A model of plant isoprene emission based on available reducing power captures responses to atmospheric CO₂

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Received: 15 November 2013
Accepted: 11 February 2014

doi: 10.1111/nph.12770

Key words: black poplar (Populus nigra), hybrid aspen (Populus tremula × P. tremulaoides), isoprene emission, light response, modelling, photosynthetic electron transport, quantum yield, volatile compounds.

Summary

- We present a unifying model for isoprene emission by photosynthesizing leaves based on the hypothesis that isoprene biosynthesis depends on a balance between the supply of photosynthetic reducing power and the demands of carbon fixation.
- We compared the predictions from our model, as well as from two other widely used models, with measurements of isoprene emission from leaves of Populus nigra and hybrid aspen (Populus tremula × P. tremulaoides) in response to changes in leaf internal CO₂ concentration (Cᵢ) and photosynthetic photon flux density (PPFD) under diverse ambient CO₂ concentrations (Cₐ).
- Our model reproduces the observed changes in isoprene emissions with Cᵢ and PPFD, and also reproduces the tendency for the fraction of fixed carbon allocated to isoprene to increase with increasing PPFD. It also provides a simple mechanism for the previously unexplained decrease in the quantum efficiency of isoprene emission with increasing Cₐ.
- Experimental and modelled results support our hypothesis. Our model can reproduce the key features of the observations and has the potential to improve process-based modelling of isoprene emissions by land vegetation at the ecosystem and global scales.

Introduction

Isoprene (2-methyl-1,3-butadiene; C₅H₈) is released into the atmosphere by its main source, terrestrial vegetation. With a total annual emission of c. 0.5 Pg C yr⁻¹ (Guenther et al., 2006, 2012; Arneth et al., 2008), this extremely volatile and reactive molecule is the most important biogenic volatile organic compound (BVOC) produced by plants.

Why do certain plants emit isoprene and others do not? What is the advantage for emitters in losing 2% or more of their assimilated carbon in the form of isoprene? What are the controls over isoprene production and emission? These questions remain largely unresolved. However, some indications have emerged in recent years thanks to advances in diverse fields from cell physiology to phylogeny (Li & Sharkey, 2012; Monson et al., 2013; Niinemets & Monson, 2013; Sharkey, 2013). Isoprene appears to protect the photosynthetic apparatus from heat and oxidative damage by enhancing membrane stability at high temperatures, and by quenching reactive oxygen species (Sharkey & Yeh, 2001; Vickers et al., 2009; Velikova et al., 2011, 2012; Possell & Loreto, 2013). Isoprene is produced in the chloroplast from its immediate precursor dimethylallyl diphosphate (DMADP), which is synthesized via the methylerythritol 4-phosphate (MEP) pathway (Lichtenthaler, 1999; Logan et al., 2000; Sharkey et al., 2008). Isoprene production is therefore controlled by the supply of DMADP, and by the activity of isoprene synthase (IspS) (Rasulov et al., 2009a,b, 2010; Vickers et al., 2010; Li et al., 2011; Monson, 2013). The metabolic controls of the MEP pathway, in relation to isoprene biosynthesis, are just beginning to be understood (Li & Sharkey, 2012; Banerjee et al., 2013; Weise et al., 2013), and the whole pathway controls cannot yet be included in isoprene emission models in a wholly mechanistic manner (Grote et al., 2013; Li & Sharkey, 2013).

In addition to its physiological interest, isoprene has sparked attention in climate science because of its impact on atmospheric chemistry and climate. Because of its abundance and reactivity, isoprene emission substantially affects the atmospheric content of tropospheric ozone, methane and secondary organic aerosols (Poissant et al., 2000; Sanderson et al., 2003; Claey s et al., 2004; Heald et al., 2008; Pike & Young, 2009; Nozière et al., 2011; Paasonen et al., 2013). To investigate the potential impact of isoprene on air quality and climate, models for isoprene emission
have been developed (Grote & Niinemets, 2008; Monson et al., 2012; Grote et al., 2013). Many recently published studies have used the MEGAN model (Guenther et al., 2012), which is based on the pioneering work of Guenther and co-workers (Guenther et al., 1991, 1993). In MEGAN, a species-specific standard isoprene emission \((I_s)\) is modified by empirical functions that account for the observed variations in isoprene emissions as a result of various environmental controls. Although simple, this approach is vulnerable to model overparameterization because of interactions among environmental drivers (Niinemets et al., 2010; Sun et al., 2012). Other models have been developed based on the available knowledge of the underlying biochemical processes. These include the models of Niinemets et al. (1999) and Martin et al. (2000), and the SIM–BIM model (Zimmer et al., 2000, 2003). Nevertheless, all isoprene emission models remain largely empirical, and the mechanistic content of current models admits considerable scope for improvement (Monson et al., 2012; Grote et al., 2013).

Although often invoked as a potential driver of isoprene production (Niinemets et al., 1999; Rasulov et al., 2010; Li & Sharkey, 2012), few studies have quantitatively explored the impact of leaf energetic status on isoprene emissions. We define the leaf energetic status as the balance (or imbalance) between the supply of photosynthetic induced reducing power and the demands of carbon fixation and photorespiration. Here, we investigate the hypothesis that the rate of isoprene biosynthesis depends on the leaf energetic status. We used observations from *Populus nigra* grown in full sun (this study) and hybrid aspen (*Populus tremula × P. tremuloides*) grown at two CO2 concentrations (Sun et al., 2012). For each dataset, the experimental protocol allowed us to study short-term variations in isoprene emission, and associated variations of the electron balance between photosynthetic supply and carbon assimilation requirements. Changes in both isoprene emission and energy balance were obtained by modifying the light and CO2 conditions of the experiments. We used these datasets to test a new modelling framework, in which changes in leaf energetic status are approximated by the difference between the light- and Rubisco-limited electron fluxes for carbon assimilation. We used the same data to test the responses of two of the better known among the published isoprene models: the Guenther et al. (1993) algorithm that underlies MEGAN, and the ‘process-based’ model developed by Niinemets et al. (1999), Niinemets (2004) and modified by Arneth et al. (2007).

Hypothesis

Isoprene is produced in the chloroplast by the MEP pathway, in which glyceraldehyde 3-phosphate (G3P) and pyruvate (Pyr) are transformed into DMADP. The process involves reduction steps that require reducing power in the form of NADPH and/or ferredoxin (Fd) (Charon et al., 1999; Hecht et al., 2001; Seemann et al., 2006; Li & Sharkey, 2012). DMADP is further transformed into isoprene by the enzyme IspS. Therefore, isoprene production is co-driven by enzymatic activity and NADPH and/or ATP availability (Lichtenthaler, 1999).

Plastid NADPH is provided by the electron transport flux generated by the light reactions of Photosystem II. As reduction steps in carbon assimilation and photorespiration consume almost all of the NADPH generated, it is common to assume that the total electron flux \((J_{\text{tot}})\) is the same as the total electron flux used in carbon assimilation \((J_{\text{CO2+O2}})\). However, in reality, \(J_{\text{tot}}\) is always somewhat larger than \(J_{\text{CO2+O2}}\). It has to be so in order to

![Fig. 1 Schematic diagrams of the processes underlying the proposed hypothesis for isoprene emission modelling.](image-url)

**Fig. 1** Schematic diagrams of the processes underlying the proposed hypothesis for isoprene emission modelling. The arrow colour scheme is as follows: red, total electron flux generated by light reaction in Photosystem II \((J_{\text{iso}})\); green, electron flux used in reactions associated with carbon assimilation and photorespiration \((J_{\text{CO2+O2}})\); hashed grey, electron flux used in the MEP pathway \((J_{\text{iso}})\); grey, electron flux used for other redox reactions. Changes in flux intensities in situations of (a) high and (b) low demand for carbon assimilation are symbolically represented by changes in arrow width. These schematic diagrams are illustrative only and the arrows are not fitted to scale. ETC, electron transport chain; MEP, 2-C-methyl-D-erythritol 4-phosphate; NADPH, nicotinamide adenine dinucleotide phosphate.
supply NADPH for additional redox reactions in the leaf (Niinemets et al., 1999; Singsaas et al., 2001; Niinemets, 2004). The reduction steps along the MEP pathway constitute some of these additional reactions. Thus, \( J_{\text{tot}} \) can be expressed as \( J_{\text{tot}} = J_{\text{CO}_2 + \text{O}_2} + J_{\text{iso}} + J_{\text{other}} \), where \( J_{\text{iso}} \) and \( J_{\text{other}} \) represent electron fluxes involved in isoprene production and other redox reactions, respectively, in the leaf. We hypothesize accordingly that the additional reducing power available for isoprene production is dependent on the extent to which the NADPH requirements of the Calvin–Benson and photorespiratory cycles are satisfied (Harrison et al., 2013; Morfopoulos et al., 2013). As illustrated in Fig. 1, the MEP pathway could be envisioned to act like a small branch circuit, with the greatest influx occurring when the demand of carbon assimilation for reducing power is least (Rosenstiel et al., 2004; Owen & Peñuelas, 2005). However, the MEP pathway alone does not have the capacity to absorb all of the excess energy generated. Thus, our hypothesis also suggests that isoprene emissions might co-vary with other, more effective energy quenching processes, including the Mehler reaction and the xanthophyll cycle.

Although the biochemical mechanisms controlling the partitioning of the NADPH fluxes inside the plastid are incompletely understood, the nature of the responses of isoprene emission to different environmental drivers suggests that this hypothesis is well founded (Morfopoulos et al., 2013). Indeed, the literature shows a persistent tendency for plants to increase isoprene emission (and the fraction of assimilated carbon transformed to isoprene) with increasing leaf energetic status. For example:

1. Isoprene emissions increase with decreasing CO2 concentration (Rosenstiel et al., 2003; Wilkinson et al., 2009; Possell & Hewitt, 2011; Sun et al., 2012).
2. The fraction of assimilated carbon transformed to isoprene increases with increasing light intensity (Sharkey & Loreto, 1993; Harley et al., 1996; Lerdau & Keller, 1997).
3. The temperature optimum for isoprene emissions is lower than that of IspS activity, and apparently co-controlled by the temperature dependences of the electron transport rate and IspS activity (Monson et al., 1992, 2012; Rasulov et al., 2010).
4. Isoprene emissions decrease in plants fed with nitrate (which consumes NADPH in the process of nitrate reduction to ammonia), but increase if fed with ammonia directly (Rosenstiel et al., 2004).
5. Isoprene emissions increase when light use efficiency decreases (Peñuelas et al., 2013).

These observations all support the hypothesis that isoprene emissions are influenced by the balance of reducing power between what can be produced by light reactions and what is needed for carbon assimilation and other major NADPH sinks.

Ideally, to represent this hypothesis quantitatively, we should model the total electron flux and the dynamics of all relevant electron sinks. However, in reality, process-based models that can simulate the total electron transport rate \( J_{\text{iso}} \) are in an early stage of development (Ye et al., 2013), the partitioning of the additional reducing power between \( J_{\text{other}} \) and \( J_{\text{iso}} \) remains enigmatic, and the nanomole scale at which isoprene emission occurs (compared with the micromole scale of electron flux) makes it unrealistic to attempt a full mass balance of the competing processes. Accordingly, our pragmatic approach is to model the energetic status of the leaf using the Farquhar model (Farquhar et al., 1980) for photosynthetic carbon assimilation, thus approximating the energetic status of the leaf as the difference between the light-limited electron flux \( J \) and the electron flux required to support Rubisco-limited photosynthesis \( J_0 \). \( J \) is an approximation of the amount of reductant that light reactions can supply, and \( J_0 \) represents the capacity of Rubisco to absorb this reducing power. Therefore, energy transfers to processes other than carbon assimilation \( \left[ J_{\text{iso}} - J_{\text{CO}_2 + \text{O}_2} = J_{\text{other}} + J_{\text{iso}} \right] \) should be correlated with the magnitude of the difference \( \left[ J - J_0 \right] \). Based on this proxy, we build a model of isoprene emissions that we describe further in the text. We test the model with data on isoprene emission as a function of internal CO2 concentration \( (C) \) and photosynthetic photon flux density (PPFD).

We further test our hypothesis by examining observed and modelled changes in the fraction of assimilated carbon allocated to isoprene production. The ratio of isoprene emission to gross carbon assimilation \( \text{ISO}/\text{A}_{\text{gross}} \) is a sensitive indicator of the allocation of reducing power to the MEP pathway vs the Calvin–Benson cycle (Niinemets et al., 2013). Under a constant leaf temperature and CO2 concentration, we would expect the fraction of assimilated carbon re-emitted as isoprene to be constant, if only enzymatic limitations are involved. However, if isoprene production depends on the energetic status of the leaves, \( \text{ISO}/\text{A}_{\text{gross}} \) would be expected to increase with increasing PPFD (Niinemets et al., 2013), as carboxylation becomes progressively Rubisco limited, whilst electron transport continues to increase.

Finally, we examine changes in the quantum efficiency of isoprene emission \( \Phi_{\text{iso}} \). Previous studies have reported changes with environmental conditions (Monson et al., 1992; Logan et al., 2000; Sun et al., 2012). Changes in the quantum efficiency of CO2 assimilation \( \Phi_{\text{CO}_2} \) cannot explain changes in \( \Phi_{\text{iso}} \). The processes controlling quantum yields for isoprene are not fully understood. We postulate that differences in the quantum efficiency of isoprene emission \( \Phi_{\text{iso}} \) are driven by the energetic status of the leaves, and can thus be related to the variation in \( \left[ J - J_0 \right] \). Thus, we expect the quantum yield of isoprene emission to be lower when the NADPH demand for carbon assimilation is higher.

We show that our energetic status model is able to reproduce changes in isoprene emission induced by changes in \( C_i \) and PPFD, the observed tendency of \( \text{ISO}/\text{A}_{\text{gross}} \) to increase with increasing PPFD and the observed increase in \( \Phi_{\text{iso}} \) with decreasing CO2 concentration.

**Materials and Methods**

**Plant material and growing conditions**

In this study, we examine results from experiments conducted on two different species: *Populus nigra* L. and hybrid aspen (*Populus tremula* L. × *P. tremuloides* Michx.).

The first set of experiments was conducted on three saplings of *P. nigra*, grown in 15-l pots with a substrate composed of peat
and sand (2:1) in a nursery (Tres Turons S.C.P., Castellar del Vallès, Catalonia, Spain). Plants were grown in a sunny environment under Mediterranean ambient conditions outdoors for 2 months before the measurement (2 May–7 July 2012). The typical Mediterranean climate is characterized by seasonal summer drought with warm temperatures and mild winters. This is reflected by the average monthly temperature of 22.8°C in August and 7.9°C in January. Mean annual precipitation and temperature are 723 mm and 15.1°C (1951–2010), respectively (Ninyerola et al., 2000). As a result of high temperature and low precipitation, the plants were under conditions of high evaporative demand. However, regular irrigation ensured that the substrate was held at field capacity throughout this period. Here, we used data from one leaf of each sapling, giving an overall dataset of three sun-adapted individuals.

The second set of experiments was conducted with 2-yr-old saplings of hybrid aspen (P. tremuloides × P. tremula) grown under two different ambient CO₂ concentrations (380 and 780 μmol mol⁻¹). These experiments, together with a full description of the materials and methods used, are reported in Sun et al. (2012, 2013b), and here only a brief summary of the methods is provided. The plants were grown in a custom-made, four-chamber, open gas exchange system. Each individual chamber experienced a 12-h photoperiod at levels of light between 500 and 800 μmol m⁻² s⁻¹, day/night air temperature of 28–30/23°C and air relative humidity of 60%. Two chambers (chambers 1 and 3) were kept at an ambient CO₂ concentration of 380 μmol mol⁻¹ (HA-G380), whereas the other two chambers were treated with an elevated CO₂ concentration of 780 μmol mol⁻¹ (HA-G780). Here, we used data from three leaves in each chamber, giving an overall dataset of six individuals grown at ambient CO₂ concentration and six individuals grown at elevated CO₂ concentration. For each dataset, the results shown are averaged values across individuals.

Foliage gas exchange analyses and isoprene emission rates

Gas exchange measurements were conducted on individuals of P. nigra using a Li-Cor LI-6400 portable photosynthesis system (an open gas exchange analyser using a 6-cm² clamp-on leaf cuvette (LI 6400; LI-COR, Inc., Lincoln, NE, USA)). The calibration of the infrared gas analyser (IRGA) was performed by the manufacturer < 1 yr before the measurements.

The exhaust tube of the IRGA measure head was connected to a Proton-Transfer-Reaction Mass Spectrometer (PTR-MS) system (Ionicon Analytik, Innsbruck, Austria), using tubing made of Siltek-passivated stainless steel (Restek, Bellefonte, PA, USA). Analyses of emission rates for isoprene were performed simultaneously with gas exchange measurements with the PTR-MS. The PTR-MS technique is based on chemical ionization, specifically non-dissociative proton transfer from H₃O⁺ ions to most of the common BVOCs, and has been fully described elsewhere (Lindinger et al., 1998). In our experiment on P. nigra, the PTR-MS drift tube was operated at 2.1 mbar and 60ºC, with an E/N (electric field/molecule number density) of c. 130 Td (Townsend) (1 Td = 10⁻¹⁷ V cm²). The primary ion signal (H₂O⁺) was maintained at c. 6 x 10⁶ counts per second. The instrument was calibrated using an aromatic mix standard gas (TO-14A; Restek) and isoprene standard gas with 100 nmol mol⁻¹ isoprene in N₂ (Abelló-Linde SA, Barcelona, Spain). Before data acquisition, the leaf cuvette was left empty in order to analyse the background concentrations of isoprene, and thereafter to calculate the foliar emission rates. No significant drift in the background of isoprene was found during the experiments.

Foliage gas exchange analyses and isoprene emission rates on hybrid aspen were obtained using a Walz GFS-3000 portable gas exchange system and a Fast Isoprene Sensor (FIS; Hills Scientific, Boulder, CO, USA). More information on the methods can be found in Sun et al. (2012, 2013b).

Before each experiment, the leaf was enclosed in the gas exchange system and left under baseline conditions until net assimilation (A), stomatal conductance (gₛ) and Cᵢ stabilized (typically 20–30 min). For P. nigra, baseline conditions were PPFD of 1000 μmol m⁻² s⁻¹, leaf temperature of 30°C, relative humidity of 50% (± 10%) and ambient CO₂ concentration of the leaf chamber (C₀) of 390 μmol mol⁻¹. For hybrid aspen, baseline conditions were PPFD of 500 μmol m⁻² s⁻¹, leaf temperature of 30°C, relative humidity of 60%, C₀ of 380 μmol mol⁻¹ for HA-G380 and C₀ of 780 μmol mol⁻¹ for HA-G780. After preconditioning the leaf as explained above, two types of response curve were created: (1) the leaf net assimilation vs internal CO₂ concentration (A/C₀); and (2) the leaf net assimilation vs PPFD (A/PPFD).

CO₂ response curves of net assimilation and isoprene emissions

Cᵢ response curves were obtained at a leaf temperature of 30°C and a quantum flux density of 1000 μmol m⁻² s⁻¹ for P. nigra and 500 μmol m⁻² s⁻¹ for hybrid aspen. The Cᵢ values used to generate the A/Cᵢ response curve were:

1. 50 → 150 → 200 → 250 → 350 → 390 → 500 → 700 → 800 → 900 → 1200 → 2000 (μmol mol⁻¹), for P. nigra;
2. 380 → 200 → 150 → 100 → 50 → 20 → 0 → 380 → 780 → 1000 → 1500 → 2000 (μmol mol⁻¹), for HA-G380;
3. 780 → 380 → 200 → 150 → 100 → 50 → 20 → 0 → 780 → 1000 → 1500 → 2000 (μmol mol⁻¹), for HA-G780.

At every Cᵢ, values of A, isoprene emission rate (Iso) and stomatal conductance (gₛ) were recorded when the gas exchange rates were stable, typically 5–10 min after the change in Cᵢ.

PPFD response curves of net assimilation and isoprene emissions

By applying sequential changes in PPFD, light response curves at different Cᵢ were obtained. Three different Cᵢ values (200, 390 and 1000 μmol mol⁻¹) were applied for P. nigra, and two different Cᵢ values (380 and 780 μmol mol⁻¹) were applied for hybrid aspen.
The following sequence of PPFD was applied:

1. 2500 → 2000 → 1750 → 1500 → 1250 → 1000 → 700 → 500 → 250 → 150 → 75 → 0 (μmol m\(^{-2}\) s\(^{-1}\)) for *P. nigra*;
2. 500 → 1500 → 1000 → 800 → 400 → 200 → 120 → 60 → 30 → 12 → 0 (μmol m\(^{-2}\) s\(^{-1}\)) for hybrid aspen.

The waiting time between each light intensity was c. 10 min. The data were logged when the rates of *A*, *g*\(_{c}\), *C*\(_{i}\) and *Iso* were in the steady state, except for hybrid aspen at PPFD higher than 1500 μmol m\(^{-2}\) s\(^{-1}\), where the values were recorded after 5–8 min to avoid the development of photoinhibition.

**Energetic status model**

Our isoprene model is modified in one small (but important) way from that introduced by Harrison *et al.* (2013) and Morfoioupolos *et al.* (2013), and deals with the issue of negative values for isoprene emission generated using the first version of the model (Supporting Information Notes S1; Table S1; Figs S1, S2). In these earlier papers, the isoprene emission rate was assumed to be linearly related to the energy status of the leaf, whereas, here, the fraction of electrons allocated to isoprene biosynthesis is linearly related to the energetic status of the leaf:

\[ \varepsilon = c_1 + c_2(J - J_e) \quad \text{Eqn 1} \]

and

\[ Iso = \varepsilon J f(G_i) f(T) \quad \text{Eqn 2} \]

where *Iso* is the isoprene emission, *f*(*G*\(_{i}\)) is a function of internal CO\(_2\) concentration, *f*(*T*) is a function of temperature, taking into account the response of enzymatic activity to temperature, *J* is the light-limited electron flux, taken to be a non-rectangular hyperbolic function of absorbed PPFD and the maximum electron flux *J*\(_{\text{max}}\), following Farquhar *et al.* (1980), and

\[ J_e = 4V_{\text{max}}(C_i + 2\Gamma^*))/\left( C_i + K_c \right) \quad \text{Eqn 3} \]

which is the electron flux required to support Rubisco-limited carbon assimilation. Γ\(^*\) is the CO\(_2\) compensation point in the absence of mitochondrial respiration in the light, *V*\(_{\text{max}}\) is the Rubisco carboxylation capacity and *K*\(_{c}\) = *K*\(_{c}\)(1 + [O\(_2\)]/*K*\(_{c}\)), where *K*\(_{c}\) and *K*\(_{c}\) are the Michaelis coefficients of Rubisco for CO\(_2\) and O\(_2\), respectively (Farquhar *et al.*, 1980). The term *ε* in Eqn 2 is not constant, but varies depending on the energetic status of the leaf, estimated by *(J - J\(_{\text{e}}\))*.

The function *f*(*G*\(_{i}\)) in Eqn 2 is chosen to take the value *G*/Γ\(^*\) when *G*\(_{i}\) ≤ Γ\(^*\) and ‘1’ otherwise, and reflects the common observation that isoprene emission ceases when *G*\(_{i}\) < Γ\(^*\) as a result of a minimum supply of carbon chains required for isoprene synthesis and/or the inhibition of the electron transport rate below Γ\(^*\) (Dietz *et al.*, 1985; Wolfertz *et al.*, 2003; Rasulov *et al.*, 2009b, 2011; Monson *et al.*, 2012; Sun *et al.*, 2012). This fall-off of isoprene at low *G*\(_{i}\) is not fully understood and is not always observed: the quantity of isoprene in CO\(_2\)-free air has been reported in a few studies (Monson & Fall, 1989; Affek & Yakir, 2003; Li & Sharkey, 2012). However, comparable conditions are not found in natural environments. Using the *G*\(_{i}\) response curves, changes in the fraction *ε* of the light-limited electron flux (*J*) allocated to isoprene production (Eqns 1, 2) were plotted against the corresponding difference between light- and Rubisco-limited electron fluxes *(J - J\(_{\text{e}}\))*.

Parameters *c*\(_{1}\) and *c*\(_{2}\) were obtained from a linear regression between *ε* and *(J - J\(_{\text{e}}\)) when *G*\(_{i}\) > Γ\(^*\) (Figs 2a, 3a,b). Because all our experiments were conducted at a leaf temperature of 30°C, we neglect here the temperature dependence caused by IsP\(_{5}\) activity, and *f*(*T*) is accordingly set equal to unity. Quantum efficiencies for isoprene production (*P*\(_{\text{iso}}\)) were calculated as the initial slope of isoprene emission vs PPFD, for PPFD < 250 μmol m\(^{-2}\) s\(^{-1}\). The bounds of uncertainty of the energetic status model displayed in the figures represent uncertainties in the estimated values of *V*\(_{\text{max}}\) and *J*\(_{\text{max}}\) in the Farquhar model.

**The G93 algorithm**

The algorithm developed by Guenther *et al.* (1993), which is the basis of the isoprene module of the MEGAN model (Guenther *et al.*, 2012), is the most widely used algorithm for the prediction of isoprene emission by plants. Hereafter, this algorithm is referred to as G93. In G93, the emission rates of isoprene are calculated by multiplying a species-specific standard emission rate (*L*) by a set of empirical equations taking into account changes in environmental variables. The standard conditions for *I*\(_{\text{s}}\) are a leaf temperature of 30°C and an incident PPFD of 1000 μmol m\(^{-2}\) s\(^{-1}\). Because, in this study, all the experiments were conducted at a constant leaf temperature of 30°C, we consider only changes driven by light intensity:

\[ Iso = I_s C_L \quad \text{Eqn 4} \]

with

\[ C_L = \alpha C_{L_1} \frac{PPFD}{\sqrt{1 + \alpha^2 PPFD^2}} \quad \text{Eqn 5} \]

where *C*\(_{L_1}\) and *α* are empirical coefficients. For each light response curve, in order to take into account the CO\(_2\) effect on the standard emission rates, the value of *I*\(_{s}\) was taken as the observed emission rate at a PPFD of 1000 μmol m\(^{-2}\) s\(^{-1}\), under the CO\(_2\) conditions of the experiment.

**The Niinemets model**

The Niinemets model (Niinemets *et al.*, 1999) is based on the quantification of the NADPH cost for isoprene synthesis. It builds on the Farquhar model of photosynthesis. The general concept is that a temperature-dependent fraction of the electron flux (*ε*\(_{\text{N}}\)) is used for isoprene production:

\[ \varepsilon_N = J_{\text{iso}} / J_{\text{tot}} \quad \text{Eqn 6} \]

where *J*\(_{\text{iso}}\) is the electron flux required in order to produce a quantity of isoprene and *J*\(_{\text{tot}}\) is the total photosynthetic electron flux, approximated by *J*, using the Farquhar model:
Fig. 2 Isoprene emissions vs internal CO2 concentration ($C_i$) at a leaf temperature of 30°C and a photosynthetic photon flux density of 1000 μmol m$^{-2}$ s$^{-1}$ for *Populus nigra*. (a) Observed changes in the fraction of electrons used for isoprene production, taken as the ratio of the isoprene emission rate to the light-limited electron flux for carbon assimilation ($ε = I_{iso}/I_0$), in response to changes in the energetic status of the leaf, taken as the difference between the light- and Rubisco-limited electron fluxes for carbon assimilation ($J - J_r$). (b) Observed (closed circles) and modelled (solid line) isoprene emission rates in response to changes in $C_i$. The grey shaded area represents uncertainties of the isoprene model as a result of uncertainties in the values of the maximum Rubisco carboxylation capacity ($V_{cmax}$) and maximum electron flux ($I_{max}$) in the Farquhar model. Error bars represent the maximum and minimum bounds of the isoprene curve.

\[ J_{tot} \approx J = A_4(4 C_i + 8 Γ^* )/( C_i - Γ^* ) \quad \text{Eqn 7} \]

where $A_4$ is the gross assimilation under electron transport-limited conditions, $C_i$ is the internal CO2 concentration and $Γ^*$ is the compensation point.

The total NADPH cost for isoprene production per mole CO2 assimilated is 1.17 times higher (2.33 NADPH per CO2) than for sugar synthesis (2 NADPH per CO2), and six molecules of CO2 must be assimilated to produce one isoprene molecule. Drawing a parallel with the Farquhar model, $I_{iso}$ is thus estimated as:

\[ I_{iso} = 6 Iso \cdot 1.17 \left( 4 C_i + 8 Γ^* \right)/( C_i - Γ^* ) \]
\[ = 7.02 Iso \left( 4 C_i + 8 Γ^* \right)/( C_i - Γ^* ) \quad \text{Eqn 8} \]

Combining Eqs 6–8, the overall model for isoprene emission becomes:

\[ Iso = ε_N J(C_i - Γ^* )/(7.02 \left( 4 C_i + 8 Γ^* \right)) = ε_N / 7.02 A_4 \quad \text{Eqn 9} \]

Because all our experiments were conducted at a leaf temperature of 30°C, we neglect the temperature dependence of $ε_N$. The effect of changes in CO2 concentration on $ε_N$ is adapted from Arneth et al. (2007):

\[ ε_N = ε_{N_0} C_{a_s}/C_s \]
\[ A = A_4 - R_d = V_{cmax}(C_i - Γ^* )/(C_i + K) - R_d \quad \text{Eqn 11} \]

where $J$ is the potential rate of electron transport. $J_r$ in turn, depends on PPFD up to a maximum $J_{max}$ (de Pury & Farquhar, 1997). For each $C_s$, the averaged value of observed $C_i$ was used for the model simulations.
Values of Michaelis–Menten constants, activation and de-activation energies, specificity for Rubisco and their temperature dependences were taken from Bernacchi et al. (2002) and Medlyn et al. (2005) (Notes S2; Table S2).

For the experiment on *P. nigra*, probably as a result of the growing conditions of the plants (Mediterranean summer sunlight), the plants adapted their maximum Rubisco capacity (*V*<sub>max</sub>) to the prevailing high levels of irradiance and temperature. As a result, under most of the experimental conditions (including a large part of the *A*/*C<sub>i</sub>* curve), the carbon assimilation was found to be limited by electron transport and not by Rubisco capacity. In order to estimate *V*<sub>max</sub>, we therefore used the light response curve for assimilation, at a *C<sub>i</sub>* of 200 μmol mol<sup>−1</sup> and PPFD ≥ 1500 μmol m<sup>−2</sup> s<sup>−1</sup>, where *A* was saturating. We calculated *V*<sub>max</sub> by minimizing the residual sum of squares (RSS) between the Rubisco-limited equation and the observations. The capacity for photosynthetic electron transport (*J*<sub>max</sub>) was obtained similarly by minimizing RSS between the light-limited equation and the assimilation data from all experiments. For hybrid aspen, *J*<sub>max</sub> and *V*<sub>max</sub> were estimated from *A*/*C<sub>i</sub>* curves by minimizing RSS between the Farquhar model and the observations.

Model parameters are summarized in Table 1. Statistical analyses were performed using the software R version 2.15.0 (http://www.r-project.org/).

### Results

#### Experiments varying *C<sub>i</sub>*

For each plant type, isoprene emissions showed a strong negative response to changes in *C<sub>i</sub>* (Figs 2b, 3c,d). For *P. nigra*, the maximum isoprene emissions were c. 33 nmol m<sup>−2</sup> s<sup>−1</sup> at low *C<sub>i</sub>* (73–174 μmol mol<sup>−1</sup>), declining to 8 nmol m<sup>−2</sup> s<sup>−1</sup> at high *C<sub>i</sub>* (1280 μmol mol<sup>−1</sup>). Maximum isoprene emission rates (at low *C<sub>i</sub>* represented up to 2.24% of assimilated carbon (Fig. S3); this percentage dropped to 0.17% at high *C<sub>i</sub>*. For hybrid aspen, averaged isoprene emissions peaked at low *C<sub>i</sub>* (105–140 μmol mol<sup>−1</sup>) with maxima of c. 21 nmol m<sup>−2</sup> s<sup>−1</sup> for HA-G380 and 25 nmol m<sup>−2</sup> s<sup>−1</sup> for HA-G780, declining below 4 nmol m<sup>−2</sup> s<sup>−1</sup>

### Fig. 3

Isoprene emissions vs internal CO<sub>2</sub> concentration (*C<sub>i</sub>* at a leaf temperature of 30°C and a photosynthetic photon flux density of 1000 μmol m<sup>−2</sup> s<sup>−1</sup> for hybrid aspen (*P. tremula × P. tremuloides*). Observed changes in the fraction of electrons used for isoprene production, taken as the ratio of the isoprene emission rate to the light-limited electron flux for carbon assimilation (*ε = Iso/J*), in response to changes in the energetic status of the leaf, taken as the difference between the light- and Rubisco-limited electron fluxes for carbon assimilation (*J* – *J*<sub>l</sub>), for hybrid aspen grown under (a) ambient CO<sub>2</sub> concentration (HA-G380; closed circles) and (b) elevated CO<sub>2</sub> concentration (HA-G780; open circles). Observed (circles) and modelled (solid line) isoprene emission rates in response to changes in *C<sub>i</sub>* for hybrid aspen grown under (c) ambient CO<sub>2</sub> concentration (closed circles) and (d) elevated CO<sub>2</sub> concentration (open circles). The grey shaded area represents uncertainties of the isoprene model as a result of uncertainties in the values of the maximum Rubisco carboxylation capacity (*V*<sub>max</sub>) and maximum electron flux (*J*<sub>max</sub>) in the Farquhar model. Error bars represent the maximum and minimum bounds of the isoprene curves. The experimental details are reported in Sun et al. (2012, 2013b).
at high $C_i$ (1400 μmol mol$^{-1}$). A decline in isoprene emissions for very low values of $C_i$ was observed whatever the growing conditions. As highlighted in Sun et al. (2012), isoprene emissions reached higher rates for individuals grown under elevated CO$_2$ concentrations, contrary to that which is usually assumed. Maximum emission rates represented a loss of assimilated carbon into isoprene of 5.6% for HA-380 and 6.6% for HA-G780; this percentage dropped to 0.09% for high values of $C_i$.

For all experiments, a very strong linear correlation was found between [(J$-\bar{J}$)] and the number of electrons $\varepsilon$ engaged in the isoprene production pathway, with $r^2 > 0.89$ (Figs 2a, 3a,b). Yet, the response of $\varepsilon$ vs [(J$-\bar{J}$)] seems to start to saturate at very negative values of [(J$-\bar{J}$)] in each dataset. This behaviour might be caused by an overall saturation of the redox state of Q$_X$ (the primary acceptor of Photosystem II) associated with a limitation of capacity of $J_{tot}$ that can be observed under high $C_i$ (Dietz et al., 1985).

With parameters obtained from the linear regression of $\varepsilon$ vs [(J$-\bar{J}$)], our model simulated isoprene emissions in response to changes in $C_i$ with excellent agreement to the observations ($r^2 = 0.94$, 0.87 and 0.93 for P. nigra, HA-G380 and HA-G780, respectively) (Figs 2b, 3c,d).

We also tested the response vs $C_i$ of the Niinemets model corrected by the empirical CO$_2$ response function proposed by Arneth et al. (2007) (Fig. S4). The Niinemets model reproduced the data reasonably well, but tended to underestimate the isoprene emissions for P. nigra, whereas it tended to overestimate the isoprene emissions for the hybrid aspen experiments. It should also be noted that, without the CO$_2$ response function proposed by Arneth et al. (2007), the Niinemets model would show an increase in isoprene emissions with increasing $C_i$, imitating the response of $A_i$.

### Experiments varying PPFD

**Isoprene emissions** For all experiments, isoprene emission rates increased with increasing PPFD, with observed maxima for isoprene emissions inversely related to $C_i$ (and consequently to $C$) – opposite to the net assimilation rates. Observed isoprene emissions vs $J$ were found to have a quadratic type of response, in line with our model (shown for hybrid aspen in Fig. S5).

For P. nigra at each $C_i$, our model captured the variations in isoprene emissions extremely well with $r^2 > 0.99$ (Fig. 4, Table 2). For $C_i$ of 200 μmol mol$^{-1}$, however, our model systematically underestimated the observed values. The Niinemets model showed comparable $r^2$ values (Table 2), consistent with the fact that isoprene emission, in both our model and that of Niinemets, is proportional to $J$. G93 was the only model with a component ($I_a$) fitted directly to the observations, yet G93 performed less well than the other two models. All models underestimated isoprene emission rates at the highest PPFD of 2500 μmol m$^{-2}$ s$^{-1}$.

For hybrid aspen, all models captured well the variation in isoprene emissions with PPFD with $r^2 > 0.88$. Yet, our model tended to systematically underestimate isoprene emissions for HA-G380 (Fig. 5).

**Isoprene: assimilation ratios (Iso/A$_{\text{gross}}$)** Observed mean Iso/A$_{\text{gross}}$ increased with increasing PPFD, regardless of $C_i$, plant type or growth conditions. However, the range of Iso/A$_{\text{gross}}$ across individuals is considerable. The fraction of assimilated carbon re-emitted as isoprene was inversely related to the CO$_2$ concentration. The high ratios of Iso/A$_{\text{gross}}$ at low $C_i$ were a result of a

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**Table 1: Model parameter values at a leaf temperature of 30°C**

<table>
<thead>
<tr>
<th>Data</th>
<th>Model</th>
<th>Parameter</th>
<th>Value</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>Populus nigra</td>
<td>Farquhar</td>
<td>$J_{\text{max}}$</td>
<td>111 ($\pm$20 – 15)</td>
<td>μmol m$^{-2}$ s$^{-1}$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$V_{\text{cmax}}$</td>
<td>169 ($\pm$35 – 32)</td>
<td>μmol m$^{-2}$ s$^{-1}$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$\gamma_{\text{CO}_2}$</td>
<td>0.27 ($\pm$0 – 0.03)</td>
<td>mol electron mol$^{-1}$ photon</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$\theta$</td>
<td>0.01 ($\pm$0.36 – 0)</td>
<td>Unitless</td>
</tr>
<tr>
<td>Hybrid aspen</td>
<td>Energetic status model</td>
<td>$c_1$</td>
<td>0.309 $\times$ 10$^{-3}$</td>
<td>Unitless</td>
</tr>
<tr>
<td>HA-G380</td>
<td>Farquhar</td>
<td>$J_{\text{max}}$</td>
<td>88 ($\pm$39 – 15)</td>
<td>μmol m$^{-2}$ s$^{-1}$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$V_{\text{cmax}}$</td>
<td>56 ($\pm$6 – 17)</td>
<td>μmol m$^{-2}$ s$^{-1}$</td>
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<tr>
<td></td>
<td></td>
<td>$\gamma_{\text{CO}_2}$</td>
<td>0.385*</td>
<td>mol electron mol$^{-1}$ photon</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$\theta$</td>
<td>0.7*</td>
<td>Unitless</td>
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<tr>
<td>Hybrid aspen</td>
<td>Energetic status model</td>
<td>$c_1$</td>
<td>0.193 $\times$ 10$^{-3}$</td>
<td>Unitless</td>
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<tr>
<td>HA-G780</td>
<td>Farquhar</td>
<td>$J_{\text{max}}$</td>
<td>95 ($\pm$29 – 24)</td>
<td>μmol m$^{-2}$ s$^{-1}$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$V_{\text{cmax}}$</td>
<td>59 ($\pm$15 – 0)</td>
<td>μmol m$^{-2}$ s$^{-1}$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$\gamma_{\text{CO}_2}$</td>
<td>0.385*</td>
<td>mol electron mol$^{-1}$ photon</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$\theta$</td>
<td>0.7*</td>
<td>Unitless</td>
</tr>
<tr>
<td>Hybrid aspen</td>
<td>Energetic status model</td>
<td>$c_1$</td>
<td>0.219 $\times$ 10$^{-3}$</td>
<td>Unitless</td>
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<tr>
<td>HA-G780</td>
<td>Farquhar</td>
<td>$J_{\text{max}}$</td>
<td>89 ($\pm$29 – 24)</td>
<td>μmol m$^{-2}$ s$^{-1}$</td>
</tr>
<tr>
<td></td>
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<td>$V_{\text{cmax}}$</td>
<td>59 ($\pm$15 – 0)</td>
<td>μmol m$^{-2}$ s$^{-1}$</td>
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<tr>
<td></td>
<td></td>
<td>$\gamma_{\text{CO}_2}$</td>
<td>0.385*</td>
<td>mol electron mol$^{-1}$ photon</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$\theta$</td>
<td>0.7*</td>
<td>Unitless</td>
</tr>
<tr>
<td>All</td>
<td>G93</td>
<td>$\alpha$</td>
<td>0.0027*</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>$C_{l1}$</td>
<td>1.066*</td>
<td>Unitless</td>
</tr>
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</table>

Farquhar model uncertainties (in parentheses) were obtained by fitting the model to the maximum and minimum bounds of the assimilation curves. Parameters not fitted to the data: $J_{\text{max}}$, maximum electron flux; $V_{\text{cmax}}$, maximum Rubisco carboxylation capacity; $\gamma_{\text{CO}_2}$, quantum yield of electron transport; $\theta$, curvature parameter of the light response curve; $c_1$ and $c_2$, parameters of our energetic status model; $\alpha$ and $C_{l1}$, parameters of the G93 algorithm.
Table 2  Isoprene emissions vs changes in photosynthetic photon flux density (PPFD) at different CO$_2$ concentrations ($C_a$)

<table>
<thead>
<tr>
<th>Data</th>
<th>$C_a$ (µmol mol$^{-1}$)</th>
<th>Energetic status model</th>
<th>$r^2$</th>
<th>$p$</th>
<th>G93</th>
<th>$r^2$</th>
<th>$p$</th>
<th>Niinemets model</th>
<th>$r^2$</th>
<th>$p$</th>
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<tr>
<td><em>Populus nigra</em></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>200</td>
<td>c. 1</td>
<td>$&lt;1e-5$</td>
<td></td>
<td></td>
<td>0.88</td>
<td>$&lt;1e-5$</td>
<td>0.99</td>
<td>$&lt;1e-5$</td>
<td></td>
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<tr>
<td></td>
<td>390</td>
<td>c. 1</td>
<td>$&lt;1e-5$</td>
<td></td>
<td></td>
<td>0.86</td>
<td>$&lt;1e-4$</td>
<td>0.98</td>
<td>$&lt;1e-5$</td>
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<td></td>
<td>1000</td>
<td>0.92</td>
<td>$&lt;1e-4$</td>
<td></td>
<td></td>
<td>0.56</td>
<td>0.311</td>
<td>0.81</td>
<td>$&lt;1e-3$</td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HA-G380</td>
<td>380</td>
<td>c. 1</td>
<td>$&lt;1e-5$</td>
<td></td>
<td></td>
<td>0.98</td>
<td>$&lt;1e-5$</td>
<td>0.94</td>
<td>$&lt;1e-5$</td>
<td></td>
</tr>
<tr>
<td>Hybrid aspen</td>
<td>380</td>
<td>0.98</td>
<td>$&lt;1e-5$</td>
<td></td>
<td></td>
<td>0.97</td>
<td>$&lt;1e-5$</td>
<td>0.93</td>
<td>$&lt;1e-5$</td>
<td></td>
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<tr>
<td>HA-G780</td>
<td>780</td>
<td>0.96</td>
<td>$&lt;1e-5$</td>
<td></td>
<td></td>
<td>0.93</td>
<td>$&lt;1e-5$</td>
<td>0.88</td>
<td>$&lt;1e-4$</td>
<td></td>
</tr>
</tbody>
</table>

With the exception of hybrid aspen at $C_a = 380$ µmol mol$^{-1}$, the Niinemets model failed to capture the changes in $\text{Isol}A_{\text{gross}}$ with changing $PPFD$, showing no relationship between $\text{Isol}A_{\text{gross}}$ and $PPFD$.

Isoprene quantum efficiencies As predicted by our hypothesis, the observed quantum efficiencies for isoprene production were dependent on the CO$_2$ concentration (Fig. 8). Higher quantum efficiencies correspond to lower $C_a$, at which the demand for reductant by the Calvin–Benson cycle is lower. Our model captured the observed decrease in $\Phi_{\text{iso}}$ with increasing $C_a$. However, the model overestimated $\Phi_{\text{iso}}$ at high $C_a$ and underestimated $\Phi_{\text{iso}}$ at low $C_a$ for *P. nigra*. The model overestimated $\Phi_{\text{iso}}$ for HA-G380 and underestimated $\Phi_{\text{iso}}$ for HA-G780.

Global results

The overall performance of each model is illustrated in Figs 9 and 10. Our energetic status model gave excellent results overall ($r^2 = 0.97$ for *P. nigra*, $r^2 = 0.94$ for hybrid aspen). No major pattern was detected in the residuals, although the model has the tendency to underestimate the observations (Figs S6, S7). Moreover, this model reproduced the following key features of the observations:

1. A decrease in isoprene emissions with increasing $C_a$.
2. An increase in isoprene emissions with increasing $PPFD$, with maxima inversely proportional to the CO$_2$ concentration.
3. An increase in the proportion of assimilated carbon diverted to isoprene production ($\text{Isol}A_{\text{gross}}$) with increasing $PPFD$.
4. A decrease in the quantum efficiency of isoprene production with increasing CO$_2$ concentration.

With $I_a$ adjusted for each experiment, G93 reproduces very well the observed variations in isoprene emission with $PPFD$, especially for hybrid aspen ($C_a$ experiments are not included for G93). For *P. nigra*, the bell-shaped pattern observed in the residuals vs fitted values plot (Fig. S6) suggests that the standard light response of G93 is not adapted to fit the observations.

With no empirical adjustment included to account for the CO$_2$ effect, the Niinemets model ($r^2 = 0.09–0.14$) failed to reproduce the observed variations in isoprene emission with $PPFD$ and $C_a$. Including a CO$_2$ effect in this model, however, caused major improvements ($r^2 = 0.97–0.89$).

combination of high isoprene emission rates and low carbon assimilation rates.

Our energetic status model can reproduce an increase in the fraction of carbon allocated to isoprene emission with increasing $PPFD$ (Figs 6, 7). It fails to reproduce absolute values of $\text{Isol}A_{\text{gross}}$; however, it should be noted that the simulated $\text{Isol}A_{\text{gross}}$ includes combined uncertainties of the isoprene model and the Farquhar model.

G93 shows versatility in the simulation of carbon allocated to isoprene emission, with simulated $\text{Isol}A_{\text{gross}}$ decreasing with $PPFD$ for *P. nigra*, but increasing for hybrid aspen.
Discussion

We used the $C_3$ and PPFD response curves of assimilation and isoprene emissions for *P. nigra* (this study) and *P. tremula* × *P. tremuloides* (hybrid aspen) (Sun et al., 2012), where changes in balance between electron supply and electron demand for carbon assimilation purposes were driven by different environmental variables. We tested against these data a new model in which isoprene production is a function of the energetic status of the leaves, alongside two widely used isoprene models: the G93 algorithm (Guenther et al., 1993) and the Niinemets model (Niinemets et al., 1999; Arneth et al., 2007). The new model showed excellent results and a visible improvement relative to the original Niinemets model (Figs 9, 10).

Our model finds its origin in the Niinemets model based on the ‘energetic requirements for isoprene synthesis and leaf photosynthetic properties’. It keeps the major advantage of its simplicity and thus the evident potential for its use in large-scale modelling, where excessive complexity is to be avoided wherever possible. Yet, the new model diverges from its prototype in two fundamental ways. First, it links isoprene emission directly to the electron flux (J) rather than to light-limited assimilation. Second, it links isoprene emission to reductant availability, and thus transcribes the original idea of Niinemets et al. (1999) of a ‘competition for electrons between isoprene synthesis and Calvin and photosynthetic cycles’. The component of electron flux generated by Photosystem II and not used for carbon assimilation and photorespiration is extremely hard to investigate experimentally (Singsaas et al., 2001). Nevertheless, our hypothesis is supported by the following: the high positive correlations found between the observations and simulations made with our energetic status model; the fact that measured Iso/$A_{gross}$ increases with increasing PPFD; the fact that observed $\Phi_{iso}$ is inversely proportional to $C_3$; strong linearity between the flux of electrons engaged in isoprene production and $[J - J_s]$; and a quadratic type of response of isoprene emission to $J$.

In fact, the first derivation of the model of Niinemets et al. (1999) and Niinemets (2004) predicted that the fraction of
electrons channelled into isoprene synthesis varies with CO\textsubscript{2} concentration, but this variation was not explicitly formalized. In the later development of this model, Arneth \textit{et al.} (2007) included this effect empirically in the emission model. Nevertheless, the reduction in isoprene emissions at intercellular CO\textsubscript{2} concentrations between 0 and 150 \textmu mol mol\textsuperscript{-1} (Loreto \& Sharkey, 1990; Rasulov \textit{et al.}, 2009b, 2011; Sun \textit{et al.}, 2012) was not considered. Wilkinson \textit{et al.} (2009) also included the CO\textsubscript{2} dependence of isoprene emission, but did not consider the declining part of the isoprene emission at low CO\textsubscript{2} concentration.

**Fig. 7** Ratios of isoprene emission to gross assimilation (A\textsubscript{gross}) vs photosynthetic photon flux density (PPFD) at a leaf temperature of 30°C for hybrid aspen (\textit{P. tremula} × \textit{P. tremuloides}) at an atmospheric CO\textsubscript{2} concentration of 380 \textmu mol mol\textsuperscript{-1} for individuals grown under (a) ambient and (b) elevated CO\textsubscript{2} concentration, and at an atmospheric CO\textsubscript{2} concentration of 780 \textmu mol mol\textsuperscript{-1} for individuals grown under (c) ambient and (d) elevated CO\textsubscript{2} concentration.

**Fig. 8** Quantum efficiencies for isoprene emission (\(\Phi_{iso}\)) modelled vs observed values at different atmospheric CO\textsubscript{2} concentrations for (a) \textit{Populus nigra} and (b) hybrid aspen (\textit{P. tremula} × \textit{P. tremuloides}) grown under CO\textsubscript{2} conditions of 380 \textmu mol mol\textsuperscript{-1} (closed circles) and 780 \textmu mol mol\textsuperscript{-1} (open circles). The solid line represents the best linear fit between the model and the data; the dashed line represents the 1 : 1 line.

**Fig. 9** \textit{Populus nigra} modelled isoprene emission rates vs observed isoprene emission rates for all the experiments. The solid line represents the best linear fit between the model and the data; the black dashed line is the 1 : 1 line. Closed circles represent our energetic status model. Squares represent the G93 algorithm, without (open) and with (closed) an adjustment of the standard emission rate to account for CO\textsubscript{2} concentration effects. Triangles represent the Niinemets model, without (open) and with (closed) a CO\textsubscript{2} effect based on Arneth \textit{et al.} (2007). Only experiments varying the photosynthetic photon flux density are represented for G93 with adjustment of the standard emission rate to account for CO\textsubscript{2} concentration effects.
concentrations. It has been shown that this reduction is associated with reduced availability of DMADP and is suggested to indicate limited NADPH or ATP availability (Rasulov et al., 2009b, 2011). Here, the model based on NADPH limitation described well the entire CO₂ response curve (Figs 2, 3), in line with the experimental observations of the variation of DMADP pool size with [CO₂].

A limitation of the present study is that experiments were conducted under constant temperature. This has the advantage of decoupling effects related to NADPH production from effects of enzyme kinetics. However, isoprene emissions also respond strongly to temperature, both instantaneously and over longer periods (Guenther et al., 1991; Pacifico et al., 2009; Laffineur et al., 2011; Sun et al., 2013a). Therefore, an improved understanding of the controls on isoprene emission for global or regional modelling purposes also requires that the hypothesis presented here be tested and analysed under variations of temperature, as well as PPFD and Cᵢ.

Following the logic of the G93 algorithm, many studies (including ours) have examined isoprene emission under the standard conditions of a leaf temperature of 30°C and a PPFD of 1000 μmol m⁻² s⁻¹. This might be a limitation, as interactions between different drivers are then neglected. As an example of the importance of this limitation, the recent study of Sun et al. (2013a) showed cancellation of the isoprene response to rapid changes in Cᵢ at higher temperature. Thus, there is a need for more complete experimental studies focusing on the interactions between the effects of simultaneous changes in temperature, PPFD and Cᵢ.

In future model development, it will also be important to consider the adaptation of model parameters to long-term variations in temperature and CO₂, and effects of changes caused by leaf ontogeny – all of which could modify the expression of the IspS gene (Monson, 2013; Rajabi Memari et al., 2013; Rosenkrantz & Schnitzler, 2013) and the pool size of DMADP (Sun et al., 2012; Rasulov et al., 2013). The consideration of such changes is needed to allow the inclusion of acclimation in isoprene emission on time scales from days to months, and thus eventually to allow the responses of isoprene emissions to global change to be modelled in a more explicitly process-based manner than has been possible so far.

Acknowledgements

The research leading to these results has received funding from the European Community’s Seventh Framework Programme (FP7 2007-2013) under grant agreement no. 238366, and from the Estonian Ministry of Science and Education (institutional grant IUT-8-3) and Estonian Science Foundation (Grant 9253).

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Fig. S2 Isoprene emissions of *Populus nigra* vs photosynthetic photon flux density (PPFD).

Fig. S3 Assimilation curves of *Populus nigra*.

Fig. S4 Isoprene response to changes in internal CO₂ concentration (Ci) using the Niinemets model modified by Arneth *et al.* (2007).

Fig. S5 Observed isoprene emission rates vs light-limited electron flux (J) for hybrid aspen.

Fig. S6 Supplementary statistics for *Populus nigra*.

Fig. S7 Supplementary statistics for hybrid aspen.

Table S1 Isoprene emissions vs changes in photosynthetic photon flux density (PPFD) at different CO₂ concentrations

Table S2 Description and values of the parameters of the Farquhar model used in standard simulations

Notes S1 Comparison of the new energetic status model with the conceptual model from Morfopoulos *et al.* (2013).

Notes S2 Additional details on the model of photosynthetic carbon assimilation.

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