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# Night-time decline in plant respiration is consistent with substrate depletion

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Understanding the response of plant respiration to climate change is key to determining whether the global land carbon sink continues into the future or declines. Most global vegetation models use a classical growth-maintenance approach, which predicts that nocturnal plant respiration is controlled by temperature only. However, recently published observations of plant respiration show a decline through the night even at constant temperature, which these global models cannot reproduce. Here we assess the role of respiratory substrates in this observed decline by evaluating an alternative model of plant respiration, in which the rate of respiration at constant temperature is instead dependent on the size of available substrate pools. We find that the observed decline in nocturnal respiration is reproduced by a model with just two substrate pools, one fast and one slow. These results demonstrate a need to change the way that plant respiration is represented in global vegetation models, moving to models based on labile pools which represent only a fraction of total plant biomass. These models naturally represent plant acclimation via changing pool-sizes and may have a significant impact on the long-term predictions of the global land carbon sink.

Plant respiration ( $R$ ) is a fundamental process that, together with photosynthesis, determines the net accumulation of carbon in growing plants. This balance between photosynthesis and respiration offsets approximately one third of the annual anthropogenic carbon contribution to the atmosphere<sup>1</sup> and is a key flux in the global carbon cycle. The current theory which underpins much of our understanding splits plant respiration into two distinct components<sup>2</sup>. These components, referred to as growth and maintenance, are generally associated with the mass and energy requirements of growing new, and maintaining existing biomass, respectively. Many models that adopt this theory do not explicitly represent plant growth<sup>3</sup>, and so growth respiration ( $R_g$ ) is typically calculated as a constant fraction ( $c$ ) of photosynthesis ( $P$ ), while maintenance respiration ( $R_m$ ) is usually a function of plant biomass ( $B$ ) and temperature ( $T$ ):

$$R = R_g + R_m = cP + f(T)B \quad (1)$$

This framework, referred to herein as the growth-maintenance respiration (GMR) paradigm, is commonly used in dynamic global vegetation models (DGVM) and land surface models<sup>4–8</sup> (LSM) due in part to its simplicity, but also its apparent consistency with observations. However, despite its prevalence within these models, the GMR paradigm has faced criticism<sup>9–12</sup>.

While it may be reasonable to split plant processes into growth and maintenance categories on a functional basis, at a biochemical level the distinction between growth and maintenance respiration is less clear<sup>9</sup>. Both maintenance and growth processes require energy as well as organic material, to either produce new, or replace old biomass (growth and maintenance respectively). In both cases, the production of energy (in the form of ATP) and the source of organic matter are the same<sup>9</sup>. From this viewpoint, attributing the two terms in equation (1) to two distinct respiratory processes is not coherent and an alternative representation of plant respiration is required.

Under controlled temperature conditions the GMR paradigm predicts that nocturnal plant respiration should remain more or less constant. Under darkness, the short-term variability of maintenance respiration is thought to be driven predominantly by temperature<sup>13,14</sup>, while the contribution of growth respiration to the total flux should be negligible given its dependence on photosynthesis. However, under these conditions plant respiration can still undergo significant short term change<sup>15</sup>. Across 31 species from both temperate and tropical biomes, respiration was found to decline through the night, with an average of 25% reduction after 8 h of darkness. This is a substantial decline that may have a large impact on global estimates of the land carbon sink, and is currently not explained by the GMR paradigm. This

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new understanding of plant respiration must be reconciled in the representation of respiration in models, in order to capture this significant process.

A hypothesis to explain nocturnal respiration decline in plants is the depletion of substrates which are used to fuel growth and respiratory processes. Plants regularly experience asynchrony between supply and demand for carbon<sup>16,17</sup>, but are able to buffer these differences by using stored pools of labile carbon to support carbon demand when instantaneous assimilation is insufficient<sup>18,19</sup>. Despite a halt in photosynthetic carbon assimilation in the dark, the demand for carbon from respiration and growth persists through the night and is supported by these stores. There is evidence that some plants can exert intricate regulation over the expenditure of stored carbon, allowing a constant supply of substrate to the required sinks at night<sup>20</sup>. Such control would not result in a reduction in respiration as substrate stores deplete over night, however, it is unclear whether this is a skill that all plants possess. In the absence of this active regulation, and under the basic assumption that utilisation rate is related to the availability of substrate, we might expect to see a decline in nocturnal respiration similar to that observed, as the stores of carbon collected throughout the day are depleted.

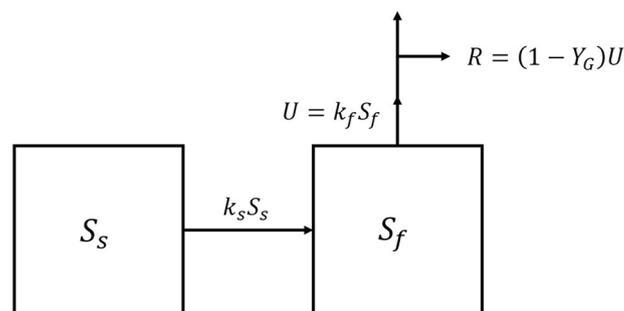
The decline in nocturnal plant respiration under constant temperature has also been shown to depend strongly on environmental growing factors. In particular, the relative decline after 8 h of nighttime has been found to be greater in sun adapted species than in shade adapted species<sup>21</sup>. We hypothesise that this difference can be explained by the coordination of leaf metabolic capacity with growth light environment. Many leaf traits including maximum photosynthetic capacity, specific dark respiration rates, and leaf nitrogen concentrations decline through forest canopies in response to the increased shading experienced by under-story leaves<sup>22</sup>. We hypothesise that the difference in nocturnal respiration decline between sun and shade adapted leaves under constant temperature is explained by this coordination of leaf nitrogen and metabolic capacity with growing irradiance, leading to differences in the rate at which substrates are depleted and therefore in the rate at which respiration declines.

We test these hypotheses here by first examining a simple model of substrate dependence in plant respiration. We assess the model's ability to capture observed patterns of nocturnal leaf respiration decline under constant temperature<sup>15</sup>, and discuss its realism and application to modelling plant respiration. We then extend the model to include daytime dynamics allowing an exploration of the effect that day-time growth light environment has on the predicted decline in nocturnal respiration, and compare the predicted model sensitivity to observations reported by ref. 21.

## Results

### Modelling nocturnal respiration decline under constant temperature

To test the ability of substrate availability to explain the observed decline in nocturnal respiration under constant temperature, we evaluate a simple model of substrate dependence in respiration against observed data. Initial tests (Supplementary Fig. 2 and Supplementary Notes 1) found that a model consisting of just a single pool of substrate was insufficient to capture the observations. The model therefore consists of two pools of substrate in which plant respiration is assumed to depend linearly on the availability of substrate in a fast turnover pool (Fig. 1). This fast turnover pool is in turn replenished by substrate from a second, and relatively slower turnover pool. At this initial stage, we make no assertions about the nature of either pools besides this qualitative statement about relative turnover rates. Since we are considering respiration under constant temperature, we do not include temperature sensitivities within the model. However, temperature is likely an important component of substrate turnover and we discuss this in the discussion section. We compare the model to observed data reported by ref. 15. These observed data are of nocturnal respiration under constant temperature, normalised by its initial value (i.e., respiration at the onset of darkness— $R_0$ ). We therefore derive an equivalent expression for this normalised rate from our model to compare with the observed data. This is



**Fig. 1 | The two-pool model of substrate dependence in plant respiration.** Substrate is drawn from a fast turnover pool ( $S_f$ ) at a rate ( $U$ ) that is linearly dependent on the amount of substrate in the pool, with coefficient  $k_f$ . A second pool ( $S_s$ ) with a slower turnover rate feeds the 'fast pool'. The flux of substrate from the slow pool to the fast pool depends linearly on the amount of substrate in the slow pool with coefficient  $k_s$ . Respiration ( $R$ ) is a constant fraction ( $1 - Y_g$ ) of the total utilisation of fast pool substrate.

given by:

$$\frac{R}{R_0}(t) = re^{-k_s t} + (1 - r)e^{-k_f t} \quad (2)$$

where,  $k_s$  ( $\text{hr}^{-1}$ ) is the specific turnover rate of carbon from the slow pool into the fast pool,  $k_f$  ( $\text{hr}^{-1}$ ) is the specific turnover rate of carbon by respiration, and  $r$  is given by:

$$r = \frac{k_s}{k_f - k_s} r_0 \quad (3)$$

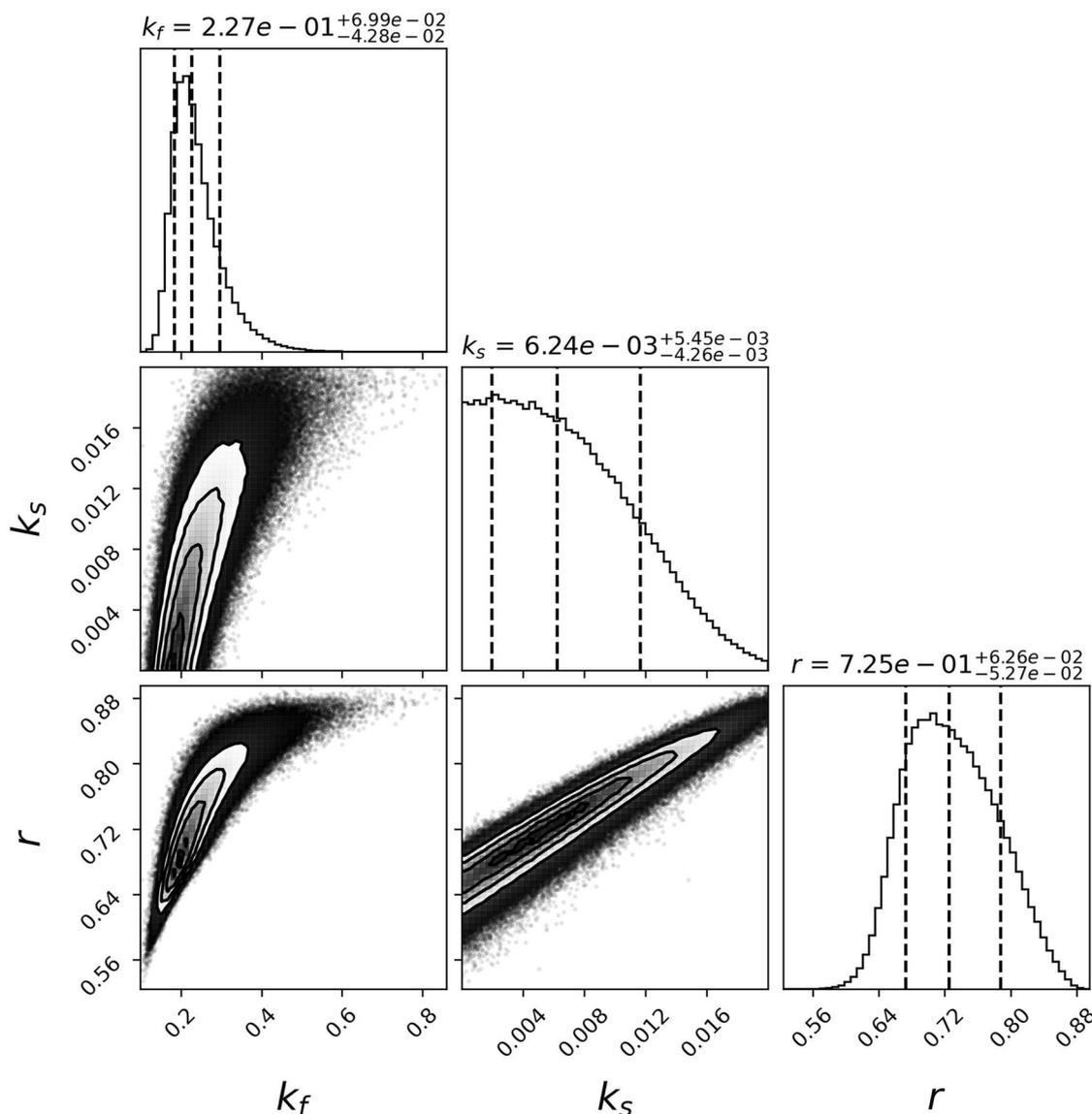
where  $r_0$  is defined as the ratio of substrate in the slow pool to substrate in the fast pool at the start of the night.

$$r_0 = \frac{S_{s_0}}{S_{f_0}} \quad (4)$$

Here, we treat  $k_f$ ,  $k_s$  and  $r$  as parameters in the model and use Markov-Chain Monte-Carlo (MCMC) to fit the model to the data (Fig. 2). This results in values of  $k_f = 0.227^{+6.99 \times 10^{-2}}_{-4.28 \times 10^{-2}} \text{hr}^{-1}$ ,  $k_s = 6.24 \times 10^{-3}^{+5.45 \times 10^{-3}}_{-4.26 \times 10^{-3}} \text{hr}^{-1}$ , and  $r = 0.725^{+6.26 \times 10^{-2}}_{-5.27 \times 10^{-2}}$ , where the central values represent the 50<sup>th</sup> percentile values of each posterior distribution, and the upper and lower bounds represent the 84<sup>th</sup> and 16<sup>th</sup> percentiles, respectively. These values allow the two-pool model to accurately capture the observed decline in normalised nocturnal respiration (Fig. 3). The close fit of the model to the observations suggests strong support for the depletion of substrate as an explanation for the observed decline in nocturnal respiration, with the substrate decline in each pool resulting in the predicted decline of respiration. The MCMC results in tight constraints on the  $k_f$  and  $r$  parameters (i.e., small range of uncertainty), however significant uncertainty remains in the value of the slow pool turnover rate ( $k_s$ ). The challenge of fitting a sum of exponentials with differing exponents to noisy data is a well studied problem and is known to be ill-conditioned in many cases e.g., ref. 23. Small changes in the empirical data can lead to large changes in the optimal parameter set, meaning that relatively small data uncertainty can result in extremely high parameter uncertainty. In addition, constraining slow processes using data at much shorter timescales is generally not possible.

### Simulating the role of leaf growing irradiance

We now test the role of growth light environment on the predicted decline in nocturnal plant respiration at constant temperature. We first consider the fact that the amount of substrate in each pool at the start of the night depends on both the amount of carbon accumulated through



**Fig. 2 | Parameter estimation through Markov-Chain Monte-Carlo.** A corner plot showing the results of the MCMC performed to fit the two-pool model to data reported by Bruhn et al.<sup>15</sup>. The figure shows the one- and two-dimensional posterior distributions for each parameter; the histograms on the diagonal show the marginalised posterior densities and the contours plots show the covariances between

each parameter pair. The 16<sup>th</sup>, 50<sup>th</sup> and 84<sup>th</sup> percentiles of the posterior samples along each axis are shown (dash black lines), representing 1σ confidence ranges around the median value. These values are shown at the top of each histogram.

photosynthesis, and the carbon lost from respiration during the preceding day. Since the carbon lost through respiration during the day also depends on the values of  $k_f$  and  $k_s$ , we cannot reasonably assume that the ratio of substrate in each pool at the onset of the night (i.e., the  $r_0$  parameter in Eqs. (3) and (4)) is independent of  $k_f$  and  $k_s$ . We therefore extend the model to include daytime dynamics and derive a new expression for the  $r_0$  parameter that depends on the  $k_f$  and  $k_s$  parameters. We then examine how changing the  $k_f$  and  $k_s$  parameters, relative to the previously fitted values, affects the predicted decline in nocturnal respiration. We explore the possible relation of these changes to growth light environment, in particular the coordination of leaf nitrogen and metabolic capacity with average leaf irradiance.

We propose that the increased nitrogen and metabolic capacity in light-adapted leaves compared to shade-adapted leaves leads to greater specific substrate utilisation rates ( $k_f$  and  $k_s$  in our model), which in turn result in greater substrate depletion, and therefore greater decline in nocturnal respiration. To test this, we examine how changes in the substrate turnover parameters relative to the 50<sup>th</sup> percentile parameters fitted in the

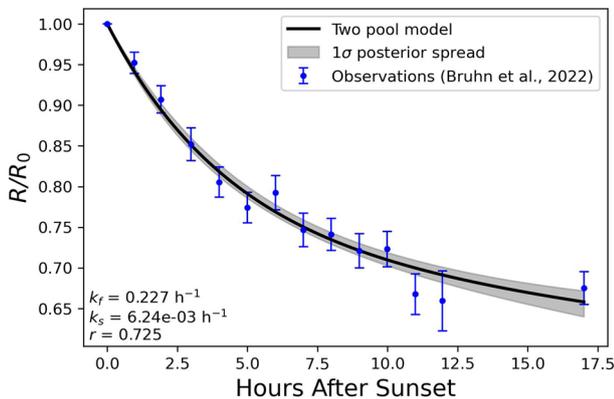
previous section affect the predicted decline in nocturnal respiration. We assume that larger values of  $k_f$  and  $k_s$  correspond to leaves grown in higher light conditions, with greater nitrogen concentrations, and therefore greater metabolic capacity.

Figure 4a shows the relative decline in nocturnal respiration under constant temperature predicted by the two-pool model for a range of parameter values. Larger values of  $k_f$  and  $k_s$  result in greater relative depletion of the substrate pools and thus greater reductions in respiration. Relating this to our assumption that higher substrate turnover rates correspond to higher nitrogen levels, and higher growth light environment, we see a qualitative match with the observations<sup>21</sup> which are also shown here in Fig. 4b.

### Discussion

A decline in nocturnal plant respiration under constant temperature<sup>15</sup> is a stark departure from the current representation of plant respiration used in most land surface and global dynamic vegetation models. Here we present

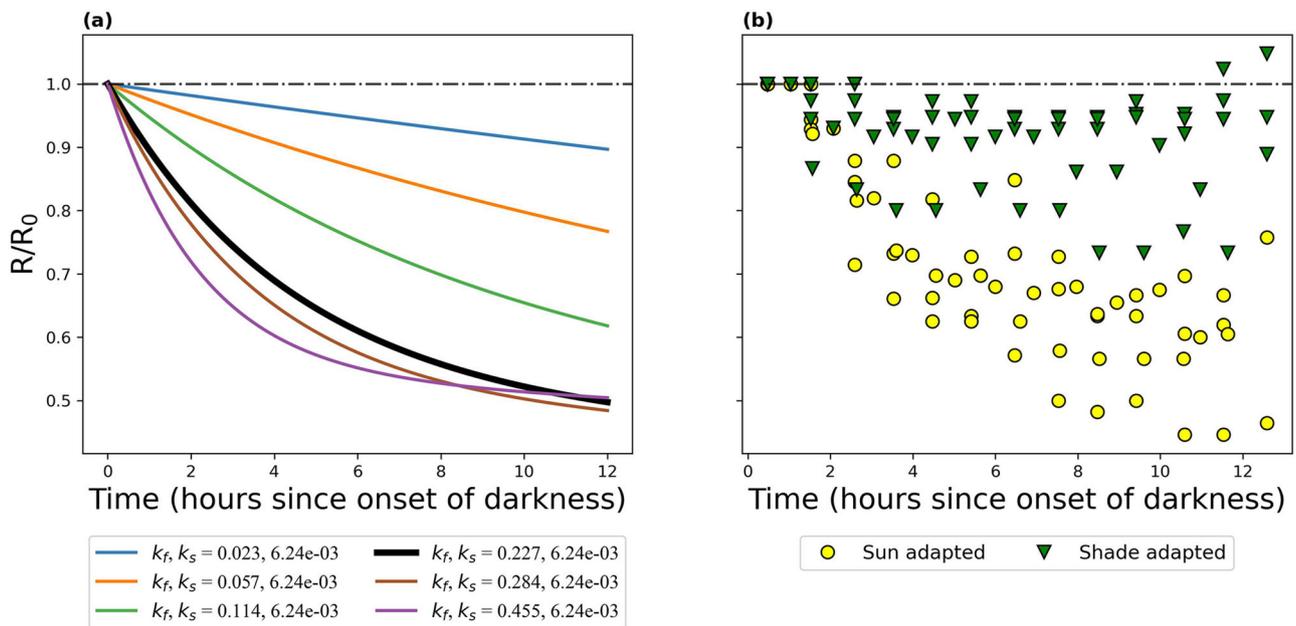
a model that using a collection of simple assumptions, most importantly that respiration depends on the amount of available respiratory substrate stored within a plant, is able to more accurately describe this decline as well as its sensitivity to growth light environment. In the absence of carbon supply from photosynthesis, plants rely on stores of substrate to fuel respiratory processes through the night. This dependence is rarely accounted for in large scale land surface models, but may have a large impact on predictions of carbon expenditure<sup>3,24–26</sup>. Our results here further highlight the importance of including substrate dependence in representations of plant respiration in LSMs and DGVMs, in particular when simulating short term dynamics.



**Fig. 3 | Predicted nocturnal leaf respiration under constant temperature.** Comparison of observed and modelled nocturnal plant respiration under constant temperature normalised to the onset of darkness value. Observations<sup>15</sup> represent a mean value across 31 species with 4–92 replicate individuals per species. Model predictions are from the two-pool substrate model using the 50<sup>th</sup> percentile parameters values derived from Markov-Chain Monte-Carlo (MCMC), for the three model parameters, ( $k_f$  &  $k_s$ , and  $r$ ). One standard deviation of the model realisations from the posterior distribution around the median is presented in grey to represent the spread of model predictions after the MCMC sampling.

Aside from the difference in their turnover rates, we have so far made no assertions about the nature of the two substrate pools in the model. A primary set of substrates used to fuel respiration in plants is sugars. Sugars are relatively fast turnover carbohydrates produced during photosynthesis and re-metabolised during respiration. A study on nocturnal sucrose depletion in *Uniculum* barley plants<sup>27</sup> estimated a specific depletion rate for sucrose of  $k = 0.13 \text{ hr}^{-1}$ . This was attributed in part to metabolism and in part to export processes. Similarly, a sucrose depletion rate of  $k = 0.15 \text{ hr}^{-1}$  was reported in barley plants<sup>28</sup>, although it should be noted that this was found to be highly sensitive to changes in day length. In the modelling literature, Dewar, Medlyn & McMurtrie<sup>29</sup>, use a value of  $0.167 \text{ hr}^{-1}$  to represent labile leaf carbon utilisation rate. Similarly, Dick & Dewar<sup>30</sup> use a value of  $0.417 \text{ hr}^{-1}$  to represent sugar utilisation in roots. These values are roughly consistent with the 50<sup>th</sup> percentile estimate ( $0.227 \text{ hr}^{-1}$ ) of the  $k_f$  parameter in the two-pool model fitted here. This may be an indication of an important role for labile sugar in the decline in observed nocturnal respiration<sup>15</sup>.

Another important respiratory carbohydrate in plants is starch. Starch is often considered a longer term form of carbon storage and known to be re-mobilised to sugar at night to support metabolism<sup>19,31,32</sup>. It is tempting therefore to assume that the slow pool in our model represents starch. Certainly, simple models of starch-sugar dynamics typically use or build upon the basic structure of our two-pool model<sup>30,33</sup>. Verifying this comparison, however, is difficult. This is due in part to the uncertainty associated with the  $k_s$  parameter of our model, and in part because few studies report fixed conversion rates of starch to sugar, as starch degradation is often found to be highly sensitive to changes in environment<sup>34,35</sup>. Nonetheless, we can examine the implied lifetime of the slow pool by considering the inverse of the fitted  $k_s$  parameter, which given the 50<sup>th</sup> percentile estimate of  $k_s = 6.24 \times 10^{-3}$ , is approximately 6 and a half days. Studies suggest that starch conversion can occur over the timescales of hours and reserves are often used almost entirely during a single night<sup>36</sup>, which is somewhat faster than the timescale implied by our model. Additionally, according to the 50<sup>th</sup> percentile parameter values fitted in our model, we calculate an implied initial nighttime ratio of starch to sugar ( $r_0$  in Eq. (3)) of 25.7. This suggests that plants store significantly more starch than they do sugar during the day. In contrast, however, most empirical studies report starch to sugar ratios



**Fig. 4 | The impact of light adaptation on nocturnal respiration decline.** **a** The decline in nocturnal respiration at a constant temperature, normalised by to the onset of darkness value, as predicted by the two-pool model for varying values of  $k_f$  and  $k_s$ . The black line represents the model predictions using the fitted parameters of

$k_f$  and  $k_s$  reported in Section “Fitting the model to observations”. **b** The effect of light adaptation on the observed decline in nocturnal respiration at constant temperature<sup>21</sup>.

much closer to, and often less than one<sup>34,37–39</sup>, implying greater storage of sugar than starch. It seems unlikely therefore that the slow pool can be characterised as a starch store.

In reality, the slow pool probably characterises several different forms of slow turnover substrates that are indistinguishable over the diurnal time-scale. The range of chemical pathways through which plants can respire is hugely diverse and the way that plants utilise these different pathways is complex and not yet well understood<sup>40</sup>. It is likely that by simulating just two pools, each with sufficiently different turnover rates, we have been able to capture the aggregate behaviour of many substrates over the time-scale of a night. Whether this approximation holds over larger time-scales is not clear, and it may be that additional substrate pools are required to capture longer-term respiration dynamics. The influence of climatic variation on substrate utilisation is also unclear owed somewhat to the uncertainty associated with measuring carbohydrate concentrations<sup>41,42</sup>. However, significant variations in the proportions of soluble sugar and starch occur with changing water availability<sup>34</sup> and it is likely that similar sensitivities apply to other respiratory substrates. What is clear though is that a single pool of substrate is insufficient to accurately capture observations. Temporal variations in the relative quantities of multiple respiratory substrates clearly play an important role in determining respiration dynamics at the diurnal time-scale<sup>43</sup> and improving our understanding of these processes should be a priority. This will allow more mechanistic models of plant respiration to be developed which will be necessary for accurate predictions of plant responses to climate change.

While the two-pool model appears to differ substantially from the traditional GMR approach used by most LSMs, the two are in fact reconcilable. If we consider the extended daytime version of the two-pool model (Eqs. (12) and (13)) and take the limit that the fast pool becomes infinitely fast (i.e.,  $k_f \rightarrow \infty$ ), we can write the rate of respiration as follows.

$$\lim_{k_f \rightarrow \infty} R(t) = (1 - Y_g) \left( \underbrace{\alpha P(t)}_{\text{growth}} + \underbrace{k_s S_s(t)}_{\text{maintenance}} \right) \quad (5)$$

In this limit, the model reduces to a two-component model of respiration that is directly comparable to the GMR approach. The first component is directly proportional to photosynthesis, while the second component is related to the influx of slow pool substrate into the fast pool. The first component is directly comparable to growth respiration which is commonly represented as a constant fraction of photosynthesis. The second component is less obviously related to maintenance respiration, but with the use of an additional assumption we find that the two can in fact become equivalent. There is evidence that recycling of structural compounds can be used to fuel respiration<sup>40</sup> and so, if we assume that the slow pool represents some component of structural biomass that is broken down and recycled into growth and respiratory substrates, this second term becomes equivalent to a maintenance respiration that is directly proportional to plant biomass. Structural biomass typically has a slow turnover rate relative to labile compounds<sup>40</sup>. In the limit that substrate turnover is slow (i.e.,  $k_s \rightarrow 0$  here) the variability of photosynthesis has little impact on the utilisation rate<sup>26</sup> and instead direct sensitivities of the turnover rate to environment drive the variability of the flux. This is the basis of Thornley's<sup>9</sup> respiration model which neatly links maintenance respiration with the energy costs of replacing old biomass with new. Viewing the two-pool model in this way provides mechanistic insight into the GMR model and suggests that there may be scenarios in which the GMR approach is a sufficient representation of plant respiration.

Over time-scales much longer than the turnover time of the fast pool (i.e.,  $\tau \gg \frac{1}{k_s}$ ), Eq. (5) is an accurate approximation of the two-pool model. For long term simulations (i.e., longer than the timescale of the fast pool) it is, therefore, reasonable to use a two-component model of respiration. Further, it is mechanistically justifiable to directly relate one of these components to photosynthesis as is traditionally done in the GMR approach. However, the

realism of relating the second component to structural biomass is uncertain. Relating maintenance respiration to biomass can lead to unrealistic predictions and the importance of more recent carbon assimilation in predicting maintenance respiration is becoming more apparent<sup>9,11,12</sup>. As we have discussed, the slow pool in our model likely approximates a large set of respiratory substrates. These substrates may behave indistinguishably over the course of a few hours but not over longer-timescales. Similarly, when slow turnover substrate pools are in equilibrium the rate of maintenance respiration may appear to be related to total plant biomass. However, changes in the availability of respiratory substrates with turnover rates much faster than that of structural compounds may result in shifts in maintenance respiration in response to long term changes in climate. This implies that while the GMR approach may be useful for predicting plant respiration under a non-changing climate, it will not be able to accurately predict the response of respiration to the changes in climate that are predicted in the future. This is clearly an issue that must be resolved before reliable estimates of the future land carbon sink can be made.

The long-term behaviour of plant respiration is an ongoing area of research. Central to this are responses of respiration to changes in environmental conditions, which we have so far not considered within our model. In particular, the metabolism of substrate is an enzyme-catalysed process and so we should expect the two fluxes of substrate in our model to be sensitive to changes in temperature. Adopting the common assumption that metabolism follows temperature according to a  $Q_{10}$  function<sup>44</sup>, and using the 50<sup>th</sup> percentile parameter values, we find that the dependence of respiration on the two substrate pools in our model results in an overall dampening of the long term temperature response when compared to the traditional GMR model (Supplementary Fig. 5 and Supplementary Notes 2). As temperature increases, so too does the utilisation of substrate resulting in a decline in substrate availability and a long term down-regulation of respiration. Similarly in periods of long-term cooling, the decreased utilisation of substrate results in an accumulation of carbon and a subsequent up-regulation in respiration. Similar behaviour is often observed in the field, with leaves subjected to periods of warming down-regulating their respiration when measured at a reference temperature and up-regulating it after periods of cooling<sup>45</sup>. This apparent shift in respiration in response to changing growth temperature, termed respiratory acclimation, can occur over time-scales of days to months<sup>46,47</sup> and is not currently explained by many LSMs that adopt the traditional GMR approach. There are many proposed mechanisms through which acclimation can occur<sup>48</sup>, however the changing availability of respiratory substrate may play an important role in this<sup>49</sup>.

The impact of respiratory acclimation on future plant carbon exchange is currently uncertain as many LSMs do not accurately represent it<sup>50</sup>. However, it is generally thought that models that do not account for acclimation likely over-estimate future plant respiration and as a result under-estimate terrestrial carbon accumulation<sup>51–53</sup>. Yet substrate dependence intricately links respiration with photosynthesis in a way that is not currently accounted for in LSMs<sup>3,26</sup>. Changes in photosynthesis, due for example to the CO<sub>2</sub> fertilisation effect, may compensate for the increased utilisation of substrate under long-term warming and prevent significant reductions in substrate and reduce the long-term dampening effect that we see in our simulations (Supplementary Fig. 5). The balance between changes in photosynthesis and changes in temperature are uncertain<sup>54</sup> and this makes the impact of models like the one we present here difficult to discern. Our two-pool model represents a simple, mechanistic way to model plant respiration and may help to reduce the uncertainty on the impact of plant respiratory acclimation on the global carbon cycle. However, substrate turnover rates are difficult to determine due to the complex nature of carbon storage, transport and utilisation in plants. Further experiments that seek to control for the effects of temperature on plant respiration are required to refine the estimation of the slow pool turnover within our model before the two-pool model presented here can be used confidently in LSMs. Modelling of plant respiration responses to climate change should be a higher priority in global vegetation and Earth system modelling.

## Conclusions

We have evaluated a simple model of substrate dependence in plant respiration against observations of declining nocturnal respiration under constant temperature conditions<sup>15</sup>. The model was able to accurately explain the observed trend, lending support to the hypothesis that declining substrate availability drives the observed decline in autotrophic respiration during the night. In addition, we found that at least two pools of substrate are required to simulate the observed trend. The model was also able to capture an apparent sensitivity in the rate of decline of plant respiration to growth light environment, which we hypothesise is related to the coordination of leaf nitrogen and metabolic capacity with growing irradiance. The existence of finite substrate pools introduces non-instantaneous sensitivities of respiration to changes in environment, implying the existence of acclimation type shifts in plant respiration.

## Methods

### Model description

Since initially we are only concerned with the nocturnal dynamics of respiration, we assume that the carbon supply from photosynthesis is negligible. Similarly, as we are considering controlled experiments in which temperature is held constant, we omit the temperature dependence of any parameters within the model. Finally, we make the assumption that respiration is a constant fraction of total night-time substrate expenditure. While we do not consider them here, this allows for other, non-respiratory processes (such as growth or substrate transport) to be included, provided that their substrate utilisation rates are directly proportional to that of respiration. This assumption forms the basis of similar substrate-based respiration models (e.g., Thornley<sup>9</sup>; Jones et al.<sup>26</sup>).

Initial tests with a model consisting of a single substrate pool were insufficient to fully capture the observed decline in respiration (results presented in Supplementary Notes 1 and Supplementary Fig. 1). We therefore evaluate a model with two pools of substrate (Fig. 1).

The slow feeding pool is assumed to turnover substrate into the fast respiring pool at a rate that depends linearly on its own availability of substrate:

$$\frac{dS_s}{dt} = -k_s S_s \quad (6)$$

where  $S_s$  ( $\text{kgCm}^{-2}$ ) is the availability of substrate in the slow pool, and  $k_s$  ( $\text{hr}^{-1}$ ) is the specific turnover rate of substrate from the slow pool to the fast pool.

Similarly, substrate is used from the fast pool, at a rate ( $U$ ) that depends linearly on the availability of substrate in the fast pool. Together with the input of substrate from the slow pool, the resulting equation for the rate of change of the fast pool is given by:

$$\begin{aligned} \frac{dS_f}{dt} &= k_s S_s - U \\ &= k_s S_s - k_f S_f \end{aligned} \quad (7)$$

where  $S_f$  ( $\text{kgCm}^{-2}$ ) is the availability of substrate in the fast pool, and  $k_f$  ( $\text{hr}^{-1}$ ) is the specific turnover rate of substrate by respiration.

Respiration is assumed to be a constant fraction ( $1 - Y_g$ ), following the notation of Thornley<sup>9</sup>) of fast pool substrate utilisation:

$$R = (1 - Y_g)U \quad (8)$$

where  $Y_g$  is a constant with the default value of 0.75<sup>55</sup>.

Solving Eqs. ((6) and (7)) leads to the following expression for the rate of nocturnal respiration normalised by its initial (respiration at the onset of darkness) value. Details of the derivation for this are given in Supplementary Notes 3 in the Supplementary Material. It is important to note that due to the normalisation of respiration by its initial value, the  $Y_g$  parameter does not

appear in our final expression.

$$\frac{R}{R_0}(t) = r e^{-k_s t} + (1 - r)e^{-k_f t} \quad (9)$$

where  $R_0$  is the initial rate of respiration (i.e.,  $R(t=0)$ ), and  $r$  is given by

$$r = \frac{k_s}{k_f - k_s} r_0 \quad (10)$$

with  $r_0$  defined as the ratio of substrate in the slow pool to substrate in the fast pool at the start of the night:

$$r_0 = \frac{S_{s_0}}{S_{f_0}} \quad (11)$$

where  $S_{s_0}$  and  $S_{f_0}$  represent the availability of substrate in the slow and fast pools respectively at the start of the night.

### Fitting the model to observations

We evaluate the model against data reported by ref. 15. The data presented in Bruhn et al.<sup>15</sup> is both collated from the literature and represent original measurements. They include a combination of field and lab based studies in which leaf respiration was measured with a gas exchange analyser more than once within a period of night-time during which leaf temperature was kept constant. The data encompasses a total of 967 nights across 31 herbaceous, shrub, grass, vine, and tree species from both tropical and temperate biomes. These data are available in ‘Suppl. Data 1’ published by Bruhn et al.<sup>15</sup>. To fit Eq. (9) to the observed data we used an error-weighted least-squares minimisation to generate an initial guess of the model parameters. This initial fit was then refined using a Markov-Chain Monte Carlo (MCMC) ensemble sampler (emcee<sup>56</sup>). MCMC methods are designed to efficiently sample from—and thereby provide sampling approximations to—the posterior probability density function, allowing us to infer uncertainty information about the parameters. The sampler was first run for a burn-in phase of 1000 steps and 500 walkers, seeded around the initial least-squares solution. The sampler was then run for 100,000 steps to fully explore parameter space and allow the MCMC chains to converge (Supplementary Fig. 4). We assumed uniform priors for each parameter between the following bounds:  $0.01 \text{ hr}^{-1} < k_f < 1.0 \text{ hr}^{-1}$ ,  $0.0 \text{ hr}^{-1} < k_s < 0.05 \text{ hr}^{-1}$ , and  $0.5 < r < 1.0$ . These prior were chosen based on expert knowledge and preliminary explorations of parameter space. The posterior distribution is thinned by taking every 100th sample prior to plotting. The posterior distributions are shown as corner plots<sup>57</sup>.

### Extension to daytime

To extend the model to the daytime, we assume that during the day there is an additional flux of carbon into each pool, coming from the accumulation of carbon by photosynthesis. We make the basic assumption<sup>33,49</sup> that accumulated carbon is split between the fast and slow pool according to a constant parameter ( $\alpha$ ) for which we assume a default value of 0.4<sup>58</sup>. The distribution of assimilate from photosynthesis between different pools within a plant is considerably more complex than this. Significant variation in starch synthesis, for example, is observed over the diurnal cycle<sup>59</sup>, and factors including light<sup>59</sup> and water<sup>34</sup> availability can cause changes in the relative quantities of labile substrates found across plant organs. However, these processes are not well understood and for the purpose of mathematical simplicity this assumption is sufficient. We also make the simple assumption that the turnover rates ( $k_f$  and  $k_s$  parameters) are the same during the day and night. As such, besides the additional input of carbon into the substrate pools that occurs during the day, we assume that the drivers of respiration change are the same during the night and day. Again, in reality complex regulation of substrate utilisation likely results in variation of specific substrate turnover over the diurnal period<sup>40,60</sup>. Accounting for this variation may be important to better capture the response, however, this

would drastically reduce the simplicity of our model which is an important feature if it is to be used efficiently within large scale land surface models. In addition, the necessary data to evaluate such processes is sparse, owing to the time consuming nature of measuring both respiration and photosynthesis continuously through the day. At present, the first order substrate dynamics used seem to represent the observed data sufficiently. The rate of change of substrate in each pool during daytime is given by:

$$\frac{dS_s^d}{dt} = (1 - \alpha)P - k_s S_s^d \quad (12)$$

and

$$\frac{dS_f^d}{dt} = \alpha P - k_f S_f^d + k_s S_s^d \quad (13)$$

where we have introduced the superscript  $d$  in order to distinguish substrate evolution in the daytime from the nighttime, and  $P$  is the rate of carbon assimilation by photosynthesis.

We then consider a plant or leaf in steady state. Here we define ‘steady state’ as the property that the contents of both the fast and slow pool at the start of each period of daytime are equal to their carbon contents at the end of each period of nighttime. In other words, the total substrate lost from each pool (to respiration, growth, transport etc.) over night is equal to the net amount of carbon regained from photosynthesis the following day:

$$\begin{aligned} S_{s_0}^d &= S_s^d(t = \tau_d + \tau_n) \\ S_{f_0}^d &= S_f^d(t = \tau_d + \tau_n) \end{aligned} \quad (14)$$

where  $\tau_d$  and  $\tau_n$  are the length of daytime and nighttime respectively, such that  $\tau_d + \tau_n = 24$  h. We assume by default that day length ( $\tau_d$ ) is 12 h. It is important to note the distinction between the parameters  $S_{s_0}^d$  and  $S_{f_0}^d$  defined here with the  $S_{s_0}$  and  $S_{f_0}$  parameters defined in Eq. (4). While those defined in Eq. (4) represent substrate availability in each pool at the start of the night, the parameters here represent the substrate availability at the start of the day.

Together with the assumption that photosynthesis is zero at the start and end of the day, this steady state assumption allows us to derive expressions for the carbon content of the slow and fast pools at the start of the night ( $S_{s_0}$  and  $S_{f_0}$  respectively), as a function of their turnover rates ( $k_f$  and  $k_s$ ), the substrate partitioning parameter ( $\alpha$ ), photosynthesis ( $P$ ), and day length ( $\tau_d$ ). Details of these derivations are given in the supplementary materials (Supplementary Notes 3).

$$S_{s_0} = \frac{1}{1 - e^{-k_s \tau}} e^{-k_s \tau_d} (1 - \alpha) \int_0^{\tau_d} P e^{k_s t} dt \quad (15)$$

$$S_{f_0} = \frac{1}{1 - e^{-k_f \tau}} e^{-k_f \tau_d} \frac{\alpha k_f - k_s}{k_f - k_s} \int_0^{\tau_d} P e^{k_f t} dt + \frac{k_s S_{s_0}}{k_f - k_s} \quad (16)$$

Finally, we make an assumption about the variation of photosynthesis throughout the day. For simplicity, we assume that photosynthesis varies sinusoidally through the day according to:

$$P(t) = P_{max} \sin\left(\frac{\pi t}{\tau_d}\right) \quad (17)$$

where  $P_{max}$  is the maximum photosynthetic rate of the leaf.

Substituting Eqs. (15), (16), (17) into Eq. (9) results in an expression for the decline in nocturnal plant respiration in terms of the substrate turnover parameters ( $k_f$  and  $k_s$ ), substrate partitioning ( $\alpha$ ), and day length ( $\tau_d$ ). To test the effect of increasing and decreasing  $k_f$  and  $k_s$ , we then multiply both parameters by a range of factors between 0 and 2. These factors are 0.1, 0.25, 0.5, 1.0, 1.25, and 2.0. Both  $k_f$  and  $k_s$  are multiplied by the same factor for

each experiment such that their ratio is always the same. Since we are assuming that these changes in  $k_f$  and  $k_s$  are related to changing leaf nitrogen we believe that this approach is justified although we acknowledge that there many other ways we could vary the parameters and the dependence of substrate utilisation on leaf nitrogen is likely more complex than a simple linear dependence.

### Data availability

The observational data used in this study are freely available in the supplementary material sections of Bruhn et al.<sup>15</sup> and Bruhn<sup>21</sup>. The model output data used to produce Figs. 2, 3, and 4 are available at <https://doi.org/10.5281/zenodo.10066241>.

### Code availability

Python code for analysis and the production of figures is available at <https://doi.org/10.5281/zenodo.10066241>.

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## Author contributions

L.M.M. and P.M.C. suggested the concept of modelling nocturnal respiration decline with substrate. S.J. and N.R. conducted the modelling study with contributions from L.M.M., P.M.C. and D.B.; S.J. prepared the manuscript with contributions from L.M.M., P.M.C., D.B., and N.R.

## Competing interests

The authors declare no competing interests.

## Additional information

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