Appendix I *Modelling net assimilation rates (A_n) for C_4 photosynthesis.*

For the calculations of net photosynthetic rates with different values for bundle sheath leakiness we used the model by Von Caemmerer and Furbank (1999) described in detail by Von Caemmerer (2000), combined with measurements. The model is based on the assumption that net assimilation steady state rates are determined by the most limiting factor. Therefore, C_4 photosynthesis rate was defined by light limitation A_j and by enzyme limitation A_c. Photosynthetic rate was determined by taking the minimum of these two rates as described in Equation 4.47 (numbers according to Von Caemmerer 2000)

A_n = minimum (A_c, A_j) (vC 4.47)

For A_c we used the measured net assimilation rates at saturating light intensity and 380 µmol mol^{-1} external CO_2 concentration (figure 2). A_j was defined by the ATP requirement of C_4 pathway CO_2 fixation in the absence of photorespiration (based on stoichiometry of chloroplastic electron transport chain, with obligatory operating Q-cycle):

$$A_j = \left(\frac{J_t}{\frac{x}{\left(2 - \beta\right)^{+R}}} - R_d \right)$$

J_t is the electron transport rate (ETR), which was obtained by fitting a non-rectangular hyperbola on the ETR measurements as a function of PFD in Figure 2. Based on the stoichiometry without leakiness, five molecules of ATP are needed for each CO_2 fixed. However, when leakiness takes a value between 0 and 1, the ATP consumption per CO_2 fixed increases accordingly, leading to a reduction in assimilation rate under light limiting conditions (analogous to equation 4.55 in the model by Von Caemmerer describing ATP requirement).

An adjustment was made to enable expression of temperature effects on net assimilation rates. We agree with the formal approach of using an Arrhenius equation on each of the rate limiting parameters proposed by Massad, Tuzet and Bethenod (2007) as well as their suggestion that mesophyll and bundle sheath conductance and Michaelis-Menten constants for O_2 and CO_2 are also temperature-dependent. However, based on measurements on *Miscanthus* at differing temperature by Naidu *et al.* (2003) for the range of measured temperatures in our study a simple descriptive Q_{10} approach was considered sufficient to describe temperature effects on the light saturated net assimilation rates as well as on the measured values of R_d.

$$\begin{cases} T\ adjusted\ A_n = A_n\, Q_{10}^{\frac{T-T_{base}}{10}} \\ T\ adjusted\ R_d = R_d\, Q_{10}^{\frac{T-T_{base}}{10}} \end{cases}$$

T_{base} was set at 22 ^{o}C based on the measurements of A_n and R_d. Q_{10} was set at 2 and T is the measured temperature within the canopy (^{o}C).

References

Massad R-S, Tuzet A, Bethenod O (2007) The effect of temperature on C4-type leaf photosynthetic parameters. Plant Cell and Environment, **30**: 1191-1204.