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OPINION PAPER

Temperature responses of substrate carbon conversion efficiencies and growth rates of plant tissues

Lee D. Hansen[a,*], Nathan R. Thomas[a] and Birgit Arnholdt-Schmitt[b]

[a]Department of Chemistry and Biochemistry, Brigham Young University, Provo, UT 84602, USA
[b]EU Marie Curie Chair, ICAM, University of Évora, 7002-554 Évora, Portugal

Correspondence
*Corresponding author,
e-mail: Lee_Hansen@BYU.edu

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Growth rates of plant tissues depend on both the respiration rate and the efficiency with which carbon is incorporated into new structural biomass. Calorespirometric measurement of respiratory heat and CO_2 rates, from which both efficiency and growth rate can be calculated, is a well established method for determining the effects of rapid temperature changes on the respiratory and growth properties of plant tissues. The effect of the alternative oxidase/cytochrome oxidase activity ratio on efficiency is calculated from first principles. Data on the temperature dependence of the substrate carbon conversion efficiency are tabulated. These data show that ε is maximum and approximately constant through the optimum growth temperature range and decreases rapidly as temperatures approach temperature limits to growth. The width of the maximum and the slopes of decreasing ε at high and low temperatures vary greatly with species, cultivars and accessions.

Introduction

Substrate carbon conversion efficiency (aka carbon use efficiency) and respiration rate are directly related to plant growth rate (Hansen et al. 1998). Therefore, understanding the relationship between plant growth and environmental conditions requires understanding the relationship between respiration, efficiency and growth and the response of respiration rate and efficiency to environmental variables. Activity of the alternative oxidase (AOX) pathway in plants affects respiration rate and the AOX/cytochrome oxidase (COX) activity ratio affects carbon use efficiency. Consequently, developing functional markers for efficient growth under defined environmental conditions and searching for gene candidates from the respiration chain for use in plant breeding seem to be well justified (Arnholdt-Schmitt et al. 2006).

In this paper, the general response of growth rate to environmental temperature patterns is described. The quantitative relationship between growth and efficiency and respiration rate is derived and used to develop a quantitative description of the effect of efficiency on both growth rate and total growth over time. The quantitative effect of the AOX/COX activity ratio on efficiency and growth rate is deduced from first principles. The basis of the calorespirometric method for the determination of substrate carbon conversion efficiency (ε) and growth rate is described. The calorespirometric method is compared with the carbon balance method for the determination of ε. Calorespirometric data on tomato and cabbage leaf tissue are used to illustrate the application of the method for the determination of both ε and growth rate as functions of temperature. Literature data on the temperature dependence of ε are tabulated. Efficiency data from calorespirometry are compared with data from the carbon balance method. The tabulated data on ε show a general pattern of the response of ε to temperature: ε is at a maximum and approximately constant over the range of normal growth temperatures and decreases rapidly as temperatures approach temperature

Abbreviations – AOX, alternative oxidase; COX, cytochrome oxidase.

limits to growth. The temperature range of maximum ε is related to the temperature range the plant is adapted or acclimated to–a broad temperature range in some plants and a narrow range in others–and therefore varies among species, cultivars and accessions.

Temperature dependence of growth rates

Natural selection produces well-adapted autochthonous plants with a curve of growth rate vs temperature congruent with the curve of time-at-temperature vs temperature. (Criddle et al. 2005) Congruency means the maxima and minima occur at the same temperatures in the two curves. Such a congruency optimizes the temperature response of growth rate to the local environment within the constraints of other environmental variables during the growth season for a given plant. Time-at-temperature curves describing the temperature distribution in stable climates with small diurnal temperature changes and in unstable climates appear as a parabola skewed to higher temperatures with a maximum near the mean temperature and zero values at the minimum and maximum temperatures (Criddle et al. 2001, Hansen et al. 2002, 2008, Summers et al. 2009, Thygerson et al. 2002, Yu et al. 2008). Examples of such curves are shown schematically in Fig. 1. In stable climates with large diurnal temperature changes, time-at-temperature curves have two maxima at the mean day and night temperatures and a minimum near the mean daily temperature (Criddle et al. 2005) and curves of growth rate vs temperature have the same shape (McCarlie et al. 2003, Matheson 2000, Ward 2007). The congruency between the curve describing the environmental temperature pattern and the curve of growth rate vs temperature for native plants demonstrates that optimizing crop yields requires matching plant temperature responses to the local climate. For example, compare growth rates of A, B, C and D in Fig. 1. In a climate with a mean temperature of 14°C, A grows fastest, B grows at about 80% of the growth rate of A, C grows at about 40% of the growth rate of A and D will not grow. In a climate with a mean temperature of 19°C, B grows fastest, C grows at about 80% of the growth rate of B, A grows at about 60% of the growth rate of B and D grows at about 30% of the growth rate of B. In a climate with a mean temperature of 24°C, C grows fastest, D grows at about 80% of the growth rate of C, B grows at about 60% of the growth rate of C and A will not grow. The response of growth rate to temperature also explains why small changes in climate temperatures can cause large changes in growth and consequent range shifts of native plant populations (Colwell et al. 2008, Lenoir et al. 2008, Pörtner and Farrell 2008, Svenning and Condit 2008). For example, for a temperature shift from 20°C to 22°C for the growth curves illustrated in Fig. 1, B and C would become equally good growers, the growth rate of D would increase slightly and A would eventually disappear.

Fig. 1 also illustrates why the results from common garden studies typically used to evaluate new cultivars or accessions of wild plants are not readily transferable

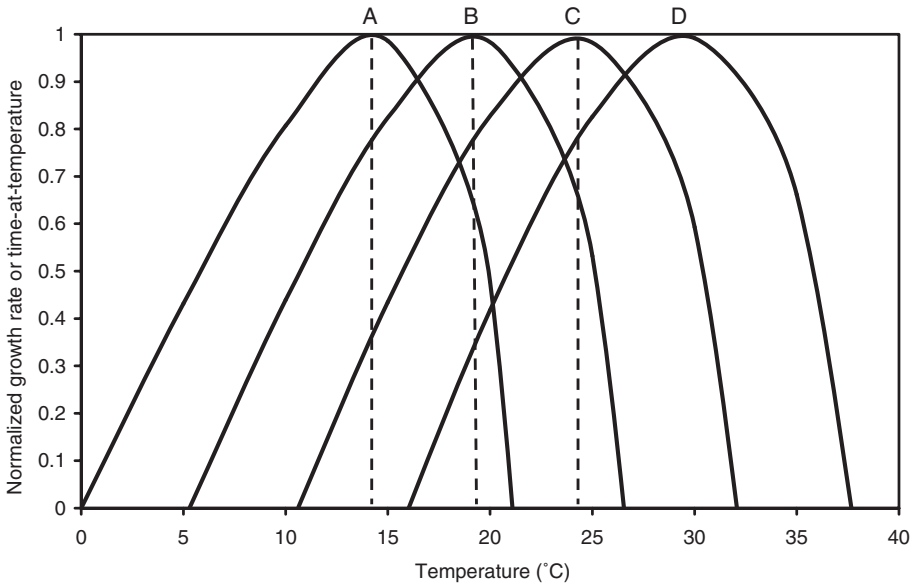

Fig. 1. Schematic curves of growth rate vs temperature for plants with different temperatures for maximum growth rate and the same temperature range for growth.

between locations, i.e. common garden growth studies can only pick the best grower at the location and temperature pattern at the garden location. Calorespirometric determination of the growth curve vs temperature (Criddle et al. 1997, Criddle and Hansen 1999, Hansen et al. 2005) enables matching the growth curve to a local temperature pattern (i.e. the curve of time-at-temperature vs temperature) without growing the plant at the actual location and temperatures it is adapted to. Calorespirometric data can thus be used to rapidly optimize productivity and avoid crop losses from extreme temperature events.

The relationship between respiratory properties and growth

Natural selection to match the growth curve to the temperature curve does not operate directly on growth rate, but instead on the determinants or genes of growth rate that are related to the respiratory properties and their response to temperature. The overall reaction describing respiration driven growth of structural biomass ($C_{biomass}$) is:

$$C_{substrate} + N, P, K, \text{etc.} + xO_2 \rightarrow \varepsilon C_{biomass} + (1-\varepsilon)CO_2 \quad (1)$$

where ε is the substrate carbon conversion efficiency or carbon use efficiency and is equal to the fraction of substrate carbon passing through respiration that is converted into structural biomass. The symbol ε is used here both to avoid the awkward use of a multiple character symbol in equations (Mills et al. 1993) and to distinguish the quantity calculated (ε) from CUE which includes total carbon, not just structural biomass carbon. Stoichiometry requires:

$$R_{biomass}/R_{CO2} = \varepsilon/(1-\varepsilon) \text{and } R_{biomass} = R_{CO2}[\varepsilon/(1-\varepsilon)] \quad (2)$$

where R indicates the rate of formation of the substance in the subscript and biomass indicates structural biomass (Hansen et al. 2004). To understand how growth rate, i.e. $R_{biomass}$, responds to temperature, the temperature responses of both R_{CO2} and $[\varepsilon/(1-\varepsilon)]$ must be measured. Note the large effect that small changes in ε have on total growth because of the functional form of Eqn 2, see Supporting information Fig. S1. Changing ε from 0.7 to 0.8 changes total growth over one unit of time by a factor of 5 and over 10 units of time by a factor of 10^7. Thus, small changes in ε exert huge changes in total growth when integrated over time.

Many measurements of R_{CO2} as functions of temperature are reported in the literature, but only few cover the full range of growth temperatures with sufficient detail; most have been made on mature non-growing tissues, and, without accompanying determinations of ε, are of little use in understanding adaptation of respiration, and thereby growth rate, to the environmental temperature pattern.

Effect of AOX activity on substrate carbon conversion efficiency

Because the AOX pathway produces only about one-third of the amount of ATP per substrate carbon oxidized to CO_2 as does the COX pathway, and because the amount of ATP required per substrate carbon incorporated into structural biomass is approximately constant and independent of the AOX/COX activity ratio, it is a simple matter of stoichiometry that changes in the AOX/COX ratio must change ε unless changes in the AOX/COX ratio are compensated for by changes in other 'futile' pathways of metabolism such as those catalyzed by ATPases and NAD(P)H dehydrogenases (Macfarlane et al. 2002, 2009, Moore et al. 2003). In the absence of such a compensation, increasing the ratio of AOX activity to COX activity decreases ε and decreasing AOX/COX increases ε (Macfarlane et al. 2002). Extending this reasoning further, if ε is constant, then AOX/COX ratio is also likely to be constant (Macfarlane et al. 2009).

Effect of AOX activity on growth rate

If there are no compensating changes in respiration rate, R_{CO2}, increasing the AOX/COX activity ratio decreases ε which decreases growth rate, and decreasing the AOX/COX activity ratio increases ε which increases growth rate; see Eqn 2 and Supporting information Fig. S1 and S2 in this paper and discussion in Macfarlane et al. (2002). Supporting information Fig. S1 shows the effect of ε on total growth over time and Fig. S2 shows the effect of ε on growth rate. Beyond the assumptions stated above, the relationships shown in Supporting information Fig. S1 and S2 are solely based on stoichiometric relationships and the law of conservation of mass, and thus must be valid so far as the assumptions are valid.

Because increasing the AOX/COX activity ratio decreases ε which decreases growth rate, it is surprising that AOX is ubiquitous in plants and plant tissues, occurs in most other kingdoms of life (McDonald et al. 2003, McDonald and Vanlerberghe 2004, 2005, 2006, McDonald 2008) and is active to a significant extent under most conditions. AOX must play a significant role in regulating plant metabolism and in metabolic responses to environmental changes, or natural selection would have deleted it from plants where growth

rate or efficiency is a selection factor. However, as discussed in previous papers (Hansen et al. 2001, 2002, Macfarlane et al. 2002, Moore et al. 2002, Stucki 1980) and in this special issue (Kapuganti et al. 2009, Rasmusson and van Dongen 2009, Vanlerberghe et al. 2009), futile cycles and pathways with differing efficiencies may be necessary for homeostasis in changing conditions.

Understanding the role of AOX in adaptation of plant growth and metabolism to environmental temperatures will require measurements of both the AOX/COX activity ratio and ε as functions of temperature in several species grown under a variety of conditions. Measurements on variants with downregulated or upregulated activities of both AOX and COX would be helpful. To date very few measurements of the AOX/COX activity ratio have been made as functions of temperature (Armstrong et al. 2008, Kruse et al. 2008, Macfarlane et al. 2009, Ribas-Carbo et al. 2005), and the results of those studies appear to disagree as to whether or not the ratio of AOX to COX activity changes with temperature. It is not clear whether the disagreement is because of differences in measurement methods, differences in measurement temperatures, choice of species or tissues or differences in growth conditions. Because of the close relationship between the AOX/COX ratio and ε (see Supporting information Fig. S2), data on the temperature dependence of ε suggest those species and conditions that would be of most interest to examine by isotope ratio mass spectrometry (Ribas-Carbo et al. 2005) or laser spectroscopy (Kerstel and Gianfrani 2008) to directly determine the AOX/COX ratio from oxygen isotope fractionation.

Methods for measuring substrate carbon conversion efficiency

Two methods are available for the determination of ε, direct measurement of the carbon balance (e.g. see Gifford 2003, Taylor et al. 1998, van Iersel and Seymour 2000, van Iersel 2003, Yamaguchi 1978) and indirect measurement through measurements of R_{CO2} and the oxygen uptake rate, R_{O2}, or equivalently, the respiratory heat production rate, R_q (e.g. see Criddle and Hansen 1999, Hansen et al. 2002, 2005, Macfarlane et al. 2002). Note that heat and oxygen rates are directly proportional as stated by Thornton's rule (Battley 1999, Hansen et al. 2004):

$$R_q = [(-455 \pm 15)\ \text{kJ mol}^{-1}](R_{O2}) \quad (3)$$

When applied to determine the temperature dependence of the substrate carbon conversion efficiency, the carbon balance method is slow, requiring days to months, and requires keeping the plants at a test temperature or temperature pattern for an extended period of time (e.g. see Gifford 2003, van Iersel 2003, Yamaguchi 1978), thus making it difficult to separate adaptation from acclimation. There appear to be only two reports of determinations of the temperature dependence of carbon use efficiency by the carbon balance method, Yamaguchi (1978) and Gifford (2003). The carbon balance method is the appropriate method for whole plants larger than seedlings (e.g. see van Iersel 2003, van Iersel and Seymour 2000) and for ecosystems (e.g. see DeLucia et al. 2007, Gifford 2003), but is not appropriate for small samples of individual tissues. In contrast, the calorespirometric method requires small samples (typically ~100 mg wet weight) of tissue, and therefore is applicable only to tissue samples or small seedlings. Calorespirometric measurements of ε are easily made as a function of tissue age and temperature (e.g. see Matheson 2004) and can be made in hours, too rapid for acclimation to occur. The results, therefore, more accurately describe the response of the plant or tissue to diurnal temperature changes than to longer-term temperature changes.

Calorespirometric measurement of substrate carbon conversion efficiency, ε, and growth rate

The calorespirometric method for the determination of ε is based on the following relations (Criddle and Hansen 1999, Hansen et al. 2002, 2004, 2005, Macfarlane et al. 2002). The metabolic heat rate is the sum of the heat rates from the two reactions (anabolism and catabolism) comprising the above overall reaction 1:

$$R_q = -\Delta H_{CO2} R_{CO2} - \Delta H_B R_{biomass} \quad (4)$$

ΔH_B is the enthalpy change for the reaction $C_{substrate} \rightarrow C_{biomass} + yO_2$, which for carbohydrate going to vegetative plant biomass equals $+(30 \pm 20)$ kJ mol^{-1} (calculated from data in Ellingson et al. 2003, Gary et al. 1995, Lamprecht 1999), and ΔH_{CO2} for carbohydrate equals -470 kJ mol^{-1}, i.e. the heat of combustion of aqueous sugars (Domalski 1972, Miller and de Pablo 2000). The value of ΔH_B can be obtained from the heat of combustion of the tissue, from Thornton's rule and elemental composition of the tissue, and from parallel measurements of growth rate and calorespirometric measurements of R_q and R_{CO2} (Ellingson et al. 2003). Because it is a state function, ΔH_B depends only on the substrate (carbohydrate is assumed here) and the elemental composition or oxidation state of the carbon in the tissue. The nature of the carbohydrate is not significant because all carbohydrates have nearly the same heat of combustion per Cmole (Domalski 1972). Nitrogen is the

only element present in sufficient amount to significantly affect the value of ΔH_B. If the substrate nitrogen is present as NH_4^+, no change in oxidation state occurs between the substrate and the tissue and there is no significant effect on ΔH_B. If the nitrogen source is NO_3^-, nitrate replaces an equivalent amount of O_2, and because the heats of reduction of NO_3^- and O_2 per equivalent are similar, this also has only a small effect on ΔH_B.

Rearranging Eqn 4 gives the equation for the rate of formation of structural biomass, the anabolic or growth or development rate:

$$R_{biomass} = (-\Delta H_{CO2} R_{CO2} - R_q)/(\Delta H_B) \quad (5)$$

Combining Eqns 2 and 4 to eliminate $R_{biomass}$ gives:

$$R_q/R_{CO2} = -\Delta H_{CO2} - \Delta H_B[\varepsilon/(1-\varepsilon)] \quad (6)$$

Defining $x = [\varepsilon/(1-\varepsilon)]$ and substituting appropriate values for ΔH_{CO2} and ΔH_B:

$$x = [\varepsilon/(1-\varepsilon)] = [(R_q/R_{CO2}) - 470]/(-30) \quad (7)$$

$$\varepsilon = x/(x+1) \quad (8)$$

Simultaneous measurements of R_q and R_{CO2} as functions of temperature thus provide the data to calculate both growth rate (Eqn 5) and ε (Eqns 7 and 8) as functions of temperature. Such measurements in triplicate take about 90 min per temperature in the MC-DSC calorimeter manufactured by TA Instruments, Lindon, UT (Hansen et al. 2005). The precision of ε values calculated from calorespirometric data is typically about ± 0.05; however, at small values of ε, a small uncertainty in the R_q/R_{CO2} ratio causes a large uncertainty in ε; at large values of ε, a large uncertainty in the R_q/R_{CO2} ratio causes only a small uncertainty in ε, see Supporting information Fig. S3.

Comparisons, both quantitative and qualitative, of directly observed growth rates with growth rates calculated from calorespirometric data demonstrate the accuracy of the method for both growth rates and efficiencies (Ellingson et al. 2003, Marcar et al. 2002, Macfarlane et al. 2005, Taylor et al. 1998).

Determination of growth and maintenance coefficients by calorespirometry

Much of the literature on the relationship of carbon use efficiency to growth rate has been discussed in terms of a growth coefficient and a maintenance rate (e.g. see Gifford 2003 and discussion and Eqn 5 in van Iersel 2003). Because calorespirometry can be used to rapidly determine both respiratory CO_2 and growth rates as functions of temperature, it immediately suggests its use to determine growth coefficients and maintenance rates, and indeed such determinations have been reported (Matheson 2004). But derivation of growth and maintenance coefficients from the slope and intercept of plots of specific respiratory CO_2 rate vs specific growth rate as has traditionally been done is problematic. Because it requires varying the age of the plant or the growth environment (Gifford 2003), such plots contain one or the other of these hidden variables. The hidden variable of increasing age is correlated with decreasing concentration of metabolically active tissue, and this 'dilution by growth' violates the assumption of constant concentration of active tissue implicit in the growth-maintenance model (Matheson et al. 2004). Varying the growth environment as the hidden variable violates the assumption of constant maintenance rate implicit in the analysis (Gifford 2003, Hansen et al. 1998, Matheson et al. 2004). Interpretation of the slope and intercept of such plots, respectively, as a growth coefficient and a maintenance rate is thus not valid. Instead, plots of specific respiratory CO_2 rate vs specific growth rate can be interpreted with Eqn 2, wherein the slope of a line from the (0,0) origin to a data point is equal to $\varepsilon/(1-\varepsilon)$.

Calorespirometric data illustrating the temperature dependence of respiration and growth rates

Fig. 2 shows data on a warm-climate, chilling sensitive plant, tomato, and a cold-climate plant, cabbage, that are characteristic of the data collected by calorespirometry. Although these are not autochthonous plants, the data are typical of curves of CO_2 production rate, metabolic heat rate, efficiency and growth rate as functions of temperature in rapidly growing vegetative tissues. Note that heat and CO_2 rates cannot have the same temperature dependence in growing tissues or growth rate would be independent of temperature, see Eqn 5.

The heat production rate, which is directly proportional to the O_2 uptake rate (Hansen et al. 2004), has typically been described by an Arrhenius function (i.e. rate $= Ae^{-\mu/T}$ where A and μ are constants, e is the natural logarithm base and T is Kelvin temperature, see Taylor et al. 1998), but the data in Fig. 2 and from several other studies show this function does not describe the data over the growth temperature range. The data at higher temperatures always fall below the function extrapolated from lower temperatures. A smooth function also cannot clearly capture the significant deviations that often occur in carefully measured data. (Uncertainties for data in Fig. 2 are given in Criddle et al. (1997).) Such deviations from a smooth function have been observed in several other studies of growing tissues

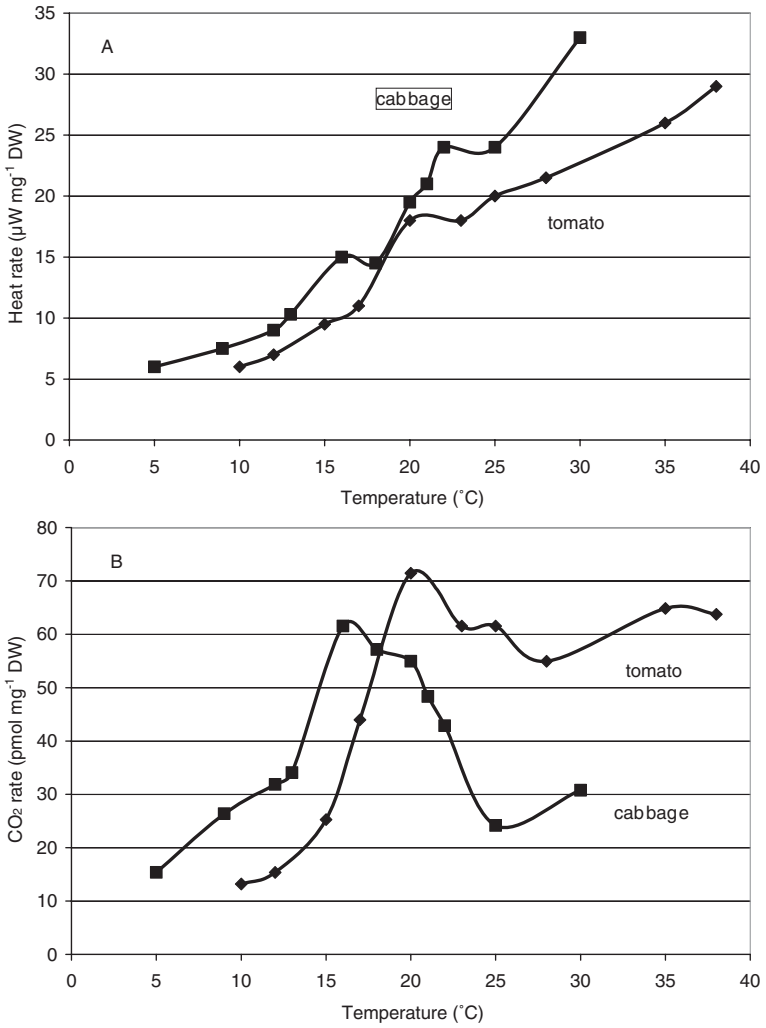

Fig. 2. Measured respiratory heat rate (A), CO_2 production rate (B), calculated growth rate (C) and substrate carbon conversion efficiency (D) for rapidly growing leaf tissue from tomato (*Lycopersicon esculentum*) and cabbage (*Brassica oleracea var. capitata*). Uncertainty is about ±0.5μW mg^{-1} DW in heat rates and ±1 pmol mg^{-1} DW s^{-1}in CO_2 production rates. Data taken from Criddle et al. (1997).

(Hansen et al. 2008, Matheson 2000, Macfarlane 2002, Smith et al. 2001, Summers et al. 2009, Ward 2007, Yu et al. 2008).

Although CO_2 rates of mature tissues can often be fit by the Arrhenius relation, the Arrhenius function is a poor description of the CO_2 production rate in growing tissues near and above the temperature of maximum growth rate. The observed rapid decrease in the CO_2 rate at temperatures above the temperature of maximum growth rate is always observed in rapidly growing tissues and occurs because there are two sources of CO_2 in growing tissues, the catabolic reaction

$$C_{substrate} + O_2 \rightarrow CO_2 \quad (9)$$

and the anabolic or growth reaction

$$C_{substrate} \rightarrow C_{biomass} + yCO_2 \quad (10)$$

In rapidly growing tissues, the latter reaction produces the majority of the CO_2, and thus the total CO_2 rate is greatly reduced at temperatures that inhibit growth. Reaction 10 is of course absent in mature tissues and much lower specific CO_2 rates are observed.

Growth rate is proportional to the difference, CO_2 rate minus the heat (or O_2) rate, see Eqn 5 (Hansen et al. 2004). The data in Fig. 2C exhibit the typical skewed parabolic shape of the response to temperature. The decrease in growth rate above the optimum temperature must be caused by reduced efficiency (ε) because the heat rate (and hence the oxygen uptake rate) continues

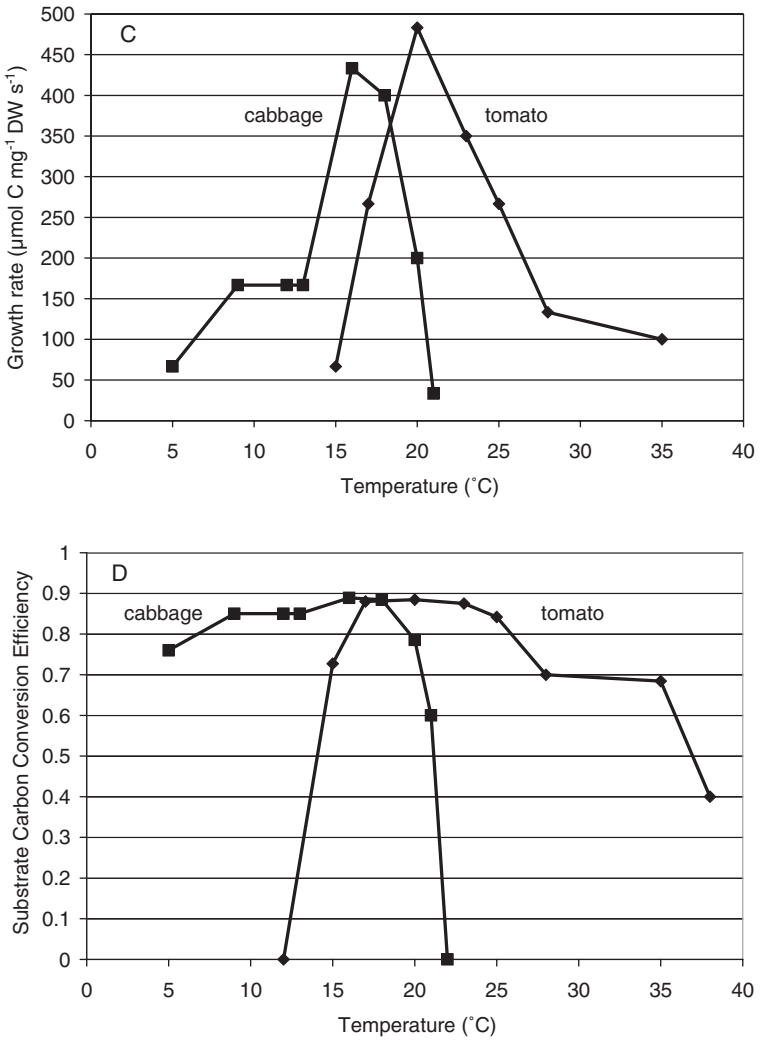

Fig. 2. Continued

to increase. AOX may play an important role in the rapid decrease in ε at high temperatures.

Efficiency is related to the ratio of heat rate to the CO_2 rate, see Eqns 6–8 and Supporting information Fig. S3. In the examples of tomato and cabbage in Fig. 2, the efficiency is relatively constant through the temperature range, 17–25°C for tomato and 8–18°C for cabbage, declines somewhat above this range in tomato and below this range in cabbage, but drops precipitously at stress temperatures <15°C for tomato and >20°C for cabbage. The calorespirometric measurements are made rapidly enough not to allow acclimation, but are representative of the rate of diurnal temperature changes. Thus, the expectation is that the ratio of AOX/COX activities is constant in tomato from 17°C to 25°C and in cabbage from 8°C to 18°C, and if change in the AOX/COX activity ratio is the cause of the decreasing efficiency, the ratio of AOX/COX activities would be expected to increase with increasing temperature above 25°C in growing tomato leaves and below 8°C in growing cabbage leaves. The precipitous drops in ε below 15°C in tomato and above 20°C in cabbage are probably not caused solely by changes in the AOX/COX ratio, but by loss of the ability to maintain homeostasis of intracellular potentials (Criddle et al. 1997). These conditions are fatal for tomato if maintained long enough and lead to death or bolting in cabbage.

Literature review of the temperature dependence of substrate carbon conversion efficiency

Supporting information Table S1 summarizes literature data on substrate carbon conversion efficiency but is

limited to reports where ε was measured at different temperatures in growing tissues. (Note that by definition, $\varepsilon = 0$ in non-growing tissues, and measurements on mature, and thus non-growing, tissues would not provide any meaningful data on ε.) Nearly all of the data are from calorespirometry. Only two sets of data for the temperature dependence of ε determined by the carbon balance method are available, Yamaguchi (1978) and Gifford (2003). For consistency, all values of ε from calorespirometric data are calculated using Eqn 7. Absolute values of ε from calorespirometry may thus be slightly inaccurate because of variable tissue composition that affects ΔH_B values. Physiological tissue age also affects the relative amount of metabolically active tissue in a sample, and thus ε tends to decrease with tissue age (e.g. see Macfarlane et al 2005, Matheson 2004, van Iersel 2003). However, because similar tissue is used across the measurement temperature range in any given calorespirometry experiment, the precision in a set of ε values and trends with temperature should not be affected by these sources of inaccuracy. Temperature stress typically reduces ε (e.g. see *Nicotiana*, #3 in Supporting information Table S1, at 10°C and 30°C), but at temperatures well outside the normal range, ε values calculated from Eqn 7 either go negative or become greater than 1 indicating that Eqn 7 no longer applies because the values of ΔH_{CO2} and ΔH_B no longer equal −470 and +30 kJ mol^{-1}, respectively. Under these conditions, Eqn 7 sometimes gives anomalously large values of ε [i.e. see *Zea mays* (Santa Ana Blue), #11 in Supporting information Table S1, at 40°C, *Avena sativa* (Edirne and Hatay), #14, at 35°C, *Eucalyptus globulus* grown at 28°, #28, at 40°C, and *Juglans regia*, #35, at 25°C and 30°C] instead of zero or negative values as expected. Measured, but negative values of ε are not reported in Supporting information Table S1.

Comparison of ε values from calorespirometry and from carbon balance

Calorespirometric values of ε range from 0 to 0.93 with values typically between 0.6 and 0.8 at temperatures in the optimal growth temperature range. Values of ε for maize seedlings determined by carbon balance range from 0.83 to 0.89 (Yamaguchi 1978) and are in agreement with the ε values for maize seedling tissue determined by calorespirometry, see #11 in Supporting information Table S1 (Smith et al. 2001, Taylor et al. 1998). This direct comparison of the two methods further demonstrates the validity of the calorespirometric method. Values of ε for forest ecosystems determined by the carbon balance method range from 0.2 to 0.8, but are typically around 0.5 (DeLucia et al. 2007, Gifford 2003, Macfarlane et al. 2005). van Iersel (2003) reported values of 0.5 to 0.6 for small lettuce plants and 0.2 to 0.3 for large plants. The generally wider range of ε values reported from calorespirometric measurements compared with values from carbon balance reflects the ability of the calorespirometric method to measure ε at conditions where plants cannot survive and to measure ε for individual tissues. The carbon balance method can only be used at conditions where plants can be grown. Also, the carbon balance method is usually applied to whole plants rather than to single tissues, so the value of ε obtained depends on the phenology of the plant, i.e. young, rapidly growing tissues have high values of ε, and, because they are not growing or growing very slowly, mature tissues have small to zero values of ε. The value of ε obtained on whole plants is thus determined by the ratio of growing to non-growing tissue. The calorespirometric method is usually applied to rapidly growing tissues and thus tends to report higher values of ε than does the carbon balance method. The effect of tissue age on ε is clearly shown in Macfarlane et al. (2005) as a plot of calorespirometric ε values vs growth rate, and hence tissue age, of *E. globulus* leaf tissue which shows an abrupt decrease of ε to zero as leaves stop growing. Similarly, van Iersel (2003) shows a linear relationship between the reciprocal of ε and the reciprocal of specific growth rate of lettuce plants, i.e. ε decreases as growth rate decreases.

Temperature dependence of ε

A general conclusion that can be drawn from the data in Supporting information Table S1 is that the temperature response of ε is highly variable among species, cultivars and accessions, e.g. see Fig. 2D, 3 and 4. In general, the curve of ε vs temperature is a flattened, inverted 'U.' The plots for tomato and cabbage in Fig. 2D are typical. As shown in Fig. 3 and 4, the width of the flattened part of the curve and the slopes of the legs on the U as well as details of the curve vary with species and with populations or cultivars of the same species that are adapted to different temperature patterns. A shift of the maximum to the most frequent temperature during the growing season and a widening or narrowing of the inverted U curve of ε to match the temperature difference between the maximum and minimum temperatures of the growth season occur in comparisons of populations of the same species. In these aspects, the curves of ε vs temperature mimic curves of growth rate vs temperature, a not surprising result in light of the significance of ε in determining both growth rate and total growth, see Supporting information Fig. S1 and S2. The ε data for Turkish oat accessions in Supporting information

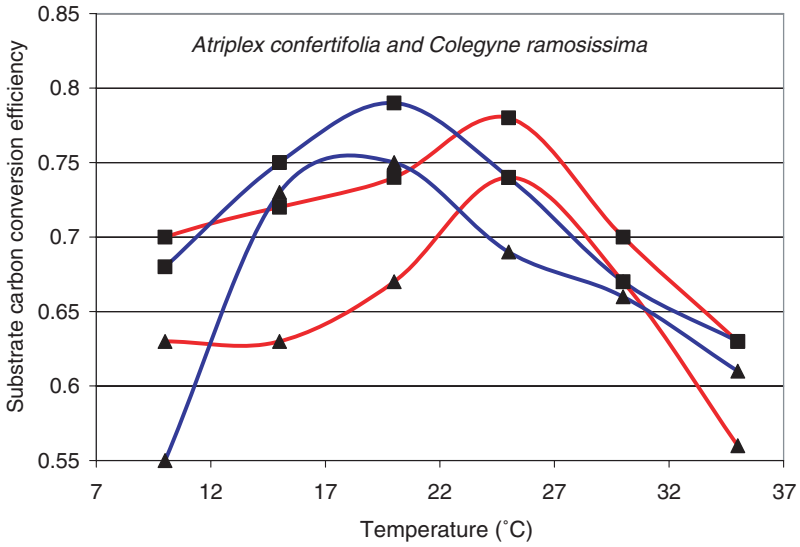

Fig. 3. Temperature response of substrate carbon conversion efficiency in leaf tissue of *Atriplex confertifolia* (square symbols, #17 in Supporting information Table S1) and *Coleogyne ramosissima* (triangle symbols, #18 in Supporting information Table S1). Red curves are for plants from lower elevations and blue curves are for plants from higher elevations within the native range.

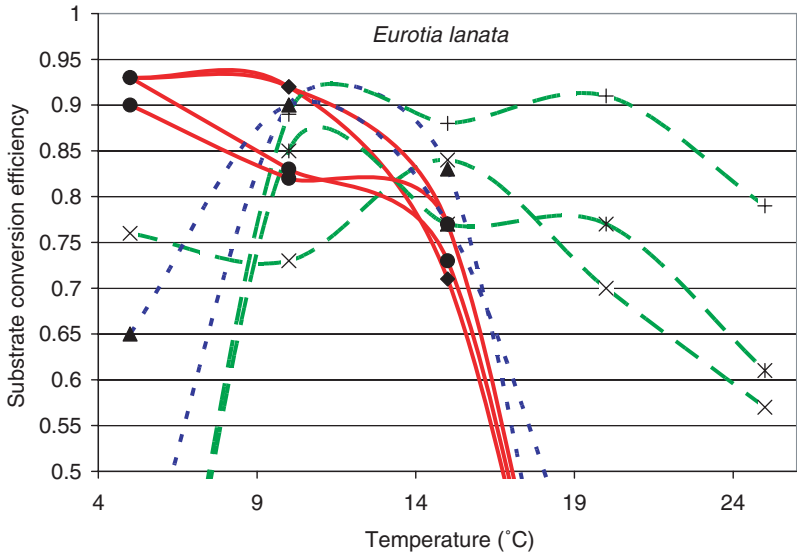

Fig. 4. Temperature response of substrate carbon conversion efficiency in *Eurotia lanata* (#21 in Supporting information Table S1) leaf tissue from different accessions. Three types of response are indicated with red solid, blue dotted and green dashed lines. The Pinebluffs accession is indicated by (♦), the Matador accession by (▲), the Sterling accession by (●), the Northside accession by (X), the Westside accession by (*) and the Southside accession by (+). The Pinebluffs, Matador and Sterling accessions were measured at two different times.

Table S1 (#14) show that efficiency may have the same bimodal response to temperature as growth curves for plants adapted to stable climates with a large diurnal temperature difference. Just as the temperature dependence of ε varies among populations of native species, it also varies among cultivars of crop plants. For example, compare *Z. mays* cv. Pioneer G17 with cv. Pioneer T10 (#11 in Supporting information Table S1). G17 has a narrow and low growth temperature range, only about 10°C wide around 15–25°C, whereas T10

has a much wider and higher growth temperature range, about 15°C wide around 20–35°C. Assuming the same respiration rate, G17 would grow six times faster than T10 at 15°C, whereas at 30°C, T10 would grow five times faster than G17 (see Eqn 2). These data show the importance of calorespirometric measurements of ε and growth rate in rapidly characterizing new cultivars.

In some plants, ε is constant over a broad temperature range (20°C or 25°C wide), i.e. *Cucurbita pepo* (pumpkin, #4 in Supporting information Table S1), *Raphanus sativus* (#7), some *Z. mays* cultivars (#11), *Hesperostipa comata* (#13), *Artemisia tridentata* (#19), *Caragana* (#20) and *Agapanthus orientalis* (#38). In the other plants in Supporting information Table S1, the range of temperatures at which ε is constant is much smaller. An approximately constant ε over the range of normal growth temperatures implies the ratio of AOX to COX activity must also be relatively constant over the same temperature range and therefore the temperature dependencies of the two pathways must be approximately equal over the range of temperatures in which ε is constant. Because ε rapidly decreases outside this range, the AOX/COX activity ratio is also expected to increase rapidly at temperatures outside the range where ε is constant. Macfarlane et al. (2009) observed closely similar temperature dependences of AOX and COX over approximately the same temperature range as near constant ε values (see *Nicotiana sativa*, #3, between 15°C and 25°C, *C. pepo*, white scalloped squash, #4, between 20°C and 35°C, and *Vicia faba*, #5, between 20°C and 30°C, in Supporting information Table S1). In contrast, Kruse et al. (2008) observed a difference in the temperature dependencies of the AOX and COX pathways in *Pinus radiata*. The very narrow temperature range of the maximum ε in these data (Supporting information Table S1, #27, at 10°C) probably explains the reason. Except for the one high value of ε at 10°C, their measurements (at 15°C and above) are apparently on the high temperature leg of the inverted U of ε values.

Because nearly all measurements of ε have been made at 5°C intervals, the data in Supporting information Table S1 are only given at 5°C intervals, and as a consequence curves of ε vs temperature near the upper and lower temperature limits are poorly defined. However, the data in Supporting information Table S1 show that ε decreases steeply as temperatures exceed the normal range the plant is adapted to. Calorespirometric data show this decrease in ε happens in less than 2 h, and therefore is probably a result of a relative decrease of COX activity, rapid activation of pre-existing AOX and/or rapid activation of other futile pathways. As they likely take longer than this, gene activation and subsequent protein synthesis are unlikely to be the cause of this rapid decrease.

The one set of data in Supporting information Table S1 in which two environmental variables were studied simultaneously, i.e. salt and temperature with the halophyte *Salicornia utahensis* (#16), shows that changes in one environmental variable can change the response to another environmental variable. Because the salt playas where *S. utahensis* grows are flushed with snowmelt in spring and the salt is concentrated by evaporation in summer, *S. utahensis* is adapted for low salt concentrations when temperatures are low and for high salt concentrations when temperatures are high (Harris et al. 2001). The efficiency data in Supporting information Table S1 reflect this adaptation, i.e. viable ε values occur at low salt/low temperature and at high salt/high temperature conditions. Another study, of *E. camaldulensis* leaf tissue, done only at 25°C and thus not included in Supporting information Table S1 demonstrated synergistic effects of salt and high pH in decreasing ε (Marcar et al. 2002). The relative rapidity with which calorespirometric measurements can be made makes such multivariable studies feasible.

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References

Armstrong AF, Badger MR, Day DA, Barthet MM, Smith PMC, Millar AH, Whelan J, Atkin OK (2008) Dynamic changes in the mitochondrial electron transport chain underpinning cold acclimation of leaf respiration. Plant Cell Environ 30: 1156–1169

Arnholdt-Schmitt B, Costa JH, Fernandes de Melo D (2006) AOX–a functional marker for efficient cell reprogramming under stress? Trends Plant Sci 11: 281–287

Battley EH (1999) The thermodynamics of microbial growth. In: Kemp RB (ed.) From Macromolecules to

Man, Handbook of Thermal Analysis and Calorimetry, Vol. 4. Elsevier Science, Amsterdam, pp 250–251
Colwell RK, Brehm G, Cardelús CL, Gilman AC, Longino JT (2008) Global warming, elevational range shifts, and lowland biotic attrition in the wet tropics. Science 322: 258–261
Criddle RS, Breidenbach RW, Fontana AJ, Henry J-M, Smith BN, Hansen LD (1996) Plant respiration responses to climate determine geographic distribution. Russ J Plant Physiol 43: 698–704
Criddle RS, Hansen LD (1999) Calorimetric methods for analysis of plant metabolism. In: Kemp RB (ed.) From Macromolecules to Man, Handbook of Thermal Analysis and Calorimetry, Vol. 4. Elsevier Science, Amsterdam, pp 711–763
Criddle RS, Hansen LD, Smith BN, Macfarlane C, Church JN, Thygerson T, Jovanovic T, Booth T (2005) Thermodynamic law for adaptation of plants to environmental temperatures. Pure Appl Chem 77: 1425–1444
Criddle RS, Smith BN, Hansen LD (1997) A respiration based description of plant growth rate responses to temperature. Planta 201: 441–445
Criddle RS, Smith BN, Hansen LD, Church JN (2001) Determination of plant growth rate and growth temperature range from measurements of physiological parameters. USDA Forest Service Proceedings RMRS-P-21, pp 251–258
DeLucia EH, Drake JE, Thomas RB, Gonzalez-Meler M (2007) Forest carbon use efficiency: is respiration a constant fraction of gross primary production? Global Change Biol 13: 1157–1167
Domalski ES (1972) Selected values of heats of formation of organic compounds containing the elements C, H, N, O, P, and S. J Phys Chem Ref Data 1, 221–277
Ellingson D, Olson A, Matheson S, Criddle RS, Smith BN, Hansen LD (2003) Determination of the enthalpy change for anabolism by four methods. Thermochim Acta 400: 79–85
Gary C, Frossard JS, Chenevard D (1995) Degree of reduction and carbon content. 3. Interrelated methods of estimating the construction cost of plant tissues. Agronomie 15: 59–69
Gifford RM (2003) Plant respiration in productivity models: conceptualization, representation and issues for global terrestrial carbon-cycle research. Funct Plant Biol 30: 171–186
Gupta KJ, Zabalza A, van Dongen JT (2009) Regulation of respiration when the oxygen availability changes. Physiol Plant 137: 383–391
Hansen LD, Breidenbach RW, Smith BN, Hansen JR, Criddle RS (1998) Misconceptions about the relation between plant growth and respiration. Bot Acta 111: 255–260
Hansen LD, Church JN, Matheson S, McCarlie VW, Thygerson T, Criddle RS, Smith BN (2002) Kinetics of plant growth and metabolism. Thermochim Acta 388: 415–425
Hansen LD, Criddle RS, Smith BN (2005) Calorespirometry in plant biology. In: Lambers H, Ribas-Carbo M (eds) Plant Respiration: From Cell to Ecosystem, Advances in Photosynthesis and Respiration. Springer, Dordrecht, pp 17–30
Hansen LD, Farnsworth LK, Itoga NK, Nicholson A, Summers HL, Whitsitt MC, McArthur ED (2008) Two subspecies and a hybrid of big sagebrush: comparison of respiration and growth characteristics. J Arid Environ 72: 643–651
Hansen LD, Macfarlane C, McKinnon N, Smith BN, Criddle RS (2004) Use of calorespirometric ratios, heat per CO_2 and heat per O_2, to quantify metabolic paths and energetics of growing cells. Thermochim Acta 422: 55–61
Hansen LD, Smith BN, Criddle RS, Church JN (2001) Responses of plant growth and metabolism to environmental variables predicted from laboratory measurements. Shrubland Ecosystem Genetics and Biodiversity: Proceedings, USDA Forest Service, RMRS-P-21, pp 259–264
Harris LC, Khan MA, Zou J, Smith BN, Hansen LD (2001) Effects of salinity and temperature on respiratory metabolism of *Salicornia utahensis* from a great basin playa. Shrubland Ecosystem Genetics and Biodiversity: Proceedings, USDA Forest Service, RMRS-P-21, pp 265–270
Hemming DJB, Meyer SE, Smith BN, Hansen LD (1999) Respiration characteristics differ among cheatgrass (*Bromus tectorum* L.) populations. Great Basin Naturalist 59: 355–360
Kerstel E, Gianfrani L (2008) Advances in laser-based isotope ratio measurements: selected applications. Appl Phys B 92: 439–449
Kruse J, Hopmans P, Adams MA (2008) Temperature responses are a window to the physiology of dark respiration: differences between CO2 release and O2 reduction shed light on energy conservation. Plant Cell Environ 31: 901–914
Lamprecht I (1999) Combustion calorimetry. In: Kemp RB (ed.) From Macromolecules to Man, Handbook of Thermal Analysis and Calorimetry, Vol 4. Elsevier Science, Amsterdam, pp 175–218
Lenoir J, Gégout JC, Marquet PA, de Ruffray P, Brisse HA (2008) A significant upward shift in plant species optimum elevation during the 20th century. Science 320: 1768–1771
Macfarlane C, Adams MA, Hansen LD (2002) Application of an enthalpy balance model of the relation between growth and respiration to temperature acclimation of

Eucalyptus globulus seedlings. Proc R Soc Lond B 269: 1499–1507
Macfarlane C, Hansen LD, Edwards J, White DA, Adams MA (2005) Growth efficiency increases as growth rate increases in shoots and roots of *Eucalyptus globulus* deprived of nitrogen or treated with salt. Tree Physiol 25: 571–582
Macfarlane C, Hansen LD, Florez-Sarasa I, Ribas-Carbo M (2009) Plant mitochondria electron partitioning is independent of short-term temperature changes. Plant Cell Environ 32: 585–591
Marcar NE, Criddle RS, Guo J, Zohar Y (2002) Analysis of respiratory metabolism correlates well with the response of *Eucalyptus camaldulensis* seedlings to NaCl and high pH. Funct Plant Biol 29: 925–932
Matheson S (2000) Metabolic Adaptation of Oats to Climatic Temperature. A University Scholar Project Thesis. Brigham Young University, Provo, Utah. 50 pages. (Available from Harold B. Lee Library, document AS 36. B752 M37835 2000.)
Matheson S, Ellingson DJ, McCarlie VW, Smith BN, Criddle RS, Rodier L, Hansen LD (2004) Determination of growth and maintenance coefficients by calorespirometry. Funct Plant Biol 31: 929–939
McCarlie VW, Hansen LD, Smith BN, Monsen SB, Ellingson DJ (2003) Anabolic rates measured by calorespirometry for eleven subpopulations of *Bromus tectorum* match temperature profiles of local microclimates. Russ J Plant Physiol 50: 183–191
McDonald AE (2008) Alternative oxidase: an inter-kingdom perspective on the function and regulation of this broadly distributed 'cyanide-resistant' terminal oxidase. Funct Plant Biol 35: 535–552
McDonald AE, Amirsadeghi S, Vanlerberghe GC (2003) Prokaryotic orthologues of mitochondrial alternative oxidase and plastid terminal oxidase. Plant Molecular Biol 53: 865–876
McDonald AE, Vanlerberghe GC (2004) Branched mitochondrial electron transport in the animalia: presence of alternative oxidase in several animal phyla. IUBMB Life 56: 333–341
McDonald AE, Vanlerberghe GC (2005) Alternative oxidase and plastoquinol terminal oxidase in marine prokaryotes of the Sargasso Sea. Gene 349: 15–24
McDonald AE, Vanlerberghe GC (2006) Origins, evolutionary history, and taxonomic distribution of alternative oxidase and plastoquinol terminal oxidase. Comparative Biochem Physiol D: Genomics Proteomics 1: 357–364
Mills I, Cvitaš T, Homann K, Kallay N, Kuchitsu K (1993) Quantities, Units and Symbols in Physical Chemistry. Blackwell Science, Oxford
Miller DP, de Pablo JJ (2000) Calorimetric solution properties of simple saccharides and their significance for the stabilization of biological structure and function. J Phys Chem B 104: 8876–8883
Moore AL, Albury MS, Crichton PG, Affourtit C (2002) Function of the alternative oxidase: is it still a scavenger? Trends Plant Sci 7: 478–481
Moore CS, Cook-Johnson RJ, Rudhe C, Whelan J, Day DA, Wiskich JT, Soole KL (2003) Identification of AtNDI1, an internal non-phosphorylating NAD(P)H dehydrogenase in *Arabodopsis* mitochondria. Plant Physiol 133: 1968–1978
Pörtner HO, Farrell AP (2008) Physiology and climate change. Science 322: 690–692
Rasmusson AG, Fernie AR, van Dongen JT (2009) Alternative oxidase: a defence against metabolic fluctuations? Physiol Plant 137: 371–382
Ribas-Carbo M, Robinson SA, Giles L (2005) The application of the oxygen-isotope technique to assess respiratory pathway partitioning. In: Lambers H, Ribas-Carbo M (eds) Plant Respiration: From Cell to Ecosystem, Advances in Photosynthesis and Respiration, Vol. 18. Springer, Dordrecht, pp 17–30
Smith BN, Walker JL, Stone RL, Jones AR, Hansen LD (2001) Temperature-dependent respiration-growth relations in ancestral maize cultivars. Shrubland Ecosystem Genetics and Biodiversity: Proceedings, USDA Forest Service, RMRS-P-21, pp 276–279
Stucki JW (1980) The optimal efficiency and the economic degrees of coupling of oxidative phosphorylation. Eur J Biochem 109: 269–283
Summers HA, Smith BN, Hansen LD (2009) Comparison of respiratory and growth characteristics of two co-occurring shrubs from a cold desert, Coleogyne ramosissima (blackbrush) and Atriplex confertifolia (shadscale). J Arid Environ 73: 1–6
Svenning J-C, Condit R (2008) Biodiversity in a warmer world. Science 322: 206–207
Taylor DK, Rank DR, Keiser DR, Smith BN, Criddle RS, Hansen LD (1998) Modelling temperature effects on growth-respiration relations of maize. Plant Cell Environ 21: 1143–1151
Thygerson T, Booth DT, Harris JM, Hansen LD, Smith BN (2001) Microcalorimetric studies on metabolic and germination response to temperature for three populations of winterfat (*Eurotia lanata*). Shrubland Ecosystem Genetics and Biodiversity: Proceedings, USDA Forest Service, RMRS-P-21, pp 283–286
Thygerson T, Harris JM, Smith BN, Hansen LD, Pendleton RL, Booth DT (2002) Metabolic response to temperature for six populations of winterfat (*Eurotia lanata*). Thermochim Acta 394: 211–217
van Iersel MW (2003) Carbon use efficiency depends on growth respiration, maintenance respiration, and relative growth rate: a case study with lettuce. Plant Cell Environ 26: 1441–1449

van Iersel MW, Seymour L (2000) Growth respiration, maintenance respiration, and carbon fixation of *Vinca*: a time series analysis. J Am Soc Hortic Sci 125: 702–706

Vanlerberghe GC, Cvetkovska M, Wang J (2009) Is the maintenance of homeostatic mitochondrial signaling during stress a physiological role for alternative oxidase? Physiol Plant 137: 392–406

Ward TA (2007) Guiding restoration efforts through metabolic properties of native grass populations and reptile abundance and species richness. M.S. Thesis. Brigham Young University, Provo, Utah. 81 pages. (Available from Harold B. Lee Library, document S20.02. W368 2007.)

Yamaguchi J (1978) Respiration and the growth efficiency in relation to crop productivity. J Fac Agric Hokkaido Univ 59: 59–129

Yu W, Hansen LD, Fan W, Zhao W, McArthur ED (2008) Adaptation of growth and respiration of three varieties of Caragana to environmental temperature. Asian J Plant Sci 7: 67–72

Supporting Information

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

Table S1. Substrate carbon conversion efficiencies measured at different temperatures.

Figure S1. Effect of ε value on growth over time.

Figure S2. The ratio of growth rate to CO_2 production rate as a function of the substrate carbon conversion efficiency, ε, according to Eqn 2.

Figure S3. Relationship between the ratio of respiratory heat rate to CO_2 production rate and the substrate carbon conversion efficiency, ε.

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