturated concentration of oxygen (Fig. 2). This happens when the difference between the dissolved oxygen concentration (*C*) at the bottom and top station ($C_{BOT_{t+\tau}}-C_{TOP_t}$) exceeds the reaeration rate K_A (Fig. 2). The results obtained with the new method (thick black line, with uncertainties, thin dashed grey lines) provide more accurate results (respiration at night) in line with expectations from the dissolved oxygen curves (Fig. 2, top panel).

metabolism. The only way to reduce the spatial uncertainty objectively is to deploy as many sondes as possible along the reach. This new method is also far less sensitive to nonlinear oxygen sensor drift that cannot be objectively corrected (Fig. S2), a common problem with Clark-type oxygen sensors (including the YSI rapid pulse system).

Corrections for the upstream zone of influence of dissolved oxygen concentrations

The assumption of homogeneity applies not only to the studied reach but also to a theoretical length of stream $L > 3u/k_2$ immediately upstream of the top station (Fig. 1). Ideally, the studied reach should be situated below this zone, but this might not always be

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possible for practical reasons, hence the need for corrections. For example, if there is a large inflow of ground water (e.g. a large spring) with $C_{g} \ll C_{s}$ just upstream of the top station and nowhere else within the studied reach, then the metabolic activity of the stream will be doubly biased (because average C_s will be underestimated and $C_{BOT_t} > C_{TOP_t}$) as 95% of C_g will equilibrate with the atmosphere along the stream system for a theoretical distance $L = 3u/k_2$. We encountered this situation at one of our sites (site 8), and both stations needed small corrections for $C_{\rm s}$. Rather than relying on $L > 3u/k_2$ (because of the presence of cascades just downstream of the source, 20 m above the top station), oxygen saturation recorded at night was plotted against distance from source, and the equilibrium was calculated with a Michaelis–Menten type equation ($r^2 = 0.98$, n = 10). The top station and bottom station observed dissolved oxygen saturations were corrected by 2.59% and 1.10%, respectively, prior to stream metabolism calculations.

Corrections for lateral inflows

Lateral inflows (Q_g) from these groundwater-fed streams are largely coming from point inflows. Groundwater oxygen concentrations (C_g) were tightly related to water temperature $(C_g = 0.0053 T^2 - 0.4908 T + 14.481; r^2 = 0.95, n = 9, P < 0.0001)$ based on measurements of C_g at nine springs (3–47 °C). We calculated C_g for all streams using the observed averaged stream water temperature. Corrections for lateral inflows in the stream metabolism calculations followed Hall & Tank (2005), a more simple method than McCutchan *et al.* (2002) and McCutchan & Lewis (2006), yielding nearly identical results (±3%, Demars unpublished). The net metabolism in the present study was therefore calculated as follows:

$$\text{NEP}_t = \left(C_{\text{AV}_{t+\tau}} - C_{\text{AV}_t} - K_{\text{At}}\right) \frac{Q}{wL} - \left(C_{\text{g}} - C_{\text{AV}_t}\right) \frac{Q_{\text{g}}}{wL}$$

Stream metabolism daily rates and uncertainties

ER was calculated from the net metabolism at night scaled to 24 h and GPP resulted from subtracting the dark from the light metabolism and averaged over 24 h (Fig. 4). Respiration during day light may be calculated with the regression method of Marzolf *et al.* (1994) if, during the dark period, early morning respiration

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differs substantially with evening respiration (such observations however were probably caused by oxygen sensor and methodological artefacts, both of which largely corrected by the new optic oxygen sensors and the new stream metabolism calculation method; Demars unpublished). The NEP was calculated as GPP minus ER, with the assumption that autotrophic and heterotrophic respirations were the same under light conditions as those measured at night.

Whole-stream metabolism was carried out with the most accurate method currently available for stream ecosystem studies, and relative uncertainties (based on 1 SD) of all measurements were propagated throughout all the calculations, including those arising from the spatial heterogeneity in dissolved O_2 in



Fig. 4 Dissolved oxygen data and net metabolism at site 2. Dashed lines are uncertainties in averaged dissolved oxygen saturation and net metabolism based on 1 SD. Red and yellow lines (top panel) are temperature and photosynthetic active radiation (PAR), respectively. Daily (24 h) ecosystem respiration and gross primary productivity are represented by the areas in red and green (see text for calculations).

small turbulent streams and from corrections of lateral inflows, elements previously overlooked in the calculations of uncertainties in whole-stream metabolism (McCutchan, Lewis & Saunders, 1998).

The relative uncertainties of the net metabolism were calculated and propagated for each time step (1-min interval) based on 1 standard deviation $(\pm \delta x)$. For sums, we took the square root of the sum of the squares of the standard deviations. For multiplications, we took the square root of the sum of the squares of the proportional errors (relative uncertainties).

ER uncertainties were simply the average of all the relative uncertainties calculated for each time step during night time. The relative uncertainty ($\delta x/x$) in daily GPP was based on every 1-min time steps *i* (1, ..., *n*) of the net metabolism (NEP) and average night respiration (ER) as follows:

$$\delta \text{GPP}/\text{GPP} = 1 - \left(\frac{\sum_{i=1}^{n} (\text{NEP}_{-1\text{SD}} - \text{ER}_{-1\text{SD}}) \div n}{\text{GPP}}\right)$$

with -1SD subscript meaning minus 1 standard deviation, and $(\text{NEP}_{-1\text{SD}} - \text{ER}_{-1\text{SD}}) = 0$ at night (PAR <1 μ mol m⁻² s⁻¹).

Light

Photosynthetically active radiation (PAR) was measured continuously in air, and averages logged every 5 min (LICOR instruments, Lincoln, NE, USA). The night period was defined as PAR <1 μ mol photon m⁻² s⁻¹. To check for bias in GPP measurements, the relationship between GPP and PAR (using 5 min time step data) was modelled with a Michaelis–Menten type equation as follows:

$$\text{GPP} = \frac{\text{GPP}_{\text{MAX}}\text{PAR}}{k_{\text{PAR}} + \text{PAR}}$$

where GPP_{MAX} is the maximum GPP and k_{PAR} is the PAR at which half the GPP_{MAX} is realised. GPP_{MAX} and k_{PAR} (Table S2) were determined with the nonlinear regression model of S-Plus 7.0 software (Insightful Corp., Seattle, WA, USA). Since GPP (24 h) was strongly correlated with *GPP*_{MAX} (r = 0.97, n = 13, P < 0.0001), light availability did

not bias GPP (24 h) estimates. The metabolic activity of a warm stream was also monitored continuously for nine days as an additional control on the stability of ER, GPP and NEP during the period of measurements (Fig. S3).

Water chemistry and nutrient cycling

Water samples were collected and filtered with Millipore 0.45 μ m pore size on the last day of field work and analysed at the Macaulay Institute as in Demars & Edwards (2007). Stream water chemistry was very similar in all streams (Table S3) as reported previously (Friberg et al., 2009). Additional wholestream nutrient cycling studies were determined in August 2006, as described by Webster & Valett (2007). Short-term nutrient additions were carried out to characterise the uptake/release dynamic equilibrium (nutrient cycling), at two warm (17.2 \pm 0.2 °C) and two cold $(11.1 \pm 1.9 \text{ °C})$ sites, in August 2006, to quantify the uptake velocity ($v_{\rm f}$, mm s⁻¹) of NH₄, NO₃ and PO₄, a measure of uptake efficiency normalised for stream velocity and depth (Peterson et al., 2001; Webster & Valett, 2007).

Stream hydraulic parameters

Water transient storage and storage exchange rate were determined using the upstream-downstream conductivity curves (10 second time step) produced by NaCl slug injections and the equations developed by Bencala & Walters (1983). The equations were solved numerically using the DISCUS method (Manson, Wallis & Hope, 2001), which is an improvement on the traditional OTIS method (Cox & Runkel, 2008). The good fit of the model simulations to the experimental data, together with relatively low Damkohler numbers (range 0.9-4.3) indicated that the model output was an accurate reflection of the actual stream processes (Hart et al., 1999). To obtain comparable measurements across streams, the cross-sectional area of the storage zone was normalised by the stream cross-sectional area (As:A). Hydraulic parameters were unrelated to temperature (Fig. S4).

Standing biomass

It is notoriously difficult to assess standing biomass in streams, especially for microorganisms living at the surface of stones, macrophytes and in the hyporheic zone. Water transient storage is a good surrogate for habitat complexity or surface area availability for microorganisms (the metabolic engines of the ecosystem). Since water transient storage was unrelated to temperature, we can assume that surface area is unrelated to temperature. Epilithic biofilm thickness was calculated from diatoms, green algae and cyanobacteria cell counts and biovolumes per unit area (Hauer & Lamberti, 2007) from eight sites sampled in August 2007. Biofilm thickness, at least for the autotrophs, was unrelated to temperature (Fig. S5). Hence, standing biomass should be unrelated to temperature.

Activation energies and absolute metabolic fluxes

We measured the temperature dependence of ER and GPP according to Arrhenius equations formally derived from the MTE (Enquist et al., 2003; Brown et al., 2004; Appendix S1). The general model for scaling biochemical kinetics from individual organisms to ecosystems was obtained with an Arrhenius plot by regressing the natural log of a metabolic rate (ER, GPP) against the temperature reciprocal 1/kT, with *k* representing Boltzmann's constant (in eV K^{-1}) and T temperature (in K) - Enquist et al., 2003. The temperature dependence is given by the slope of the linear regression describing the activation energy E (minimum amount of energy necessary for a chemical reaction to occur; in eV; 1 eV = 96.5 KJ mol⁻¹) of the metabolic rate given here as E_r and E_p for ER and GPP, respectively. The intercepts $\ln(c_r)$ for ER and $\ln(c_p)$ for GPP represent the absolute metabolic fluxes which are the product, $\ln(b_0C)$, of a normalisation parameter for individual metabolism (b_0 , mass^{1/4} carbon time⁻¹) and the sizes and abundances of individual organisms per unit area (C, mass^{3/4} carbon length⁻²). In other words, according to the MTE, the absolute metabolic flux represents the product of mitochondrial density (through b_0) and total biomass (through C) - Gillooly et al. (2006). In the studied streams, E_r approximates the activation energy E_h of heterotrophic respiration (HR), and c_r approximates the absolute metabolic flux c_h of HR, because of the very minor role of autotrophic respiration (Appendix S1).

Here, the absolute metabolic flux (intercept) was normalised to a reference temperature (as in Gillooly *et al.*, 2001) to be biologically more meaningful. The MTE equation used on the Arrhenius plot was therefore as follows:

$$\ln(B_{\rm e}) = \ln(c) - E\frac{1}{k}\left(\frac{1}{T} - \frac{1}{T_{\rm c}}\right)$$

with $B_{\rm e}$ the total ecosystem metabolic flux per unit area (eV), ln (*c*), normalised absolute metabolic flux (eV), $T_{\rm c}$, reference temperature (here 288 K, equivalent to 15 °C).

While the MTE has been criticised, there is no alternative quantitative theory to make *a priori* predictions regarding the metabolic balance of ecosystems to change in temperature. To facilitate the reading of the results to those not familiar with Arrhenius plots, log scale metabolic activities (g $O_2 \text{ m}^{-2} \text{ day}^{-1}$) were displayed against temperature (°C) and the normalised reciprocal of temperature is simply indicated at the top of the graphs.

Data analyses

Metabolic activities were In-transformed prior to regression and correlation analyses to normalise the data and reduce heteroscedasticity or because the biological response to an environmental variable was known to be exponential from previous work (Enquist et al., 2003; Allen et al., 2005; Lopez-Urrutia et al., 2006). Normality of the data was tested with the Shapiro-Wilk test. We then used ordinary and nonlinear least square regression models. We used the *t*-statistics for testing whether the coefficients were significantly different from zero (S-Plus 7.0). The level for statistical significance was $\alpha = 0.05$, except for the linear regression of ln(NEP) as a function of temperature where we applied $\alpha = 0.1$, owing to inevitable large uncertainties in NEP. Because of the small sample size, the means of the nutrient cycling parameters (v_{f} , U) between the two pairs of cold and warm streams were considered significantly different when there was no overlap in between the two means ± SEM. All uncertainties reported in the text are based on ±1 SD and were propagated throughout the calculations.

Results

ER exceeded GPP in all cases, ranging from -3 to -67 g O_2 m⁻² day⁻¹ and 2–28 g O_2 m⁻² day⁻¹, respec-

tively, among streams (Table S2). In terms of carbon, this is equivalent to -1 to -25 g C m⁻² day⁻¹ for ER and 1–10 g C m⁻² day⁻¹ for GPP, assuming a respiratory quotient of 1. The relative uncertainty in ER and daily average GPP were generally around 50% (38–86%, excluding one outlier) and 20% (1–57%), respectively (Table S2).

Both Log-transformed ER ($r^2 = 0.44$, P = 0.013) and GPP ($r^2 = 0.31$, P = 0.039) were linearly related to daily average stream water temperature (Figs 5 and 6). The observed activation energies (temperature dependence) of ER and GPP were $E_r = 0.67 \pm$ 0.23 eV and $E_p = 0.54 \pm 0.24$ eV, respectively. The normalised absolute metabolic flux of GPP or GPP_{15 °C}, 8(7–10) g O₂ m⁻² day⁻¹, was statistically ($\pm 68\%$ CI) lower than that of ER_{15 °C}, 14(12–18) g O₂ m⁻² day⁻¹. ER predicted NEP significantly (log-log plot, $r^2 = 0.67$, n = 13, P = 0.0006) contrary to GPP (log-log plot, $r^2 = 0.21$, n = 13, P = 0.13).



Fig. 5 'Natural experiment'. Two of the thirteen geothermal streams studied (site 7 and 8) discharging in the River Hengladalsá and their diel change in net metabolic activity. The net metabolism is negative when respiration activity exceeds the rate of photosynthesis.

As a consequence, negative NEP increased exponentially with temperature (Fig. 7, $r^2 = 0.23$, n = 13, P = 0.099) owing to the combined effect of the



Fig. 6 Stream metabolism and stream water temperature at thirteen sites. Ecosystem respiration (ER) response to temperature, $\ln(ER) = \ln(c_r) - E_r \frac{1}{k} \left(\frac{1}{T} - \frac{1}{T_c}\right)$, $r^2 = 0.44$, P = 0.013; and gross primary productivity (GPP) response to temperature, $\ln(GPP) = \ln(c_p) - E_p \frac{1}{k} \left(\frac{1}{T} - \frac{1}{T_c}\right)$, $r^2 = 0.31$, P = 0.039. Error bars are 1 SD and represent the cumulated errors in ER and GPP estimates. The normalised temperature reciprocal $1/k(1/T-1/T_c)$, used to calculate the activation energies E_r and E_p (slopes) and absolute metabolic fluxes c_r , c_p (intercepts), is also displayed with *k* Boltzmann's constant (in eV K⁻¹) and *T* temperature (in Kelvin degrees).

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differential in activation energies and absolute metabolic fluxes (ER > GPP whatever the temperature), although only at the significance level $\alpha = 0.1$. From this empirical exponential equation, NEP ranged from $-1.4 \text{ g } \text{O}_2 \text{ m}^{-2} \text{ day}^{-1}$ at 5 °C to $-12.2 \text{ g } \text{O}_2 \text{ m}^{-2} \text{ day}^{-1}$ at 25 °C, equivalent to -0.5 and $-4.6 \text{ g C m}^{-2} \text{ day}^{-1}$, respectively.

The whole-stream nutrient uptake velocity ranged over one to two orders of magnitude ($v_f = 0.02-1 \text{ mm s}^{-1}$) and was significantly faster in the warm streams than in the cold streams for NH₄ ($0.72 \pm 0.39 \text{ v}$. 0.06 ± 0.06) and NO₃ ($0.46 \pm 0.13 \text{ v}$. 0.05 ± 0.01) but not for PO₄ ($0.09 \pm 0.04 \text{ v}$. 0.04 ± 0.02) mm s⁻¹. This translated into much higher areal uptake rates (*U*) in the warm streams than in the cold streams: NH₄-N ($19.6 \pm 2.5 \text{ v}$. 4.8 ± 1.4), NO₃-N ($11.6 \pm 1.1 \text{ v}$. 2.4 ± 0.5), and even PO₄-P ($7.1 \pm 2.3 \text{ v}$. 1.2 ± 1.6) mg m⁻² h⁻¹ (Fig. 8).

Discussion

New calculation method

The example given in the demonstration (Fig. 3) was an extreme case of spatial heterogeneity in dissolved oxygen concentrations, and so respiration had very large relative uncertainties (208%). Most of this uncertainty was attributable to spatial heterogeneity (130%) and the remaining (78%) to oxygen calibration



Fig. 7 Negative net ecosystem production (NEP) exponential response to stream temperature. $r^2 = 0.23$, n = 13, P = 0.099. Dotted lines represent the 68% confidence interval. Error bars are 1 SD and represent the cumulated errors of ecosystem respiration and gross primary productivity.



Fig. 8 Nutrient gross uptake rate (= cycling rate) is faster in the warm streams than in the cold streams.

and other measurement errors such as reaeration, discharge, mean travel time and lateral inflows. Without this new calculation method, the results of the present study would have been very different. For example, the previously published methods (e.g. Hall & Tank, 2005; McCutchan & Lewis, 2006) underestimated respiration by 29% at site 7 and overestimated respiration by 41, 47 and 400% at sites 10, 11 and 14, respectively. While site 14 had extremely large relative uncertainties (but moderate absolute uncertainties owing to low metabolic activities), this was not the case for the other sites. The superiority of the new method was also demonstrated under low reaeration coefficient ($k_2 = 0.02 \text{ min}^{-1}$) with the oxygen sensor drift example (Fig. S2). This is because reaeration is not only a function of k_2 but also function of the degree of deficit or supersaturation in oxygen. This means that spatial heterogeneity matters, irrespective of the turbulence of the stream (see also Reichert et al., 2009).

Stream metabolism and nutrient cycling

The metabolic activities in the 13 studied streams (ER, GPP) span the full range of values reported in a recent global synthesis (Battin *et al.*, 2008). This is perhaps not surprising considering the natural 20 °C temper-

ature gradient of these geothermal streams. The observed activation energy ($E_r = 0.67 \pm 0.23 \text{ eV}$) of ER was similar to stream ecosystem heterotrophic respiration from suspended sediments and epilithic biofilms (Sand-Jensen et al., 2007; Acuña et al., 2008), and very close to observations from other ecosystems $(E_r \approx 0.65 \text{ eV}, \text{ Allen } et al., 2005; \text{ Enquist } et al., 2003;$ Yvon-Durocher *et al.*, 2010; in press; and $E_{\rm h} = 0.56 \pm$ 0.02 eV Lopez-Urrutia et al., 2006). This similarity in temperature dependence is not trivial considering the different thermal history of these ecosystems (or communities) and supports the 'universal temperature dependence' at the ecosystem scale (Gillooly et al., 2001; Perkins et al., in prep.). The activation energy of photosynthesis (E_p) at 0.54 ± 0.24 eV was not statistically (owing to large uncertainties) lower than E_r or different from theoretical predictions or observations from many other studies (≈0.32 eV, Allen et al., 2005; Lopez-Urrutia et al., 2006; Yvon-Durocher et al., 2010, in press). The temperature dependence can also be expressed into the more familiar Q₁₀ (rate of metabolic change over an increase of 10 °C). The Q₁₀ of ER and GPP were 2.6 and 2.1, respectively, within the range of observed temperature (5-25 °C).

Observed E_p may partially result from the intimate relationship between GPP and ER (log-log plot, r = 0.85, n = 13, P = 0.0002), which we explain in terms of nutrient cycling. A higher-than-expected temperature dependence of GPP (E_p) has previously been reported from freshwater mesocosm experiments (≈0.45 eV, Yvon-Durocher et al., 2010). Two other reasons might explain why the observed temperature dependence of GPP (E_p) may be higher than expected. First, E_p might have been slightly overestimated because the estimation of GPP relied on the standard assumption that respiration during the day is the same as that measured at night (Odum, 1956), since it is currently not possible to separate daytime autotrophic and heterotrophic respiration (Marzolf et al., 1994; Williams & del Giorgio, 2005; Yvon-Durocher et al., 2010). Second, since the studied streams have relatively high alkalinity and low excess pCO_2 (HCO₃ ≈ 1.5 mg L⁻¹, $epCO_2 \approx 5$ times atmospheric pressure; Demars, unpublished), photosynthesisers may significantly rely on HCO₃ assimilation via carbon-concentrating mechanisms (e.g. Demars & Trémolières, 2009). This would alleviate the increase in photorespiration with increasing temperature compared to the classic C3 photosynthesis assumed in the derivation of E_p (Allen *et al.*, 2005).

NEP was $-1.2(-1.8 \text{ to } -0.8) \text{ g C m}^{-2} \text{ day}^{-1} \text{ at } 13 \text{ }^{\circ}\text{C}$ (summer global land surface average temperature; National Climatic Data Centre, 2010), the same value as the latest independent global average estimate of stream NEP (-1.2 ± 0.15 g C m⁻² day⁻¹, Battin *et al.*, 2008, 2009b), suggesting that the magnitude of the metabolic fluxes observed in our study is comparable to those in other stream ecosystems. This suggests that if we scale up our findings using global stream area estimates (based on discharge $<500 \text{ L s}^{-1}$ or ≤5 th order; Battin et al., 2008; Wollheim et al., 2008), we may be able to provide the first global approximation of expected changes in stream NEP attributable to warming using our empirical relationship. According to our results, warming from 13 °C to 18 °C would nearly double global stream NEP (Battin et al., 2008, 2009b) to -0.21(-0.33 to -0.15) Pg C year⁻¹, which will efflux to the atmosphere as respired CO₂ (Cole et al., 2007). This predicted change in NEP $(0.09 \text{ Pg C year}^{-1})$ represents, for comparison, only 1% of the current global carbon sources to the atmosphere (Le Quéré et al., 2009) and so does not represent the same magnitude of potential threat as in marine (Lopez-Urrutia et al., 2006) or subarctic terrestrial ecosystems (Dorrepaal et al., 2009). Note that changes in stream NEP may be faster in the tropics than in high latitudes because of the nonlinear response of stream metabolism to temperature (see Dillon, Wang & Huey, 2010). An important caveat to consider when interpreting the above prediction is that the NEP-temperature relationship was relatively weak ($r^2 = 0.23$, n = 13, P = 0.099), likely due to the inevitable large uncertainties (cumulated errors) in ER and GPP estimates (Fig. 3, Table S2), as well as residual variation not accounted for by temperature such as standing biomass (e.g. Acuña et al., 2008) constrained by stream surface area availability (hyporheic zone, macrophytes; Demars & Manson, in prep.). The other obvious limitation is the exclusion of potential seasonal complexities in these metabolic fluxes.

ER and GPP within the warm streams in our study were among the highest yet reported (Battin *et al.*, 2008), despite these streams having some of the world's lowest nutrient concentrations (Table S3; *cf* Perakis & Hedin, 2002; Demars & Edwards, 2007). This may be partly attributable to the high oxygen and

carbon supply rate from reaeration and alkalinity, respectively, and high mixing rates (McIntire, 1966, 1968; Spicer & Gaston, 1999). There seemed to be a paradox, however, with data previously collected in August 2004, showing evidence of N and P limitation from short-term nutrient diffusive substrate experiments on algal accrual over 28-day assays (Friberg et al., 2009), but this can be explained by the faster cycling rate of key nutrients in the warm streams. It implies a positive feedback loop between ER and GPP in the mature biofilm (under steady-state conditions), which is supported by the tight relationship between the two processes (logGPP-logER plot, r = 0.85, n = 13, P = 0.0002). This difference in GPP response to nutrient supply by young versus mature biofilm has also been noted elsewhere (Stutter, Demars & Langan, 2010). GPP is likely to be more dependent on ER, however, because ER exceeded GPP in all streams $(GPP/ER = 0.6 \pm 0.2)$. The higher-than-predicted activation energy of GPP may therefore partly result from the increased rate of inorganic nutrient supply by ER with warming. ER was also probably supported by the DOC because the average groundwater DOC $(1.0 \pm 0.2 \text{ mg L}^{-1}, \text{ independently of the temperature})$ exceeded the average stream DOC $(0.41 \pm$ 0.16 mg L^{-1}). This is a significant difference considering that most streams are only tens of metres long. Night measurements of DOC concentration at the source and outlet of sites 7 and 8 (pictured in Fig. 5) in May 2009 showed a much lower loss of carbon in the cold stream $(0.31 \pm 0.23 \text{ g C m}^{-2} \text{ day}^{-1})$ than in the warm stream (15.4 \pm 11.7 g C m⁻² day⁻¹), in agreement with ER measurements at those sites (2.6 ± 1.3) and 25 ± 17 g C m⁻² day⁻¹, respectively). These independent preliminary results (with high uncertainties owing to high analytical errors at low DOC concentrations) on DOC degradation rates confirmed previous GPP/ER findings from other groundwater-fed streams (e.g. Logue et al., 2004; see introduction).

Hence, this study advances considerably our understanding of both stream metabolism and nutrient cycling along a large (20 °C) temperature gradient in a unique natural experiment, and its potential consequences for quantifying ecosystem services, in terms of carbon sequestration and release and nutrient cycling (Sweeney *et al.*, 2004). Our results suggest that warming could reduce the supply of nutrients (N, P) to downstream ecosystems (large rivers, floodplain lakes, estuaries and marine coastal waters) during the

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summer, thereby reducing the potential for eutrophication, but at the cost of increased release of CO_2 to the atmosphere: this interaction between warming and nutrient fluxes could create a new, and previously unanticipated, dilemma for society.

There are several caveats and limitations to the current study that merit consideration and further work in the future, including: (i) our estimates of GPP and ER did not consider the carbon excreted as EPS; (ii) while we used the metabolic theory of ecology as a rational (null model), our approach cannot test its mechanistic basis, which has been criticised; (iii) our absolute metabolic fluxes and activation energies are only strictly valid for sites at or near steady state at similar latitude (Enquist et al., 2003) and should ideally be repeated under different successional stages and locations (Acuña et al., 2008; Anderson-Teixeira, Vitousek & Brown, 2008; cf Sand-Jensen et al., 2007); (iv) a range of other factors (e.g. UV light, Kelly, Bothwell & Schindler, 2003; riparian cover, Sweeney et al., 2004; Roberts, Mulholland & Hill, 2007), extreme events (Uehlinger, 2000; Acuña & Tockner, 2010) and their potential interactions also contribute determining stream metabolism; (v) our metabolic estimates of ER, GPP and, especially, NEP still have very large uncertainties, despite the state-ofthe-art methods used in this study (tracer studies, optic oxygen sensors, new calculation method).

While nutrient availability can have profound effects on the ecology of Arctic streams (Slavik *et al.*, 2004), it did not prevent mature biofilms from reaching high metabolic rates in the warm streams. Consequently, the increase in stream metabolism with temperature might serve to buffer the effects of downstream nutrient enrichment by increasing denitrification and nutrient retention (Mulholland *et al.*, 2008), during the summer. However, this will be at the cost of increased CO_2 flux back to the atmosphere, not only in the Arctic where climate change effects are expected to be especially pronounced (Brittain *et al.*, 2008; Vincent & Laybourn-Parry, 2008), but also in the tropics, because of the exponential metabolic response to temperature (Dillon *et al.*, 2010).

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Metabolic theory of ecology and autotrophic respiration.

Figure S1. Long term temperature data from Hengill streams.

Figure S2. New stream metabolism method performs well against oxygen sensor drift.

Figure S3. Daily stream metabolism at site 1 during 9 days of continuous measurements.

Figure S4. Independence of temperature and stream hydraulic parameters.

Figure S5. Biofilm thickness is unrelated to temperature ($r^2 = 0.004$, n = 8, P = 0.88).

Figure S6. Heterotrophic respiration (HR) in the studied streams is unrelated to the autotrophic carbon use efficiency (ϵ).

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Table S1. Location and stream parameters.

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Appendix 1. Metabolic theory of ecology and autotrophic respiration

The metabolic theory of ecology (MTE, Brown *et al.*, 2004) provides a useful integrated framework for making general predictions regarding the metabolic balance of carbon in ecosystems (Allen *et al.*, 2005; Lopez-Urrutia *et al.*, 2006).

Model description

The general model for scaling biochemical kinetics from organisms to ecosystems is (Enquist et al., 2003):

$$\ln(B_e) = -E/kT + \ln(b_0C) \tag{1}$$

with B_e the total ecosystem metabolic flux per unit area (eV),

E, the activation energy of the limiting biochemical process,

k, Boltzmann's constant (in eV K^{-1})

T, temperature (K)

 b_0C , the absolute metabolic flux, resulting from the product of the normalisation parameter for individual metabolism (b₀, mass^{1/4} carbon time⁻¹) and the sizes and abundances of individual organisms per unit area (C, mass^{3/4} carbon length⁻²)

This model is similar in form to an Arrhenius plot where $\ln(B_e)$ is linearly regressed against to 1/kT. The slope of the linear regression gives *E* and the intercept *c* gives $\ln(b_0C)$.

This model can be further simplified for the purpose of this study as:

$$\ln(B_e) = -E/kT + \ln(c) \tag{2}$$

The model can also be written as follows:

$$B_e = c \exp(-E/kT) \tag{3}$$

The net ecosystem production is:

$$NEP = GPP - ER \tag{4}$$

which can be written after substituting GPP and ER by the general Eq (3):

$$NEP = c_p \exp\left(-E_p / kT\right) - c_r \exp\left(-E_r / kT\right)$$
(5)

with E_p , E_r and c_p , c_r the activation energies and absolute metabolic fluxes (as defined above) of *GPP* and *ER* respectively.

Since ER is the sum of autotrophic (AR) and heterotrophic respiration (HR) we have,

$$NEP = GPP - (AR + HR) \tag{6}$$

It has been shown by Allen *et al.* (2005) that the effective activation energy of AR is the same as GPP ($\approx 0.32 \text{ eV}$) and different from HR ($\approx 0.65 \text{ eV}$). Since our measurements cannot differentiate AR from HR, it is important to try to see whether AR may affect significantly our predictions regarding *ER*. Allen *et al.* (2005) demonstrated that under steady state conditions:

$$AR = (1 - \varepsilon)GPP \tag{7}$$

with $(1-\varepsilon)$ the fraction of photosynthate respired by autotrophs,

and so

$$NEP = \varepsilon GPP - HR \tag{8}$$

equivalent to:

$$NEP = \mathcal{E}_p \exp\left(-E_p / kT\right) - c_h \exp\left(-E_h / kT\right)$$
(9)

with E_p , E_h and c_p , c_h the activation energies and absolute metabolic fluxes (as defined above) of *GPP* and *HR* respectively.

Predictions on the role of autotrophic respiration

Without knowing the sizes and abundances of individual organisms per unit area, we cannot predict quantitatively the normalisation parameters for individual metabolism and the autotrophic carbon use efficiency ε (Lopez-Urrutia *et al.*, 2006). However we can make qualitative predictions about the absolute metabolic fluxes and activation energies. Since *ER*>*GPP* in streams independently of temperature, $0 < \varepsilon < 1$, and $E_h > E_p$, *NEP* change to temperature increase should essentially be driven by HR, independently of the carbon use efficiency by autotrophs (ε), and so $E_r \rightarrow E_h$ and $c_r \rightarrow c_h$. Hence, for streams, predictions based on *ER* will approach what is predicted for *HR* by the metabolic theory of ecology. In streams, we predicted E_r ($\approx 0.65 \text{ eV}$) > E_p ($\approx 0.32 \text{ eV}$, formally derived from terrestrial C3 plant photosynthesis model (Allen et al., 2005) and confirmed empirically for marine phytoplankton – Lopez-Urrutia *et al.*, 2006) and $c_r > c_p$, independently of stream water nutrient supply (see text).

Empirical test

We can now use our data (see results) to test those predictions by varying the autotrophic carbon use efficiency ε .

We have

$HR = ER - (1 - \varepsilon)GPP$

so, knowing ER and GPP we can calculate HR for a range of ε values (0.1 to 0.9) and calculate the resulting E_h (and c_h) using Arrhenius plots. Finally the range of activation energies and absolute metabolic fluxes of *HR* can be plotted against ε . The prediction is that there should be no significant changes in E_h and c_h with varying ε . This is confirmed by our data (Figure S6). The resulting NEP is therefore far less sensitive to the carbon use efficiency by autotrophs (ε) than to temperature (Figure S7). This was especially the case in our study because observed E_p was relatively high (\approx 0.54 eV) compared to predictions ($E_p \approx 0.32$ eV).



Figure S1. Long term temperature data from Hengill streams. a. Continuous (4 hourly) data from near the source of site 1 and 7. **b.** Spot measurements at seven sites for which long term data has been collected. Colour symbols relate to daily averaged stream water temperature in August 2008 (blue <10, yellow 10-15, orange 15-20, red >20°C).



Figure S2. New stream metabolism method performs well against oxygen sensor drift. In this example, the oxygen sensor of the bottom station had small non-linear drifts (rapid downward drift followed by slow upward drift) despite cross calibration within 1% oxygen saturation before and after sensor deployment (top panel). Despite the small reaeration coefficient (k_2 =0.02 min⁻¹), it is clear that the new method (thick black line) of stream metabolism calculation performed better than previously published methods (purple line). In this case, GPP would have been seriously under estimated by previous methods, while the new method reported GPP more accurately judging from the dissolved oxygen saturation curves. The error due to the oxygen sensor drift contributed to 30% of the reported uncertainties at night (±1 s.d., thin dashed black lines).



Figure S3. Daily stream metabolism at site 1 during nine days of continuous measurements.



Figure S4. Independence of temperature and stream hydraulic parameters. a. Water transient storage (A_s) normalised by stream cross section area (A) – temporary storage of water within quiescent zones; linear regression, r^2 =0.17, n=13, P=0.16. b.; Storage exchange rate – average water residence time in the transient storage zones; linear regression after log transformation of A_s: α A, r^2 =0.0003, n=13, P=0.96.







Figure S6. Heterotrophic respiration (*HR*) in the studied streams is unrelated to the autotrophic carbon use efficiency (ε). a. Activation energy of *HR*. b. Absolute metabolic flux of *HR*, (Appendix 1). Vertical error bars are ± 1 s.d.



Figure S7. NEP is more a function of temperature than autotrophic carbon use efficiency (ε). Dots are observed data and lines are fitted models based on the MTE equation: $NEP = \varepsilon c_p \exp(-E_p / kT) - c_h \exp(-E_h / kT)$, (Appendix 1).

Table S1. Location and stream parameters.

| | | | stream | stream | | reach | wetted | | discharge | lateral | mean travel | |
|------|-------------|-------------|-------------|-----------|-----------|--------|--------|-------|-------------------|--------------------|----------------|-------------------|
| site | N | w | temperature | range | elevation | length | width | depth | Q _{BOT} | inflows | time | k ₂ |
| | 0 | 0 | °C | °C | m | m | cm | cm | L s ⁻¹ | % Q _{BOT} | min | min ⁻¹ |
| 1 | 64°05"396' | 21°30''484' | 18.9 | 15.9-23.2 | 374 | 40 | 108 | 14 | 17 | 2 | 5.8 | 0.077 |
| 2 | 64°05''580' | 21°30"538' | 19.9 | 19.1-21.1 | 370 | 42 | 51 | 16 | 3 | 3 | 21.3 | 0.067 |
| 3 | 64°05''635' | 21°30''434' | 22.6 | 21.6-24.0 | 371 | 31 | 68 | 12 | 7 | 1 | 6.5 | 0.067 |
| 4 | 64°05''656' | 21°30"342' | 10.1 | 7.1-15.2 | 372 | 44 | 25 | 3 | 1 | 1 | 4.2 | 0.269 |
| 5 | 64°05''562' | 21°30"656' | 20.6 | 19.9-21.7 | 369 | 47 | 105 | 11 | 29 | 6 | 3.2 | 0.157 |
| 6 | 64°05''669' | 21°30"681' | 19.6 | 19.0-20.5 | 372 | 30 | 95 | 7 | 7 | 8 | 4.5 | 0.236 |
| 7 | 64°05"738' | 21°30''846' | 7.2 | 6.4-8.3 | 371 | 28 | 161 | 3 | 4 | 10 | 4.3 | 0.386 |
| 8 | 64°05''745' | 21°30"865' | 25.0 | 24.9-25.3 | 368 | 36 | 175 | 9 | 37 | 8 | 2.7 | 0.408 |
| 9 | 64°05"782' | 21°30''895' | 18.2 | 16.7-20.7 | 376 | 27 | 63 | 8 | 3 | 16 | 9.0 | 0.291 |
| 10 | 64°05"831' | 21°31"198' | 4.9 | 4.5-6.3 | 392 | 17 | 113 | 9 | 4 | 17 | 7.0 | 0.184 |
| 11 | 64°05''881' | 21°31"338' | 12.8 | 10.8-16.5 | 416 | 30 | 138 | 3 | 5 | 22 | 4.7 | 0.506 |
| 12 | 64°04"801' | 21°28"706' | 13.5 | 12.3-15.2 | 353 | 51 | 359 | 14 | 50 | 0 | 8.5 | 0.020 |
| 14 | 64°06"031' | 21°31"896' | 9.0 | 7.9-10.5 | 422 | 29 | 147 | 6 | 14 | 2 | 3.2 | 0.167 |

Note that site 13 was excluded from this study as lateral inflows were too large (79% Q_{BOT}).

 Table S2. Metabolism and light parameter.

| site | ER g O₂ m ⁻² day ⁻¹ | ER δx/x | GPP g O₂ m ⁻² day ⁻¹ | GPP δx/x | GPP _{MAX} g O₂ m ⁻² day ⁻¹ | k _{PAR} µmol photon s ⁻¹ m ⁻² |
|------|----------------------------------------------|------------|-----------------------------------------------|-------------|--------------------------------------------------------------|-----------------------------------------------------------------|
| 1 | 28.2 | 0.38 | 20.3 | 0.01 | 62.0 | 334 |
| 2 | 18.8 | 0.40 | 14.3 | 0.01 | 43.1 | 478 |
| 3 | 17.1 | 0.58 | 4.9 | 0.42 | 22.9 | 971 |
| 4 | 2.5 | 0.85 | 2.2 | 0.07 | 8.5 | 591 |
| 5 | 38.0 | 0.46 | 27.6 | 0.07 | 85.8 | 214 |
| 6 | 18.3 | 0.46 | 16.8 | 0.05 | 66.0 | 392 |
| 7 | 7.0 | 0.50 | 4.4 | 0.55 | 12.5 | 200 |
| 8 | 66.8 | 0.66 | 13.6 | 0.57 | 56.4 | 911 |
| 9 | 25.5 | 0.39 | 15.7 | 0.38 | 46.0 | 365 |
| 10 | 23.9 | 0.43 | 10.4 | 0.01 | 29.3 | 166 |
| 11 | 9.7 | 0.86 | 9.2 | 0.19 | 28.6 | 453 |
| 12 | 11.4 | 0.76 | 4.2 | 0.21 | 11.0 | 120 |
| 14 | 4.1 | 4.29 | 2.2 | 0.39 | 6.3 | 162 |

 $\delta x/x,$ relative uncertainty based on 1 standard deviation

 Table S3. Physico-chemistry of filtered water samples.

| site | pН | EC | DOC | NH₄-N | NO ₃ -N | total N | PO ₄ -P | total P | Ca | CI | к | Mg | Na | Si | s | SO ₄ -S | Cu | Fe |
|------|------|---------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|
| | | µS cm ⁻¹ | μg L ⁻¹ | mg L ⁻¹ | μg L ⁻¹ | μg L ⁻¹ |
| 1 | 7.81 | 294 | 767 | 9 | 3 | 62 | 3 | 15 | 30.8 | 6.5 | 1.4 | 7.6 | 29.0 | 18.6 | 6.6 | 4.9 | 0.37 | 4.9 |
| 2 | 7.97 | 281 | 424 | 10 | 2 | 36 | 6 | 24 | 32.9 | 6.5 | 2.0 | 7.1 | 37.1 | 16.8 | 6.5 | 4.9 | 0.21 | 4.4 |
| 3 | 7.92 | 275 | 226 | 13 | 4 | 19 | 9 | 28 | 29.5 | 6.4 | 1.8 | 6.5 | 29.9 | 19.6 | 7.9 | 6.4 | <0.03 | 10.7 |
| 4 | 7.67 | 153 | 465 | 8 | 6 | 41 | 1 | 8 | 20.3 | 7.6 | 0.3 | 5.1 | 7.4 | 9.8 | 4.4 | 2.9 | 0.51 | 71.2 |
| 5 | 7.98 | 282 | 427 | 6 | 6 | 32 | 2 | 19 | 31.1 | 6.3 | 2.2 | 6.7 | 36.8 | 19.8 | 6.2 | 4.8 | 0.03 | 3.7 |
| 6 | 8.12 | 283 | 317 | 11 | 2 | 16 | 7 | 28 | 29.8 | 6.5 | 2.2 | 6.4 | 37.0 | 19.7 | 5.9 | 4.6 | 0.05 | 2.5 |
| 7 | 7.63 | 110 | 208 | 10 | 1 | 12 | 12 | 25 | 14.1 | 7.7 | 0.5 | 4.1 | 10.6 | 7.0 | 2.6 | 1.5 | 0.1 | 6.7 |
| 8 | 8.14 | 300 | 330 | 9 | 6 | 14 | 12 | 31 | 28.2 | 6.3 | 2.6 | 5.5 | 37.5 | 20.2 | 4.5 | 3.5 | <0.03 | <0.4 |
| 9 | 8.08 | 262 | 263 | 8 | 4 | 36 | 18 | 36 | 26.9 | 6.6 | 1.5 | 6.6 | 34.6 | 17.9 | 3.8 | 2.7 | 0.03 | 4.3 |
| 10 | 7.65 | 129 | 314 | 6 | 2 | 54 | 10 | 18 | 20.1 | 6.6 | 0.3 | 4.7 | 11.0 | 6.0 | 5.0 | 3.8 | <0.03 | 7.4 |
| 11 | 8.01 | 624 | 581 | 9 | 1 | 85 | 1 | 15 | 32.4 | 5.7 | 2.5 | 29.5 | 123.8 | 25.4 | 1.2 | 0.2 | 0.16 | 0.6 |
| 12 | 7.86 | 223 | 618 | 12 | 5 | 98 | 1 | 11 | 25.1 | 7.0 | 1.4 | 7.7 | 19.6 | 17.6 | 3.8 | 2.6 | 0.26 | 80.8 |
| 14 | 8.10 | 254 | 403 | 10 | 3 | 123 | 1 | 10 | 35.9 | 6.4 | 0.5 | 8.5 | 17.5 | 11.4 | 7.1 | 5.8 | 0.19 | 8.7 |

EC=electric conductivity at 25°C, DOC=dissolved organic carbon