Hemp (Cannabis sativa L.) leaf photosynthesis in relation to nitrogen content and temperature: implications for hemp as a bio-economically sustainable crop

KAILEI TANG1,2, PAUL C. STRUIK1, STEFANO AMADUCCI2, TJEERD-JAN STOMPH1 and XINYOU YIN1

1Centre for Crop Systems Analysis, Department of Plant Sciences, Wageningen University & Research, PO Box 430, Wageningen, 6700 AK, The Netherlands, 2Department of Sustainable Crop Production, Università Cattolica del Sacro Cuore, via Emilia Parmense 84, Piacenza, 29122, Italy

Abstract

Hemp (Cannabis sativa L.) may be a suitable crop for the bio-economy as it requires low inputs while producing a high and valuable biomass yield. With the aim of understanding the physiological basis of hemp’s high resource-use efficiency and yield potential, photosynthesis was analysed on leaves exposed to a range of nitrogen and temperature levels. Light-saturated net photosynthesis rate ($A_{\text{max}}$) increased with an increase in leaf nitrogen up to 31.2 ± 1.9 μmol m$^{-2}$ s$^{-1}$ at 25 °C. The $A_{\text{max}}$ initially increased with an increase in leaf temperature ($T_L$), levelled off at 25–35 °C and decreased when $T_L$ became higher than 35 °C. Based on a C3 leaf photosynthesis model, we estimated mesophyll conductance ($g_m$), efficiency of converting incident irradiance into linear electron transport under limiting light ($\jmath_{\text{2LL}}$), linear electron transport capacity ($J_{\text{max}}$), Rubisco carboxylation capacity ($V_{\text{cmax}}$), triose phosphate utilization capacity ($T_p$) and day respiration ($R_d$), using data obtained from gas exchange and chlorophyll fluorescence measurements at different leaf positions and various levels of incident irradiance, CO$\text{}_2$ and O$\text{}_2$. The effects of leaf nitrogen and temperature on photosynthesis parameters were consistent at different leaf positions and among different growth environments except for $\jmath_{\text{2LL}}$, which was higher for plants grown in the glasshouse than for those grown outdoors. Model analysis showed that compared with cotton and kenaf, hemp has higher photosynthetic capacity when leaf nitrogen is < 2.0 g N m$^{-2}$. The high photosynthetic capacity measured in this study, especially at low nitrogen level, provides additional evidence that hemp can be grown as a sustainable bioenergy crop over a wide range of climatic and agronomic conditions.

Keywords: Hemp (Cannabis sativa L.), model, nitrogen, photosynthesis, sustainable crop, temperature

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Introduction

The multiple societal challenges such as climate change, natural resource scarcity and environmental pollution have fuelled interest in bio-economy (Jordan et al., 2007). Previous comprehensive research programmes indicated that hemp (Cannabis sativa L.) fits well in the concept of bio-economy (McCormick & Kautto, 2013; Amaducci et al., 2015). Hemp has the potential to produce up to 27 Mg ha$^{-1}$ biomass yield (Tang et al., 2016) at relatively low inputs (Struik et al., 2000; Amaducci et al., 2002) and has a positive impact on the environment (Bouloc & Van der Werf, 2013; Barth & Carus, 2015). Its stem contains high-quality cellulose (De Meijer & Van der Werf, 1994), the seeds contain high-quality oil (Oomah et al., 2002), and the inflorescence contains valuable resins (Bertoli et al., 2010). From specialty pulp and paper to nutritional food, medicine and cosmetics, there are as many as 50 000 uses claimed for hemp products derived from its stem, seed and inflorescence (Carus et al., 2013; Carus & Sarmento, 2016). Recent research demonstrated that hemp is also a suitable feedstock for bioenergy production (Rice, 2008; Kreuger et al., 2011; Prade et al., 2011).

Although once an important crop for the production of textiles and ropes, hemp has not been subjected to the intensive research that has driven great improvements in major crops in the last 50 years (Amaducci et al., 2015; Salentijn et al., 2015) due to the continuous decrease in hemp acreage after the Second World War and its slow revival in the last couple of decades.
(Wirthshafter, 2004; Allegret, 2013). To advance research needed to consolidate and expand the market of hemp renewable materials, within the frame of the EU funded project MultiHemp (www.multihemp.eu), it was proposed to develop a process-based hemp growth model similar to the successful models for major staple crops (Bouman et al., 2007). With the aim of understanding the physiological basis of hemp’s high resource-use efficiency and yield potential using a modelling approach, this study focuses on analysing leaf photosynthesis of hemp as a primary source of biomass production.

Very few studies report on leaf photosynthesis of hemp. De Meijer et al. (1995) reported a light-saturated rate of leaf photosynthesis for hemp of 30 kg CO$_2$ ha$^{-1}$ h$^{-1}$ (equivalent to 19 µmol m$^{-2}$ s$^{-1}$) under field conditions. Chandra et al. (2008, 2011a,b, 2015) showed the response of leaf photosynthesis of hemp to irradiance intensity, CO$_2$ concentration and temperature by measuring gas exchange of leaves from greenhouse-grown plants. Marija et al. (2011) found that nitrogen fertilization significantly affected different aspects of photosynthetic photochemistry, as shown by chlorophyll $a$ fluorescence analysis. To the best of our knowledge, a comprehensive analysis of the relation between leaf nitrogen status and photosynthesis rate is not yet available for hemp.

Leaf photosynthesis rate depends on both nitrogen nutrition status and environmental conditions (Sinclair & Horie, 1989). Thanks to a thorough understanding of the biochemical mechanisms of leaf photosynthesis, the response of leaf photosynthesis to irradiance intensity and CO$_2$ concentration can be modelled (Farquhar et al., 1980; Yin et al., 2006; Von Caemmerer et al., 2009). Such a model dissects net leaf photosynthesis into mesophyll conductance ($g_m$), linear electron transport capacity ($J_{\text{max}}$), RuBisCo carboxylation capacity ($V_{\text{cmax}}$), triose phosphate utilization capacity ($T_p$) and day respiration ($R_d$). The effects of leaf nitrogen status and temperature on leaf photosynthesis are considered through their effects on these photosynthetic parameters (Hikosaka et al., 2016). Experimental protocols for parameterizing the biochemical photosynthesis model have been well documented (Sharkey et al., 2007; Yin et al., 2009; Bellasio et al., 2015), and the model has been successfully embedded as a submodel in process-based crop growth models for upscaling to canopy photosynthesis and crop production (Yin & Struik, 2009), such as the GECROS crop model (Yin & Van Laar, 2005). Therefore, parameterizing the photosynthesis model for hemp is an excellent opportunity to understand its photosynthetic resource-use efficiency, as well as to provide essential information for modelling hemp growth.

The first objective of this study was to analyse leaf photosynthesis of hemp as affected by irradiance intensity, CO$_2$ concentration, temperature and nitrogen status. Secondly, this study aimed to parameterize a widely used $C_3$ leaf photosynthesis model (Farquhar et al., 1980; Yin et al., 2006) for hemp. In the final section, the photosynthetic capacity of hemp is compared with that of two other bio-economic crops, cotton (Gossypium hirsutum L.) and kenaf (Hibiscus cannabinus L.), using a modelling method. Cotton and kenaf were chosen because they are bio-economically important crops and, in particular, kenaf is considered as an alternative for hemp in tropical and subtropical climates (Lips & van Dam, 2013; Patanè & Cosentino, 2013; Alexopoulou et al., 2015).

Materials and methods

Plant growth and data collection

Three independent experiments were carried out at the research facilities of the Università Cattolica del Sacro Cuore (45.0°N, 9.8°E, 60 m asl; Piacenza, Italy). Seeds of hemp (cv. Futura 75) were received from the Fédération National des Producteurs de Chanvre, Le Mans, France. The plants were grown outdoors in 2013 and 2014 and in a glasshouse in 2015.

An experiment on the effect of nitrogen on leaf photosynthetic capacity (N-trial)

Seeds were sown in 18 containers (40 × 40 × 30 cm$^3$) placed outdoors on 9 May 2014. Each container was filled with 23 kg of soil (dry weight) that contained 0.22% total nitrogen and had a clay–silt–sand ratio of 30:43:27. After seedling emergence, the plants were hand-thinned to 18 plants per container and three levels of urea fertilization were applied (0, 1.0 and 2.0 g N per container, respectively). There were six containers for each fertilization level. Other nutrients (e.g. phosphate and potassium) were assumed not limiting factors according to historic experience in the field from which the soil was collected. The same applies to the other two trials. During plant growth, all containers were positioned randomly and tightly in one block surrounded by a green shading net (transmitting 3% of the light). The net height was adjusted daily according to the increment of plant height. The plants were well watered during the entire experiment. The daily temperature and global radiation during the growth period are presented in Fig. S1.

Photosynthetic measurements were started on 46 days after sowing (the 6th–8th pair of leaves had appeared) in a growth chamber with the temperature set at 25 °C. The container was moved into the growth chamber 2 hrs before measurements. On one representative plant in each container, the middle leaflets of the youngest, fully expanded top leaf and of the middle leaf (i.e. two nodes below the top leaf) were measured. Simultaneous gas exchange (GE) and chlorophyll fluorescence (CF) measurements were implemented in situ using a portable open gas exchange system with a 1.7-cm$^2$ clamp-on leaf chamber (CIRAS-2, PP Systems international, Inc., Amesbury, MA, USA).
combining with FMS2 (Hansatech Instruments Ltd, King’s Lynn, Norfolk, UK). The system set-up of the combined CIRAS-2 and FMS2 for performing simultaneous GE and CF measurements was implemented according to the instructions provided by PP Systems International, Inc., USA. Light response curve of net photosynthesis rate (A) (A-\(I_{\text{inc}}\)) and its CO\(_2\) response curve (A-C\(_{\text{a}}\)) were assessed for each leaf under ambient O\(_2\) (i.e. 21%) conditions. The A-\(I_{\text{inc}}\) curves were assessed by decreasing incident light intensity (\(I_{\text{inc}}\)) as 2000, 1500, 1000, 500, 300, 200, 150, 100, 60 and 30 \(\mu\)mol m\(^{-2}\) s\(^{-1}\); while keeping leaf chamber CO\(_2\) concentration (C\(_a\)) at 400 \(\mu\)mol mol\(^{-1}\). At the end of assessing the A-\(I_{\text{inc}}\) curve, the light source was turned off for 15 min to measure leaf respiration in darkness (\(R_{\text{dark}}\)). The A-C\(_{\text{a}}\) curves were assessed by changing C\(_a\) as 400, 250, 150, 80, 70, 60, 50, 400, 400, 600, 800, 1000 and 1500 \(\mu\)mol mol\(^{-1}\); while keeping \(I_{\text{inc}}\) at 1000 \(\mu\)mol m\(^{-2}\) s\(^{-1}\). Leaf temperature (\(T_L\)) and vapour pressure of supplying air during measurements were set constant at 25 °C and 2 kPa, respectively. The response curves were started when the leaf had adapted to the condition at the first \(I_{\text{inc}}\) or C\(_a\) level for 30 min. Data were recorded programmatically with 2-min interval for A-\(I_{\text{inc}}\) curves and 3-min interval for A-C\(_{\text{a}}\) curves. Premeasurements indicated these time intervals were sufficiently long for A to reach a steady state. Three plants were measured for each fertilization level.

To obtain a calibration factor that can properly convert fluence-based PSII efficiency into linear electron transport rate, parts of A-\(I_{\text{inc}}\) and A-C\(_{\text{a}}\) curves were also assessed under 2% O\(_2\). This condition was realized by supplying the CIRAS-2 with a humidified mixture of 2% O\(_2\) and 98% N\(_2\). To avoid O\(_2\) leakage, the air-in pump in the CIRAS-2 was replaced by a sealed one according to the manufacturer’s instruction. The curves for 2% O\(_2\) were assessed in accordance with the ones for ambient O\(_2\), but the A-\(I_{\text{inc}}\) curves were only assessed at \(I_{\text{inc}}\) ≤150 \(\mu\)mol m\(^{-2}\) s\(^{-1}\) and the A-C\(_{\text{a}}\) curves were only assessed at C\(_a\) ≥600 \(\mu\)mol mol\(^{-1}\). These particular \(I_{\text{inc}}\) and C\(_a\) conditions are required for obtaining the calibration factor (Yin et al., 2009), that is to ensure that A is limited by electron transport.

When the photosynthetic measurements were completed, SPAD, a proxy for chlorophyll concentration, was measured using a SPAD-502 (Minolta, Japan). Leaf area was determined from scans using IMAGEJ (version 1.49; https://imagej.nih.gov/). Dry weight was measured after drying at 75 °C until constant weight. Total leaf nitrogen concentration was analysed using a CN analyser (Vario Max CN Analyzer; Elementar Americas, Inc., Hanau, Germany). Specific leaf nitrogen (SLN; g N m\(^{-2}\)) was calculated for each measured leaf using the leaf dry weight, leaf area and nitrogen concentration. CO\(_2\) leakage of the CIRAS-2 was assessed by performing A-C\(_{\text{a}}\) curves on three heat-killed leaves. Based on these measurements, values of A and the intercellular CO\(_2\) concentration (C\(_i\)) of A-C\(_{\text{a}}\) curves were recalculated using the CIRAS-2 built-in formulae.

**An experiment on leaf photosynthesis in response to fluctuating temperature under different leaf nitrogen levels (TN-trial)**

Seeds were sown in 18 containers (60 × 20 × 18 cm\(^3\)) placed outdoor on 5 August 2013. Each container was filled with 10 kg of soil that contained 0.11% of total nitrogen and had a clay–silt–sand ratio of 15:22:63. After seedling emergence, the plants were hand-thinned to 10 plants per container and three levels of urea fertilization were applied (0, 0.78 and 1.95 g N per container, respectively). Each fertilization level had six containers. The plants were well watered during growth. Because of very late sowing, a halogen lamp (54 Watt) that was mounted at 50 cm from the top of canopy was turned on for 16 hrs per day to prevent plants from flowering. The daily temperature and radiation during the growth period are presented in Fig. S1.

Starting on 50 days after sowing (the 8th – 10th pair of leaves had appeared), GE measurements were conducted outdoors on three representative plants for each nitrogen level. A-\(I_{\text{inc}}\) and A-C\(_{\text{a}}\) curves were assessed on the middle leaflet of the youngest, fully expanded leaf. The levels of light for the A-\(I_{\text{inc}}\) curves were identical to those in the N-trial under ambient O\(_2\), while the A-C\(_{\text{a}}\) curves were assessed by increasing C\(_a\) as: 50, 60, 70, 80, 150, 250, 400, 650, 1000 and 1500 \(\mu\)mol mol\(^{-1}\) while keeping \(I_{\text{inc}}\) at 1000 \(\mu\)mol m\(^{-2}\) s\(^{-1}\). During measurement, \(T_L\) and vapour pressure were set at 5 min for A-\(I_{\text{inc}}\) and ~ 5 min for A-C\(_{\text{a}}\). SPAD, SLN and gas leakage were analysed using the procedures described for the N-trial.

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Model analysis

The photosynthesis model of Farquhar et al. (1980) coupled with CO₂ diffusion model, as described in Yin & Struik (2009), was used in this study.

Modelling net leaf photosynthesis rate at the carboxylation sites of Rubisco

The net leaf photosynthesis rate \( (A, \mu \text{mol m}^{-2} \text{s}^{-1}) \) was modelled as the minimum of the Rubisco-limited rate \( (A_{c}) \), the electron transport-limited rate \( (A_{l}) \) and the triose phosphate utilization-limited rate \( (A_{p}) \):

\[
A = \min(A_{c}, A_{l}, A_{p})
\]  

(1)

\( A_{c} \) is described, following the Michaelis–Menten kinetics, as:

\[
A_{c} = \frac{(C_{c} - \Gamma)^{+}\text{V}_{\text{max}}}{C_{c} + K_{\text{mc}}(1 + O/K_{\text{mao}})} - R_{d}
\]  

(2)

where \( C_{c} \) (mol⁻¹) and \( O \) (mol mol⁻¹) are the CO₂ and O₂ levels at the carboxylation sites of Rubisco; \( \text{V}_{\text{max}} \) (mol m⁻² s⁻¹) is the maximum rate of carboxylation; \( K_{\text{mc}} \) (mol⁻¹) and \( K_{\text{mao}} \) (mol⁻¹) are Michaelis–Menten constants of Rubisco for CO₂ and O₂, respectively; \( R_{d} \) (mol m⁻² s⁻¹) is the day respiration (respiratory CO₂ release other than by photorespiration); and \( \Gamma^{+} \) (mol⁻¹) is the CO₂ compensation point in the absence of \( R_{d} \).

\( A_{l} \) is described as:

\[
A_{l} = \frac{(C_{c} - \Gamma)^{+}J}{4C_{c} + 8\Gamma^{+}R_{d}}
\]  

(3)

where \( J \) (mol m⁻² s⁻¹) is the potential linear e⁻ transport rate that is used for CO₂ fixation and photorespiration, and it is described as:

\[
J = \frac{\kappa_{\text{LL}}L_{\text{inc}} + J_{\text{max}} - \sqrt{(\kappa_{\text{LL}}L_{\text{inc}} + J_{\text{max}})^{2} - 4J_{\text{max}}\kappa_{\text{LL}}L_{\text{inc}}}}{20}
\]  

(4)

where \( J_{\text{max}} \) (mol m⁻² s⁻¹) is the maximum value of \( J \) under saturated light; \( L_{\text{inc}} \) is the incident light (mol m⁻² s⁻¹); \( \kappa_{\text{LL}} \) (mol⁻¹) is the conversion efficiency of incident light into \( J \) at strictly limiting light; and \( \theta \) (dimensionless) is convexity factor for the response of \( J \) to \( L_{\text{inc}} \).

\( A_{p} \) is described as:

\[
A_{p} = 3T_{p} - R_{d}
\]  

(5)

where \( T_{p} \) (mol m⁻² s⁻¹) is the rate of triose phosphate export from the chloroplast.

The \( T_{l} \) response of \( R_{d} \), \( T_{p} \) and kinetic properties of Rubisco (involving \( \text{V}_{\text{max}}, K_{\text{mc}}, K_{\text{mao}} \) and \( \Gamma^{+} \)) are described using an Arrhenius function normalized with respect to their values at 25 °C (Eqn 6) while the response of \( J_{\text{max}} \) is described using a peaked Arrhenius function (Eqn 7):

\[
X = X_{298}\exp\left[\frac{E_{c}(T_{l} - 25)}{298R(T_{l} + 273)}\right]
\]  

(6)

\[
X = X_{298}\exp\left[\frac{E_{c}(T_{l} - 25)}{298R(T_{l} + 273)}\right]\left[1 + \exp\left(\frac{298S - D_{c}}{T_{l} + 273S - D_{c}}\right)\right]
\]  

(7)

where \( X_{298} \) is the value of each parameter at 25 °C (i.e. \( R_{d}, \text{V}_{\text{max}}, K_{\text{mc}}, K_{\text{mao}}, \Gamma^{+} \) and \( \text{I}_{\text{max}} \)); \( E_{c} \) and \( D_{c} \) are the energies of activation and deactivation (i.e. \( E_{\text{Kmc}}, E_{\text{Kmao}}, E_{\text{Kinc}}, E_{\text{TP}}, E_{\Gamma^{+}}, \text{I}_{\text{max}} \) and \( D_{\text{I}_{\text{max}}} \); all in J mol⁻¹); \( S_{t} \) is the entropy term (\( S_{\text{max}} \) in J K⁻¹ mol⁻¹); and \( R \) is the universal gas constant (8.314 J K⁻¹ mol⁻¹).

Modelling mesophyll conductance for CO₂

The CO₂ concentration at intercellular space (\( C_{i} \)) was taken from gas exchange measurement whereas the estimation of \( C_{i} \) relies on proper estimation of mesophyll conductance (\( g_{m} \)). \( g_{m} \) calculated by the variable \( J \) method (Harley et al., 1992a), appeared to vary with CO₂ and irradiance levels (see section Result). Whether or not \( g_{m} \) varies with CO₂ and irradiance levels is debatable (Flexas et al., 2007, 2012). We used the model of Yin et al. (2009) that is able to deal with both constant and variable \( g_{m} \) models, and have a similar form as Eqn (8):

\[
g_{m} = g_{m0} + \delta(A + R_{d})
\]  

(8)

where \( g_{m0} \) (mol m⁻² s⁻¹) is the minimum \( g_{m} \) if irradiance approaches zero; parameter \( \delta \) (dimensionless) in this model defines the \( C_{c} : C_{i} \) ratio at saturating light as \( (C_{c} - \Gamma^{+})/(C_{i} - \Gamma^{+}) = 1/(1 + \delta) \). Any positive value of \( \delta \) predicts a variable \( g_{m} \) pattern in response to \( C_{c} \) and \( I_{\text{inc}} \), and a higher \( \delta \) implies higher \( g_{m} \) and therefore a higher \( C_{c} : C_{i} \) ratio. If \( \delta = 0 \), Eqn (8) predicts an independence of \( g_{m} \) on \( C_{i} \) and \( I_{\text{inc}} \) (i.e. \( g_{m} = g_{m0} \)), equivalent to the constant-\( g_{m} \) model.

Model parameterization and validation

The data collected in the N-trial was used to assess the effect of leaf nitrogen on the values of model parameters at 25 °C. The data collected in the T-trial were used to assess the effect of leaf temperature on the values of (peaked) Arrhenius model parameters. The parameterized model was validated against the data collected in the TN-trial. In the model, Rubisco kinetic property-related parameters (i.e. \( K_{\text{mc}}, K_{\text{mao}} \) and \( \Gamma^{+} \)) and \( \theta \), convexity factor for the response of \( J \) to \( L_{\text{inc}} \), are conserved among \( C_{j} \) species (von Caemmerer et al., 2009). Thus, the value of \( \theta \) was set to 0.7 (Ögren & Evans, 1993); the values of \( K_{\text{mc}}, K_{\text{mao}} \) and \( \Gamma^{+} \) at 25 °C were set to 272 μmol mol⁻¹, 165 mmol mol⁻¹ and 37.5 μmol mol⁻¹ (at 21% O₂), respectively (Bernacchi et al., 2002). The energies of activation \( E_{\text{Kmc}}, E_{\text{Kmao}}, \) and \( E_{\Gamma^{+}} \) were adapted from the values of Bernacchi et al. (2002) as \( E_{\text{Kmc}} = 80990 \) J mol⁻¹, \( E_{\text{Kmao}} = 23720 \) J mol⁻¹ and \( E_{\Gamma^{+}} = 24460 \) J mol⁻¹.

Model parameterization with data collected in the N-trial: nitrogen effect

The stepwise parameterizing procedures described by Yin et al. (2009) were adapted in this study. Specifically:

Step 1: Estimating electron transport parameters (\( \text{I}_{\text{max}} \) and \( \kappa_{\text{LL}} \)) and \( R_{d} \)
According to Yin et al. (2009), the observed $A_i$ under non-photorespiratory conditions can be expressed using Eqn (9):

$$A_i = \frac{slinc\Phi_2}{4} - R_d$$

(9)

$$s = \frac{\beta(1 - f_{\text{pseudo(b)}})}{1 - f_{\text{cyt}}}$$

(9a)

where $s$ is a lumped parameter; $\Phi_2$ is PSII operating efficiency, usually assessed from the chlorophyll fluorescence measurements, indicating quantum efficiency of PSII $e^-$ flow on PSII-absorbed light basis; $\beta$ is leaf absorbance; $f_{\text{cyt}}$ is proportion of absorbed $l_{\text{inc}}$ partitioned to PSII; and $f_{\text{pseudo(a)}}$ and $f_{\text{pseudo(b)}}$ are the fraction of cyclic and basal pseudocyclic electron transport, respectively. Thus, a simple linear regression can be performed for the observed $A$ against $(l_{\text{inc}}\Phi_2/4)$ using data of the $e^-$ transport-limited range under nonphotorespiratory conditions (measurements conducted at 2% $O_2$). The slope of the regression yields an estimate of the calibration factor $s$, and the intercept gives an estimate of $R_d$ under 2% $O_2$ condition. The estimated $s$ allowed the conversion of CF-based PSII operating efficiency into the actual rate of linear electron transport as:

$$J = slinc\Phi_2$$

(10)

Thus, $J_{\text{max}}$ and $\kappa_{2\text{LLI}}$ can be estimated from fitting Eqn (4) to the values of $J$.

The same linear regression for the observed $A$ against $(l_{\text{inc}}\Phi_2/4)$ using data of the $e^-$ transport-limited range may be applied as well to photorespiratory conditions (i.e. ambient $O_2$) for estimating $R_d$ although the slight variation in $C_i$ with $l_{\text{inc}}$ can have bearing under these conditions (Yin et al., 2009, 2011).

Step 2: Parameterization of the $g_{\text{sm}}$ model and $V_{\text{cmax}}$ and $T_p$  
Combining Eqn (8) with Eqn (2) and Eqn (3), and replacing $C_e$ with $(C_i - A/\delta_{\text{sm}})$ yields (Yin et al., 2009):

$$A_i = \frac{-b - \sqrt{b^2 - 4ac}}{2a}$$

(11)

where

$$a = x_2 + \Gamma^* + \delta(C_i + x_2)$$

$$b = \left\{ (x_2 + \Gamma^*) (x_1 - R_d) + (C_i + x_2)[g_{\text{sm}}(x_2 + \Gamma^*) + \delta(x_1 - R_d)] + \delta[x_1(C_i - \Gamma^*) - R_d(C_i + x_2)] \right\}$$

$$c = [g_{\text{sm}}(x_2 + \Gamma^*) + \delta(x_1 - R_d)] [x_1(C_i - \Gamma^*) - R_d(C_i + x_2)]$$

with $x_1 = \left\{ \begin{array}{ll} V_{\text{cmax}} & \text{for} A_c \\ \frac{1}{4} & \text{for} A_i \end{array} \right.$

and $x_2 = \left\{ \begin{array}{ll} \frac{K_{\text{m}}(1 + \frac{J}{K_{\text{m}}})}{2\Gamma^*} & \text{for} A_c \\ \frac{1}{2\Gamma^*} & \text{for} A_i \end{array} \right.$

Thus, $V_{\text{cmax}}, T_p$ and $\delta$ (or $g_{\text{sm}}$) can be estimated simultaneously by fitting Eqn (1), Eqn (4), Eqn (5) and Eqn (11) to $A_{\text{inc}}$ and $A_{-\text{C}_i}$ using pre-estimated $J_{\text{max}}, \kappa_{2\text{LLI}}$ and $R_d$ as input.

As it is uncertain if $g_{\text{sm}}$ varies with CO$_2$ and irradiance levels, $g_{\text{sm}}$ was first assessed according to the $f$ method (Harley et al., 1992a):

$$g_{\text{sm}} = \frac{A}{C_i - \frac{E_{\text{gRL}}(1 + K_{\text{R}})\Gamma^*}{f_{\text{cyt}}(A + R_d)}}$$

(12)

where $A$ and $C_i$ were taken from gas exchange measurements and $J$ was calculated by Eqn (10). If $g_{\text{sm}}$ does vary in response to changing $C_i$ and $I_{\text{inc}}$, we could fit only $\delta$ by fixing $g_{\text{sm}}$ to 0 (Yin et al., 2009). In such a case, $g_{\text{sm}}$ can be calculated as:

$$g_{\text{sm}} = \frac{A + \delta(A + R_d)}{C_i - \Gamma^*}$$

(13)

Model parameterization with data collected in the T-trial: temperature effect

By assuming the value of $\delta$ is independent of leaf temperature, the values of $J_{\text{max}}, \kappa_{2\text{LLI}}, v_{\text{cmax}}$ and $T_p$ at each leaf temperature were solved from Eqn (1), Eqn (4), Eqn (5) and Eqn (11) by simultaneously fitting $A_{\text{inc}}$ and $A_{-\text{C}_i}$ curves. Subsequently, the parameter values at different $T_L$ were fitted to either Eqn (6) for estimating $E_{\text{RLD}}, E_{\text{Vcmax}}, E_{\text{TPR}}$, or Eqn (7) for estimating $E_{\text{Imax}}$, $D_{\text{Vcmax}}$, $D_{\text{TPR}}$ and $S_{\text{Imax}}$

Model validation

The parameterized model was validated against the data obtained in the TN-trial. The model parameters $R_d, J_{\text{max}}, v_{\text{cmax}}$ and $T_p$ at 25 $^\circ$C were derived from their linear relationships with SLN (see section Result), and the effect of $T_L$ on the values of these parameters was quantified through Eqn (6) or Eqn (7) with the estimated $E_{\text{RLD}}, E_{\text{Vcmax}}, E_{\text{TPR}}, E_{\text{Imax}}$, $D_{\text{Vcmax}}, D_{\text{TPR}}$ and $S_{\text{Imax}}$

Comparison of hemp leaf photosynthetic competence with that of cotton and kenaf

To illustrate the leaf photosynthetic competence of hemp in comparison with cotton and kenaf, $A_{-C_i}, A_{-\text{I}_{\text{inc}}}, A_{-\text{C}_i}$ and $A_{-\text{SLN}}$ curves were constructed for hemp using the validated model while those of cotton and kenaf were constructed using the FvCB models and corresponding parameters reported in Harley et al. (1992b) for cotton (cv. Coker 315) and in Archontoulis et al. (2011) for kenaf (cv. Everglades 41).

Statistics

Simple linear regression was performed using Microsoft Excel. Nonlinear fitting was carried out using the GAUSS method in PROC NLIN of SAS (SAS Institute Inc., Cary, NC, USA). If parameters were proven independent from leaf nitrogen or temperature, the dummy variables method was used to estimate one common value (Yin et al., 2009). The goodness of fit was assessed by calculating the coefficient of determination ($r^2$) and the relative root mean square (rRMSE). The effect of leaf position on parameter values was tested by performing ANOVA test considering leaf nitrogen as covariance.
Results

Results of the N-trial: nitrogen-dependent photosynthetic capacity

Measurements to assess the effect of leaf nitrogen on leaf photosynthetic capacity of hemp (N-trial) were conducted on leaves having an average SLN of 0.87 g N m⁻², 1.25 g N m⁻² and 1.75 g N m⁻² at the top of the canopy, or 0.65 g N m⁻², 0.78 g N m⁻² and 1.22 g N m⁻² at the middle of the canopy, for the three N treatments, respectively. Examples of A-I inc and A-Ci curves at different SLN levels are shown in Fig. 1. The \( R_{d} \) (µmol m⁻² s⁻¹; leaf respiration in the dark) and light-saturated net photosynthesis rate (\( A_{\text{max}} \) measured at 2000 µmol m⁻² s⁻¹) increased linearly with increasing SLN, and these linear relationships did not differ between the top and middle leaves (Fig. 2).

Using the data of electron transport-limited range under nonphotorespiratory conditions (i.e. at 2% O₂, \( C_{i} \geq 600 \) µmol mol⁻¹ in the A-Ci curve and \( I_{\text{inc}} \leq 150 \) µmol m⁻² s⁻¹ in the A-I inc curve), parameter \( s \) was estimated as the slope of a linear regression of \( A \) against \( I_{\text{inc}} \) (Fig. 2a). The value of \( s \) was independent of SLN and canopy position (\( P > 0.05 \); see Fig. 2a). Thus, a common \( s (0.33 \pm 0.01) \) was estimated from pooled data. \( k_{2 LL} \) and \( I_{\text{max}} \) were estimated from fitting Eqn (4) to the data on calculated \( J \) from Eqn (10). A preliminary estimation indicated that \( k_{2 LL} \) was unlikely to change with SLN and canopy position (\( P > 0.01 \); Fig. 2b). Thus, a common \( k_{2 LL} (0.21 \pm 0.004 \) mol mol⁻¹) was estimated together with \( I_{\text{max}} \) using the dummy variable method. The \( I_{\text{max}} \) ranged from 116.1 µmol m⁻² s⁻¹ to 316.4 µmol m⁻² s⁻¹ and increased linearly with an increase in SLN at the rate of 132.9 µmol s⁻¹ (g N⁻¹) (Fig. 3a). The relationship between \( I_{\text{max}} \) and SLN was independent of canopy position (\( P > 0.05 \)).

The estimated \( R_{d} \) values at 21% O₂ were roughly in line with the ones at 2% O₂ (see Fig. S3). Although the latter were on average 25% lower, a test of covariance indicated that \( R_{d} \) did not differ significantly between the different O₂ levels (\( P = 0.17 \)). At 21% O₂, \( R_{d} \) ranged from 0.29 µmol m⁻² s⁻¹ to 1.61 µmol m⁻² s⁻¹, increasing linearly with SLN at a rate of 0.85 µmol s⁻¹ (g N⁻¹) (Fig. 3b). The \( R_{d} \)-SLN relationship did not differ much between the middle and top leaves (\( P > 0.05 \)).

The \( g_{m} \) calculated using the variable \( J \) method, Eqn (12), indicated that it varied with changing \( I_{\text{inc}} \) and \( C_{i} \) (Fig. 4a, b). A preliminary analysis indicated that the value of \( g_{m0} \) in Eqn (8) was close to zero. By fixing \( g_{m0} \) to zero, a common value of \( \delta (2.12 \pm 0.09) \) was estimated together with \( V_{c\text{max}} \) and \( T_{p} \) using the dummy variable method. With the estimated \( \delta \), Eqn (13) estimates that \( g_{m} \) changes with \( I_{\text{inc}} \) and \( C_{i} \) in a similar trend as observed for the \( g_{m} \) calculated using Eqn (12); the latter, however, was 38% lower (Fig. 4a, b), probably as a result that the variable \( J \) method assumes the limitation on photosynthesis by electron transport over the full range of A-I inc and A-Ci curves (Yin et al., 2009). The estimated \( g_{m} \) with Eqn (13) increases with an increase in SLN (Fig. 4c). The estimated \( V_{c\text{max}} \) ranged from 53.7 µmol m⁻² s⁻¹ to 163.2 µmol m⁻² s⁻¹ and increased linearly with an increase in SLN at the rate of 76.2 µmol s⁻¹ (g N⁻¹) (Fig. 3c). The estimated \( T_{p} \) ranged from 6.9 µmol m⁻² s⁻¹ to 11.5 µmol m⁻² s⁻¹ and increased linearly with an increase in SLN at the rate of 4.2 µmol s⁻¹ (g N⁻¹) (Fig. 3d). The effects of SLN on \( V_{c\text{max}} \) and \( T_{p} \) were independent of leaf position (\( P > 0.05 \)). With the estimated \( R_{d} \), \( k_{2 LL} \), \( I_{\text{max}} \), \( \delta \), \( V_{c\text{max}} \) and \( T_{p} \), the \( r^{2} \) and rRMSE of the model description of the measured \( A \) in the N-trial were 0.99 and 18.5%, respectively.

Fig. 1 The net leaf photosynthesis (\( A \)) in response to incident irradiance (\( I_{\text{inc}} \); Panel a) and intercellular CO₂ concentration (\( C_{i} \); Panel b) under different leaf nitrogen levels. Data presented were measured at 21% O₂ on the top leaves in the N-trial. N1, N2 and N3 correspond to nitrogen treatments, resulting in average specific leaf nitrogen values of 0.87 g N m⁻², 1.25 g N m⁻² and 1.75 g N m⁻², respectively. The bars indicate standard errors of the mean (\( n = 3 \)).

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3.2 value of parable with those derived from the N-trial (Fig. 3). The T served at different levels of J while the A N-trial (i.e. J but significantly higher than the value estimated in the J common method. The J increase in J levelled off at 25–35 °C higher than 35 °C. A preliminary analysis indicated that (while those in Panel c and Panel d were obtained in the T-trial while those in Panel c and Panel d were obtained in the T-trial. The bars in panels c and d indicate standard errors of the mean (n = 3).

Results of T-trial: temperature-dependent photosynthetic capacity

The R4k increased continuously from 0.9 μmol m⁻² s⁻¹ to 4.1 μmol m⁻² s⁻¹ at increasing T_L from 15 to 40 °C while the A max initially increased with increasing T_L, levelled off at 25–35 °C and decreased when T_L became higher than 35 °C (Fig. 2c, d).

The estimated R_d increased continuously with an increase in T_L, ranging from 0.3 μmol m⁻² s⁻¹ until 3.2 μmol m⁻² s⁻¹ (Fig. 5a). The k_2 LL, J_max, V_c max and T_p were estimated simultaneously by assuming δ = 2.12 (estimated in N-trial) at each T_L. With the constant δ, the model predicted that g_m changed with an increase in T_L following a similar trend as A_max (cf. Figs 2d and 4d). A preliminary analysis indicated that k_2 LL was conserved at different levels of T_L (P > 0.05; see Fig. S2c) but significantly higher than the value estimated in the N-trial (i.e. k_2 LL = 0.21 ± 0.004 mol mol⁻¹). Thus, a common k_2 LL (0.37 ± 0.01 mol mol⁻¹) was estimated together with J_max, V_c max and T_p using the dummy variable method. The J_max, V_c max and T_p at 25 °C were comparable with those derived from the N-trial (Fig. 3). The value of T_p increased consistently with an increase in T_L from 15 to 30 °C (Fig. 5d). When T_L was higher than 30 °C, the curve fitting failed to assess T_p properly because the triose phosphate utilization is not limited at such high temperatures (Sage & Kubien, 2007; Busch & Sage, 2016). Therefore, T_p limitation was excluded to estimate J_max and V_c max at 35 and 40 °C. The V_c max increased continuously at increasing T_L from 15 to 40 °C while the value of J_max peaked at 30–35 °C (Fig. 5b, c).

By fitting the R_d-T_L, V_c max-T_L and T_p-T_L to Eqn (6), the activation energies E_k T_d, E_v c max and E_T_p were estimated at 21634.8 ± 4085.5 J mol⁻¹, 63042.7 ± 1562.2 J mol⁻¹ and 34417.8 ± 5297.7 J mol⁻¹, respectively. By fitting J_max-T_L to Eqn (7), the values of E_J max, D_J max and S_j max were estimated at 67292.1 ± 35985.5 J mol⁻¹, 114701.0 ± 28709.6 J mol⁻¹ and 375.6 ± 82.3 J K⁻¹ mol⁻¹, respectively. With the estimated parameters, the model described well the response of A to changing I_inc and C_i at different T_L (r² = 0.94 and r RMSE = 24.1%).

Model validation

The measurements in the TN-trial were conducted on leaves with SLN ranging from 0.63 g N m⁻² to 1.44 g N m⁻². During the measurement, the T_L ranged from 21 to 33 °C, and VPD ranged from 0.61 kPa to 2.61 kPa.

The parameterized model was validated against the data obtained in the TN-trial. The measured A was overestimated with either the k_2 LL derived in the N-trial.
Fig. 4  Illustration of mesophyll conductance ($g_{\text{m}}$) in relation to changing incident irradiance ($I_{\text{inc}}$; Panel a), intercellular CO$_2$ concentration ($C_i$; Panel b), specific leaf nitrogen (SLN; Panel c) and leaf temperature ($T_L$; Panel d). In panels a and b, the data presented were obtained from the leaves at the middle of the canopy in the treatment without nitrogen fertilization in the N-trial; the open (○) and closed (●) circles were calculated using the variable $J$ method of Harley et al. (1992a) (see Eqn 12 in the text) and the method of Yin et al. (2009) (see Eqn 13 in the text), respectively. In Panel c, the data presented were obtained at $I_{\text{inc}} = 1000$ μmol m$^{-2}$ s$^{-1}$ and $C_a = 400$ μmol mol$^{-1}$ in the N-trial; the open (○) and closed (●) circles represent data obtained from leaves from the middle and the top of the canopy, respectively. In Panel d, the data presented were obtained at $I_{\text{inc}} = 1000$ μmol m$^{-2}$ s$^{-1}$ and $C_a = 400$ μmol mol$^{-1}$ in the T-trial; the bars indicate standard errors of the mean ($n = 3$). Note the differences in scale along the y-axes.
(\(k_{21L} = 0.21\ \text{mol}\ \text{mol}^{-1}\)) or in the T-trial (\(k_{21L} = 0.37\ \text{mol}\ \text{mol}^{-1}\)) (Fig. 6a, b). The rRMSE reduced significantly with decreasing value of \(k_{21L}\) until 0.13 mol mol\(^{-1}\) (Fig. 6c). Assuming \(k_{21L} = 0.13\ \text{mol}\ \text{mol}^{-1}\) for the TN-trial, the \(r^2\) and rRMSE were 0.94 and 26\%, respectively; the error of model prediction distributed evenly across measured SLN and \(T_L\) (see Fig. S4).

Leaf photosynthetic competence of hemp in comparison with kenaf and cotton

Comparison of leaf photosynthetic competence of hemp with kenaf and cotton is presented in Fig. 7. The values of the main parameters are summarized in Table 1. In this illustration, we considered the uncertainty in estimated values of parameters (i.e. \(R_d\), \(J_{\text{max}}\), \(V_{\text{cmax}}\) and \(T_p\)) for their linear relationships with SLN and nonlinear relationships with \(T_L\) (presented as the shaded area). The modelled values of \(A\) for hemp are shown using lower and upper bounds of 95\% confidence interval of these parameter values. Given that there was a large variation in the value of \(k_{21L}\) among different growth environments and each estimate of \(k_{21L}\) had a very small standard error (Table 1), the lower bounds were combined with \(k_{21L}\) of 0.21 mol mol\(^{-1}\) (derived from N-trial) while the upper bounds were combined with \(k_{21L}\) of 0.37 mol mol\(^{-1}\) (derived from T-trial).

For the response to \(C_i\), these three crops had similar \(A\) at the current atmosphere CO\(_2\) level (Fig. 7a). In case of a further increase in CO\(_2\) level in the future, kenaf may become more productive than hemp. For both crops, there was a large uncertainty in the responses of \(A\) to \(I_{\text{inc}}\) and \(T_L\) (Fig. 7b, c) because these curves are affected by the value of \(k_{21L}\). When using \(k_{21L}\) of 0.37 mol mol\(^{-1}\), a value close to that of healthy C\(_3\) leaves (presented as dashed black lines), the calculated \(A\) for hemp was similar to that for kenaf across different \(I_{\text{inc}}\) levels, but was slightly higher than for cotton at intermediate \(I_{\text{inc}}\). Reducing \(k_{21L}\) to 0.21 mol mol\(^{-1}\) (presented as solid black lines) resulted in a reduction of \(A\) under light limiting condition and in a reduction of the optimal temperature. For the response to leaf nitrogen, the leaf photosynthetic competence of hemp, including its 95\% confidence interval, was consistently higher than that of cotton and kenaf at SLN < 2.0 g N m\(^{-2}\), which is close to the maximum SLN measured in this study (Fig. 7d).

Discussion

Hemp is considered an ideal annual crop for the bioeconomy as it has the potential to produce a high multipurpose biomass yield while requiring little inputs (Finnan & Burke, 2013; Tang et al., 2016). However, very limited information is available on the physiological basis of hemp resource-use efficiency. With the aim of
understanding the response of leaf photosynthesis capacity of hemp to leaf nitrogen status and environmental factors and setting the basis for a hemp growth model, this study presents the results of extensive hemp leaf photosynthetic measurements and parameterization of a widely used photosynthesis model.
### Table 1  List of model parameters (± standard errors if available) of hemp, cotton and kenaf

| Parameter       | Unit                  | Hemp       | Cotton†    | Kenaf§     |
|-----------------|-----------------------|------------|------------|------------|
| Respiration     |                       |            |            |            |
| $R_d$-SLN       | Slope                 | $\mu$mol s$^{-1}$ (g N$)^{-1}$ | 0.85 ± 0.15 | 0‡         | 0.80       |
|                 | Intercept             | $\mu$mol m$^{-2}$ s$^{-1}$ | 0.03 ± 0.19 | 0.82‡       | −0.37      |
| $E_{Rd}$        | J mol$^{-1}$          | 21634 ± 4085 | 84450      | 83440      |
| e$^{-}$ transport parameters |                     |            |            |            |
| $J_{\text{max}}$-SLN | Slope                 | $\mu$mol s$^{-1}$ (g N$)^{-1}$ | 132.9 ± 14.6 | 98.1       | 122.1      |
|                 | Intercept             | $\mu$mol m$^{-2}$ s$^{-1}$ | 54.4 ± 18.8 | −4.6       | −47.6      |
| $E_{J\text{max}}$ | J mol$^{-1}$          | 67292 ± 35986 | 79500      | 28149      |
| $D_{J\text{max}}$ | J mol$^{-1}$          | 114701 ± 28710 | 210000     | 474614||
| $S_{J\text{max}}$ | J K$^{-1}$ mol$^{-1}$ | 375 ± 82    | 650        | 1482||
| $k_{2LL}$       | mol mol$^{-1}$        | 0.21 ± 0.004 (N-trial) | 0.24*      | 0.28       |
|                 |                       | 0.37 ± 0.01 (T-trial) |            |            |
| $\theta$        | -                     | 0.70*       | 0.83*      | 0.63       |
| Rubisco parameters |                     |            |            |            |
| $V_{c\text{max}}$-SLN | Slope                 | $\mu$mol s$^{-1}$ (g N$)^{-1}$ | 76.2 ± 9.8 | 60.0|| | 66.7|| |
|                 | Intercept             | $\mu$mol m$^{-2}$ s$^{-1}$ | 12.6 ± 12.5 | −9.6|| | 26.0|| |
| $E_{V_{c\text{max}}}$ | J mol$^{-1}$          | 63024 ± 1562 | 116300     | 61812      |
| TPU parameters  |                       |            |            |            |
| $T_{p}$-SLN     | Slope                 | $\mu$mol s$^{-1}$ (g N$)^{-1}$ | 4.2 ± 0.4 | 5.1|| | NA         |
|                 | Intercept             | $\mu$mol m$^{-2}$ s$^{-1}$ | 4.3 ± 0.6 | 0.6|| | NA         |
| $E_{Tp}$        | J mol$^{-1}$          | 34417 ± 5298 | 53100      | NA         |
| $\gamma_m$ parameters |                     |            |            |            |
| $\delta$        | -                     | 2.12 ± 0.09 | NA         | NA         |
| $\delta_m$      | mol m$^{-2}$ s$^{-1}$ | 0*         | NA         | NA         |

NA: not estimated or not available.

*Parameter values are derived from Harley et al. (1992b) with plants grown at an ambient [CO$_2$] of 35 Pa; the parameter values of temperature response are converted to fit Eqn (6) or Eqn (7) in the text; the value of $\theta$ is converted to fit Eqn (4) in the text.

†$R_d$ was held constant at different nitrogen levels and equal to 0.82 $\mu$mol m$^{-2}$ s$^{-1}$.

‡Parameter values are derived from Archontoulis et al. (2011). In their paper, the value of $E_{Rd}$ is a function of SLN. The value presented here is derived at SLN = 2.0 g N m$^{-2}$. Slopes of $R_d$-SLN are calculated from simulation of $R_d$ against SLN using original model.

¶Note that the absolute value of these parameters may be lower than the presented one when $\gamma_m$ is considered.

§The optimum temperature $I_{\text{max}}$ was not observed, so its $I_{\text{max}}$ was fitted to the Arrhenius Eqn (6); thereby, $D_{J\text{max}}$ and $S_{J\text{max}}$ were not estimated. The presented value gave equal temperature sensitivities, but it was rejected by the authors due to a high standard error of the estimate.

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**Parameterization of the leaf photosynthesis model for hemp**

Theoretically, the method to estimate $R_d$ (day respiration) works best for the NPR (nonphotorespiratory) condition (Yin et al., 2011). The estimated $R_d$ in this study did not differ significantly between PR (photorespiratory) and NPR conditions ($P > 0.05$). This result suggests that estimating $R_d$ from Eqn (9) is practicable even under PR condition (Yin et al., 2009, 2011). Note that assessing the true $R_d$ is somewhat difficult and the estimated $R_d$ differs according to methodologies. A comparison of the method used in this study with other ones to estimate $R_d$ is discussed in Yin et al. (2011). The estimated $R_d$ values were on average 20% lower than $R_{dk}$ values (respiration in the dark) in line with other reports (Brooks & Farquhar, 1985; Yin et al., 2009, 2011). An *in vivo* metabolic study (Tcherkez et al., 2005) indicated that the main inhibited steps were the entrance of hexose molecules into the glycolytic pathway and the Krebs cycle. Nevertheless, detailed mechanism of this difference still needs further research (Tcherkez et al., 2012).

Both $R_d$ and $R_{dk}$ increased monotonically with an increase in SLN and $T_L$ (Figs 2, 3 and 5) within the tested ranges. The result agrees with those of Yin et al.
(2009, 2011), but does not support those in Harley et al. (1992b) for cotton, where a constant $R_d$ was considered at changing nitrogen and temperature. For hemp, Chandra et al. (2008, 2011a) reported that $R_d$ levelled off or slightly decreased with an increase in temperature from 30 to 40 °C. This was not confirmed in the present study, although the highest $R_d$, measured at 25 °C in our study is comparable with the value observed in Chandra et al. (2008, 2011a). The reason for such discrepancy of $R_d$ in response to $T_L$ is not clear. It is probably due to an artefact of different protocols or due to changes in thermal sensitivity of respiration at different growth environments and plant status (e.g. drought, nutrient availability and sugar concentration) (Atkin et al., 2005; Katja et al., 2012). If an increase of respiration with increasing $SLN$ and $T_L$ is proven for hemp, it could counteract, at least partly, the positive effects of $SLN$ and $T_L$ on $A$ (net photosynthesis rate) when considering at daily basis.

Based on the findings that the maximum quantum yields (the initial slopes of the response of CO$_2$ uptake to photon absorption) were conserved across age classes within species or across the mature photosynthetic organs of different species (Long et al., 1993), $\kappa_{2LL}$ was often fixed as a constant across different growth environments and species in studies of plant photosynthesis (Harley et al., 1999b; Medlyn et al., 2002). However, very different values have been assumed in different studies without clear explanation, ranging from 0.18 mol mol$^{-1}$ until 0.39 mol mol$^{-1}$ (Harley et al., 1992b; Wullschleger, 1993; Medlyn et al., 2002; Yamori et al., 2010). The estimated $\kappa_{2LL}$ in the present study did not change with $SLN$ and with $T_L$, but it was not constant across growth environments (0.21 mol mol$^{-1}$ for the N-trial; 0.37 mol mol$^{-1}$ for the T-trial and 0.13 mol mol$^{-1}$ resulted in the best prediction of measurements in the TN-trial), in line with Archontoulis et al. (2011) who observed that cardoon (Cynara cardunculus) had a higher $\kappa_{2LL}$ in the cold season than in the warm season. The reason for the variation in $\kappa_{2LL}$ in different environments is still not fully understood. We speculate that the low $\kappa_{2LL}$ in the N-trial and the TN-trial in comparison with the $\kappa_{2LL}$ in the T-trial is a consequence of photoinhibition that occurs naturally in field plants grown in West Europe when the temperature is low and the sky is clear (Long et al., 1994). The plants of the N-trial and the TN-trial were grown outdoors, with fluctuations in temperature and irradiance; particularly, the plants in the TN-trial experienced a sudden drop of temperature five days before measuring (Fig. S1). These conditions could have resulted in severe photoinhibition (Long et al., 1983; Powles et al., 1983) causing a reduction in $\Phi_{PSII}$ (PSII quantum use efficiency under strictly limiting light) and an increase in the fraction of alternative electron transport (i.e. $\frac{\Phi_{PSII}}{1-\Phi_{cyc}}$ ; cf. Eqn 9a) (Curviet & Van Rensen, 1993; Murata et al., 2012), hence a low $\kappa_{2LL}$. In contrast, the plants of the T-trial were grown in the glasshouse where both light intensity and temperature were controlled at a condition free of photoinhibition. Thus, the value of $\kappa_{2LL}$ (0.37 mol mol$^{-1}$) was high and close to the range for healthy C$_3$ leaves (between 0.32 mol mol$^{-1}$ and 0.35 mol mol$^{-1}$) (Hikosaka et al., 2012 and their references). Moreover, the variation in $\kappa_{2LL}$ could be partly attributed to the change in $b$ (leaf absorbance; cf. Eqn 9a) as a result of environmental acclimation (Archontoulis et al., 2011). A higher $\beta$ in the T-trial than in the N-trial and the NT-trial is reflected by the higher SPAD values when considered at the same $SLN$ (Fig. S5). Given that the value of $\kappa_{2LL}$ varied significantly across different environments and that it affected significantly the prediction of photosynthesis when electron transport was limited (i.e. $A$) (Fig. 7), caution is needed when modelling photosynthesis rate using a value of $\kappa_{2LL}$ derived from different environments, particularly if these include both glasshouse and open field conditions. To improve modelling of crop growth in field conditions, further study should be conducted to investigate the mechanisms underlying variation in $\kappa_{2LL}$ during the whole growth season.

The relationships $I_{max}SLN$, $V_{cmax}SLN$ and $T_pSLN$ were consistent across canopy positions and growth environments whereas linear regression of these relationships resulted in negative intersections at the $x$-axis (Fig. 3), in line with Akita et al. (2012) but different from Archontoulis et al. (2011) and Braune et al. (2009) where the intersection of linear extrapolating resulted in a minimum $SLN$ required for photosynthesis ($SLN_b$). Given that it is not physiologically possible to have a negative $SLN_b$, the results in this study indicate that the relationships $I_{max}SLN$, $V_{cmax}SLN$ and $T_pSLN$ for hemp may not be perfectly linear. Further study would be needed to elucidate the relationship between these parameters and $SLN$ at $SLN$ levels approaching zero.

It is well recognized that $g_m$ is not infinite (Bernacchi et al., 2002). Using both the variable $j$ method and the modelling method, our analysis for hemp (Fig. 4) supports that $g_m$ varies with changing $C_i$ and $I_{inc}$ (Flexas et al., 2007, 2012; Yin et al., 2009), which is in contrast with the assumption that $g_m$ is independent of $C_i$ and $I_{inc}$ (Bernacchi et al., 2002). This highlights an important uncertainty in the present understanding of CO$_2$ diffusion processes in leaves. The $g_m$ obtained from Eqn (13) with a constant $\delta$ changed in line with $A$ (cf. Figs 2 and 4), confirming the assumption of Piel et al. (2002) and Ethier et al. (2006) that $g_m$ is correlated with $A$. The value of $\delta$ (2.12) is lower than that of wheat (2.54) (Yin et al., 2009) but higher than that of rice (0.45–1.57) (Gu et al., 2012).
Does hemp have high photosynthetic competence?

The observed \( A_{\text{max}} \) was observed to be levelled off at 25–35 °C (Fig. 2d) that is comparable with the 27 °C reported in Cosentino et al. (2012) and the 30 °C reported in Chandra et al. (2011a) for hemp leaf photosynthesis. The wide range of optimal temperature for leaf photosynthesis confirms the fact that hemp has been cultivated from the tropic (Tang et al., 2012) to the polar circle (Pahkala et al., 2008).

The highest \( A_{\text{max}} \) (light-saturated net photosynthesis rate) at 25 °C was measured at 31.2 ± 1.9 \( \mu \text{mol m}^{-2}\text{s}^{-1} \) (Fig. 2b). This value is higher than the highest value reported for hemp in De Meijer et al. (1995) and (Chandra et al., 2008, 2011a), which were 19.0 \( \mu \text{mol m}^{-2}\text{s}^{-1} \) and 24.0 \( \mu \text{mol m}^{-2}\text{s}^{-1} \), respectively. The highest \( A_{\text{max}} \) in this study is comparable with that of other C3 bioenergy crops. Archontoulis et al. (2011) reported that the highest \( A_{\text{max}} \) of kenaf, sunflower (Helianthus annuus L.) and cardoon ranged between 30 \( \mu \text{mol m}^{-2}\text{s}^{-1} \) and 35 \( \mu \text{mol m}^{-2}\text{s}^{-1} \) under optimum temperature.

As direct comparison of \( A_{\text{max}} \) among crops is difficult due to the variation in experimental protocols and plant status, we constructed \( A_{\text{CLT}}, A_{\text{max}}, A_{\text{Tl}}, \text{ and } A_{\text{SLN}} \) curves for hemp, cotton and kenaf with the same values of variables (i.e. \( A_{\text{CLT}}, I_{\text{SAT}}, \text{ and } \text{SLN} \)) (Fig. 7). The comparison highlighted that hemp has higher leaf photosynthesis rate than cotton and kenaf at a low nitrogen condition (i.e. \( \text{SLN} < 2.0 \text{ g N m}^{-2} \)). This was presumably because hemp has a relatively low \( \text{SLN}_{0} \). Analysis of newly senesced hemp leaves resulted in a nitrogen content of 0.25 ± 0.01 g N m\(^{-2}\). This value is at the low range of \( \text{SLN}_{0} \) among C3 crops and weeds (average value = 0.31 ± 0.03 g N m\(^{-2}\)) and is considerably lower than the estimation for kenaf (0.39 ± 0.13 g N m\(^{-2}\)) (Archontoulis et al., 2011).

The high photosynthesis rate of hemp at low nitrogen condition is in line with its observed high productivity at low nitrogen input (Struik et al., 2000; Finnan & Burke, 2013) and puts hemp ahead of cotton and kenaf from a perspective of bio-economy. However, our model approach has limitations. Firstly, the comparison was based on parameters derived from different studies conducted in different environments. Secondly, even though the FvCB model is biochemically based and the relationships \( I_{\text{SAT}} - \text{SLN}, V_{\text{max}} - \text{SLN} \) and \( T_{\text{p}} - \text{SLN} \) were consistent in this study across canopy positions and growth environments (Fig. 3), increasing evidences show that the model parameters may change when plant acclimates to growing environments. For example, Harley et al. (1992b) reported that the slope of \( V_{\text{max}} - \text{SLN} \) decreased with an increase in CO\(_2\) concentration in the growth environment. The present study also indicated that the value of \( K_{\text{LL}} \) may differ among growth environments. Thirdly, variation in photosynthetic competence among cultivars has been reported for hemp (Chandra et al., 2011b). As only one cultivar was studied, it is not clear whether the advantage of photosynthetic competence of hemp is persistent across cultivars. Therefore, to consolidate the potential of hemp as a bioeconomic sustainable crop, further study is needed to compare hemp leaf photosynthetic competence with those of cotton, kenaf and other bioenergy crops in the same growing environment with multiple cultivars.

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Supporting Information

Additional Supporting Information may be found online in the supporting information tab for this article:

**Figure S1.** The daily temperature and global radiation during the period from sowing to the end of the experiment for plants grown in the open field (i.e. TN-trial in 2013 and N-trial in 2014).

**Figure S2.** Dependence of lumped parameter \( s \) in Eqn (9) on leaf nitrogen (SLN) and dependence of the efficiency of converting incident irradiance into linear electron transport under limiting light \( (\kappa_{LL}) \) on SLN and leaf temperature \( (T_L) \).

**Figure S3.** The estimated day respiration under photorespiratory condition, i.e. at 21% \( O_2 \) against that under non-photorespiratory condition, i.e. at 2% \( O_2 \).

**Figure S4.** The error of model validation against leaf nitrogen (SLN) and temperature \( (T_L) \).

**Figure S5.** The effect of growth environment on the relationship between SPAD values and leaf nitrogen (SLN).