Interactions between nitrogen nutrition, canopy architecture and photosynthesis in rice, assessed using high resolution 3D reconstruction

Alexandra J. Burgess1*, Tiara Herman2*, Asgar Ali2**, Erik H. Murchie1**

1Division of Plant and Crop Science, School of Biosciences, University of Nottingham Sutton Bonington Campus, Leicestershire LE12 5RD, United Kingdom
2School of Biosciences, University of Nottingham Malaysia, Jalan Broga, Semenyih, 43500 Selangor Darul Ehsan, Malaysia

*These authors contributed equally to the work
**Corresponding authors

Erik Murchie; erik.murchie@nottingham.ac.uk; Division of Plant and Crop Science, School of Biosciences, University of Nottingham Sutton Bonington Campus, Leicestershire LE12 5RD, United Kingdom
Asgar Ali; asgar.ali@nottingham.edu.my; School of Biosciences, University of Nottingham Malaysia, Jalan Broga, Semenyih, 43500 Selangor Darul Ehsan, Malaysia
Abstract

Increasing nitrogen use efficiency (NUE) is a key target for yield improvement programmes. Here we identify features of rice canopy architecture during altered N availability and link them to photosynthetic productivity. Empirical mathematical modelling, high-resolution 3-dimensional (3D) reconstruction and gas exchange measurements were employed to investigate the effect of a mild N deficiency versus surplus N application on canopy architecture, light and photosynthesis distribution throughout development. Three contrasting rice lines: two Malaysian rice varieties (MR219 and MR253) and a high-yielding indica cultivar (IR64) were cultivated. 3D reconstruction indicated key N-dependent differences in plant architecture and canopy light distribution including changes to leaf area index (LAI), tiller number, leaf angle and modelled light extinction coefficients. Measured leaf photosynthetic capacity did not differ substantially between the high and reduced N treatments, however, modelled canopy photosynthesis rate indicated a higher carbon gain per unit leaf area for the reduced N treatment but a higher carbon gain per unit ground area for the high N treatment. This is a result of altered canopy structure leading to increased light distribution under reduced N which partially offsets the reduced LAI. Within rice, altered N availability results in the development of full photosynthetically functional leaves, but leads to altered canopy architecture, light distribution and overall productivity suggested that N availability can be fine-tuned to optimise biomass production. We propose wider use of 3D reconstruction to assess canopy architecture and productivity under differing N availabilities for a range of species.
1. Introduction

Increased crop yield per hectare will be needed to sustain the growing global population. However yield barriers are imposed by the decreasing availability of land and resources combined with a rapidly changing climate (Ray et al., 2012; Challinor et al., 2014). Nitrogen (N) is one of the most costly agricultural inputs, in terms of finance and environmental impact, despite being one of the most important mineral nutrients required to sustain yields. Field grown crops therefore require an external input of N as fertiliser but strategies for application vary substantially (Peng et al., 2006; Peng et al., 2010). Large amounts of N fertilisers are used to increase yield and to prevent fluctuating resources from affecting production (Kant et al., 2011), however growing concerns over the environmental consequences of mineral N use, and its potential contamination when not used efficiently, has led to the need for research in the interactions between availability and crop growth (Peng et al., 2010).

Rice is a staple food in many countries, accounting for more than 40% of global food production. The impact of rice on health and livelihoods is even greater in South East Asia, where rice provides the main source of nutrition as well as income and employment (Makino, 2011; GRiSP, 2013). Evidence suggests that in recent years the average local rice yield in some rice growing countries is less than half (30-50%) of achievable potential based on local verification trials (e.g. those performed by Malaysian Agricultural and Development Institute (MARDI); Omar, 2008). The current average rice yield has been reported at 4.5-5 t ha⁻¹, however this average is mostly as a result of application of more than the recommended dose of N (Nori et al., 2008). Nevertheless, increases in yield by 50% are estimated to be required in all rice growing countries to meet demand by 2050 (Sheehy & Mitchell, 2013). The most productive systems are those which contain irrigated rice, accounting for approximately 45% of rice cultivation area, where multiple harvests occur per year and yield is high (Redfern et al., 2012). The potential for expanding crop area under cultivation is limited within most countries, with a reduction in the rate of expansion in irrigated land, damage to current cultivated land (e.g. salinization and intensification-induced degradation of soil) plus transfer of cultivated land to other uses. Therefore, increases in rice yield must come with a concurrent reduction in the amount of land under cultivation.

There has been a general trend for increased use of N fertiliser consumption in SE Asia (FAOStat). However, the use of N fertilisers is not economical and increased N levels do not
necessarily improve yield or the crop’s tolerance to uncertain climatic conditions (Kropff et al., 1993; Murchie et al., 2009; Peng et al., 2010). Furthermore, studies indicate that at any given soil N content, significantly lower yields were achieved towards the end of the 21st century than the preceding three decades (Cassman, 1999). Application of N fertiliser in excess of that required can even lead to negative effects including mutual shading, lodging and pest damage (Peng et al., 2006; Peng et al., 2010). Thus, understanding the crop response to a change in N levels, and selecting varieties that are capable of outperforming others will be critical to reduce overreliance on fertilisers.

As a primary constituent of essential proteins and enzymes that are involved in important plant metabolic processes, N is essential in the formation of the plant canopy and increasing photosynthetic leaf area. Photosynthetic components are a significant sink for leaf N: chloroplasts account for up to 80% of total leaf N with Rubisco being the dominant enzyme (Makino & Osmond, 1991). Leaf photosynthetic capacity and Rubisco content per unit leaf area is highly correlated with leaf N both within and between species (Evans, 1989; Theobald et al., 1998). N affects a number of developmental traits including plant height, panicle number, leaf size and spikelet number, all of which contribute to the yield potential of the crop. As a key requirement for cell division and expansion, N is integral for development, growth and final organ size (Wann and Raper, 1979). During the vegetative growth stage, absorbed N primarily promotes early growth and increases the number of tillers (Mae, 1997). For the formation of dense canopies, large concentrations of N are required (Connor et al., 2011). In N deficient conditions, the plant counterbalances the lack of N by producing a lower number of tillers; a compensations step that allows for fewer but fully functional leaves (Chen et al., 2003). Consequently, N deficiency generally reduces leaf area index, intercepted radiation, plant height and canopy photosynthesis rate (Connor et al., 2011). Even a mild N deficiency can make moderate changes to plant structure that will have a large impact on the light distribution and thus productivity of canopies but little is known about the changes in 3D structure (and hence light dynamics) in crop canopies with differing N content.

Assessing the productivity of crops is confounded by heterogeneous nature of plant and crop canopies; they commonly consist of multiple plants exhibiting different growth and developmental patterns (Kozłowska-Ptaszyńska, 1993; Godin, 2000). Therefore, understanding plant response to changes in N levels requires experimental data combined with high-resolution
information on the physiological characteristics associated with a particular canopy architecture. This could be achieved through modelling approaches that can make more accurate predictions of the canopy light environment, and thus the influence of architecture, compared to manual measurements. Monitoring plant growth and estimating canopy photosynthesis rate and efficiency in the field on a large scale is a complex task. Whilst some research has been carried out to study the effects of varying N treatments on crop systems (e.g. Harasim et al., 2016 for wheat) and on isolated rice varieties (Mae, 1997; Herman et al., 2015), few studies exist to investigate how different varieties respond to varying N treatments in terms of changes to their canopy architecture. Using photosynthesis measurements alongside the three-dimensional (3D) modelling of crop canopies, we can explore plant structure and estimate crop productivity at the whole canopy scale, which would not be feasible using manual measurements (Song et al., 2013; Burgess et al., 2017).

Here we employ such methods (Pound et al. 2014) to investigate the effect of N availability on three rice lines. Because N is an integral component in photosynthetic machinery and in forming structural tissue we hypothesise that the differences in both canopy architecture and photosynthesis from alteration of N content will influence not only the vertical light gradient and the spatio-temporal variation in light. Further we hypothesise that specific N-dependent changes in architecture such as leaf angle will influence this canopy light distribution. This work will identify whether the optimal canopy architecture for reduced N conditions differs to that of high N conditions.
2. Materials and Methods

2.1 Plant Material and experimental design

Two Malaysian rice varieties, MR219 and MR253, both from MARDI, were selected for study due to their potential biotic and abiotic resistance (e.g. MR253 in resistant to leaf blast) and performance in marginal soils. A high yielding IRRI cultivar, IR64, was also chosen as a control due to its high yielding potential, tolerance to multiple diseases and pests plus wide adaptability, as well as previous studies on its response to varied N application (Morris et al., 1989; Diekmann et al., 1996). Seeds were sown into module trays containing Levington Module compost with sand in the ‘FutureCrop’ Glasshouse facilities, University of Nottingham Sutton Bonington Campus, UK on the 8th May 2014. The seedlings were transplanted into soil beds at the appearance of the third true leaf. These glasshouses are ‘agronomy’ glasshouses which permit the sowing of entire crop stands in sunken concrete pits under controlled glasshouse conditions, described in full in Hubbart et al. (2018). The three rice varieties were assigned in a completely randomised design. The experimental plot was divided into 18 microplots, with each microplot containing 42 plants of the same variety (7 x 6 plants). We imposed a mild or moderate nitrogen deficiency as follows. The high nitrogen plots at the start of the experiments contained 350 kg N ha⁻¹ and the low nitrogen plots contained 250 kg N ha⁻¹. Additional fertiliser was not supplied throughout the duration of the experiment. Irrigation was supplied using drippers for 15 minutes, twice daily. Metal halide lamps provided supplementary lighting when an external light sensor detected intensity (Photosynthetic photon flux density, PPFD) below 300 μmol m⁻² s⁻¹. A 12-h photoperiod (07:00 to 19:00) was maintained in the glasshouse using blackout blinds with a constant temperature of 30°C and relative humidity (RH) of 50–60%.

Unless otherwise stated, measurements were performed at five growth stages (GS) throughout development as follows: Vegetative phase- GS1: 18 days after transplanting (DAT), GS2: 35 DAT; Reproductive phase- GS3: 49 DAT, GS4: 63 DAT and GS5: 77 DAT; where GS5 represents full canopy development in the Ripening phases (Knowledgebank, IRRI).

2.2 Composition and Morphology

Five replicate measurements per plot for plant heights and SPAD measurements were obtained weekly, from 20 days after transplanting (DAT) until the start of the flowering stage (100 DAT). Five replicate measurements per plot were also taken for tiller numbers between 14 and 35 DAT.
Soil-plant analyses development (SPAD) measurements were taken in situ using the Minolta 502 Plus Chlorophyll Meter (Spectrum Technologies, USA), to obtain the total available chlorophyll within a specified leaf area. Chlorophyll a and b content were determined spectrophotometrically. Frozen leaf samples of known area were ground in 80% acetone. The samples were then centrifuged for 5 minutes at 300 rpm and the absorbance (at 663 and 645 nm) of the supernatant was measured using a spectrophotometer. Chlorophyll a and b content were calculated using the protocol of Porra et al. (1989).

Leaf thickness was measured at the major and minor veins using leaf sectioning. Sections of the penultimate leaf on the main stem were cut from the widest part of the leaf using a sharp razor blade, for mounting on microscope slides. After mounting, the leaf sections were cleared using 85% (w/v) lactic acid saturated with chloral hydrate. The slides were heated in a hot water bath (70°C) for an hour. After clearing, the leaf sections were washed with distilled water and stained using 1% toluidine blue dye in 1% (w/v) disodium tetraborate. A few drops of glycerol were added to the leaf sections to preserve the samples before being viewed under a calibrated light microscope and images captured using a digital camera (Nikon DXM 1200). Stomatal density and length were determined using leaf impressions of both the adaxial and abaxial surfaces on the widest part of the flag leaf. Impressions were made using Coltène® PRESIDENT Plus silicone-based impression putty. Clear nail varnish was then applied to the hardened putty and later peeled and mounted on microscope glass slides for view under a 40x-magnification confocal light microscope. Images of six fields of view were taken for each variety under each treatment for analysis (Hubbart et al., 2012). All images for leaf thickness and stomata were analysed using the analytical software ImageJ.

### 2.3 Leaf Nitrogen

Leaf nitrogen analysis was carried out by Lancrop Laboratories, York, United Kingdom. Three plants per plot were chosen at random for this analysis. For each plant, leaves were clipped at the top portion of the canopy (including the flag leaf, at 10 cm height) to make up 200 grams of fresh plant material. Samples were labeled and couriered to the laboratory on the same day.
2.4 Gas Exchange

Data was taken from the glasshouse grown rice in plots in the same weeks as the imaging for reconstruction (below). Leaves were not dark-adapted prior to measurements. Light response curves (LRC) and ACi curves were taken with a LI-COR 6400XT infra-red gas-exchange analyser (LI-COR, Nebraska). The block temperature was maintained at 30 °C using a flow rate of 500 ml min\(^{-1}\) and light was provided by a combination of in-built red and blue LEDs. For LRC, illumination occurred over a series of 7 PPFD values, prior to flowering and a series of 12 values post flowering, between 0 and 2000 \(\mu\text{mol m}^{-2}\ \text{s}^{-1}\), with a minimum of 2 minutes at each PPFD. The light-response curves were taken at two different canopy heights; designated top and bottom, where the top layer refers to the last fully expanded leaf and the bottom layer refers to a fully expanded leaf in the bottom half of the canopy that did not show signs of senescence. An additional middle canopy layer was included at full canopy development (GS5) to better capture any spatial differences in large, fully grown, plants. For the ACi curves, leaves were exposed to 1000 \(\mu\text{mol m}^{-2}\ \text{s}^{-1}\) throughout. They were placed in the chamber at 400 ppm CO\(_2\) for a maximum of 2 min and then CO\(_2\) was reduced stepwise to 40 ppm. CO\(_2\) was then increased to 1500 ppm, again in a stepwise manner. At least one replicate was taken per treatment plot but with 5 replicates taken for each of the 6 treatments.

Analysis of variance (ANOVA) and Tukey’s multiple comparison tests were carried out using GenStat for Windows, 17\(^{th}\) Edition (VSN International Ltd.). All individual and interaction terms were considered in the model. Data was checked to see if it met the assumption of constant variance and normal distribution of residuals.

2.5 3D Reconstruction and Ray Tracing

3D analysis of plants was made according to the protocol of Pound et al. (2014) and Burgess et al. (2015). Every two weeks and following photosynthesis measurements, the rice plants (roots and shoots) were carefully removed from the plots, placed into pots and moved to the imaging studio located next to the glasshouse to prevent excessive movement and damage to leaves. Roots were supplied with water to prevent wilting. It was found that this process did not alter the key architectural features of the plants. They were imaged within 10 minutes using three fixed Canon 650D cameras, with a minimum of 40 images per plant. Images were captured using a revolving turntable, including a calibration target of set width (397mm). An initial point cloud was obtained using the PMVS software (Furukawa and Ponce, 2010; Wu, 2011). The PMVS photometric-
consistency threshold (Furukawa & Ponce, 2010: Eq. 2) was set at 0.45 to optimise the amount
of plant material recognised in the point cloud. Default parameters were used within the
Reconstructor software, except for maximum cluster size and boundary sample rate that were
changed to 120 and 15, respectively. One plant per plot was removed at each growth stage leading
to three replicates per line; at least two of these were used to form the final canopies. As only
one plant was removed per plot, per growth stage, removal was expected to have minimal effect
on the remaining plants however, to ensure this; care was taken to leave a buffer plant (i.e. the
edge plant) next to removal sites. Previous work has validated the reconstruction process,
indicating the in silico plants represent differ between 1-4 % in area compared to that of measured
plants and accurately capture of leaf angles (Pound et al., 2014; Burgess et al., 2015). Duplicating
and randomly rotating the individual reconstructed plants into a 3x3 grid with 10 cm within and
between rows formed reconstructed canopies.

Reconstructed canopies consist of a number of 2D triangles within a mesh. Total light per unit
leaf area for each triangle at a given time point was predicted using a forward ray-tracing
algorithm implemented in fastTracer (fastTracer version 3; PICB, Shanghai, China; Song et al.,
2013). Latitude was set at 3 (for Kuala Lumpur, Malaysia), atmospheric transmittance 0.5, light
reflectance 7.5%, light transmittance 7.5%, day set at the day of the imaging. The diurnal course
of light intensities over a whole canopy was recorded in 30 minute intervals. The ray tracing
boundaries were positioned within the outside plants so as to reduce boundary effects. The
software fires rays through a box with defined boundaries: when they exit one boundary (i.e. the
side) they enter again from the opposite side.

2.6 Modelling
All modelling was carried out using Mathematica (Wolfram).

Cumulative leaf area index (cLAI; leaf area per unit ground area as a function of depth) was
calculated from each of the canopy reconstructions. For each depth ($d$; distance from the highest
point of the canopy, i.e. the highest point on the $z$ axis), we found all triangles with centres lying
above $d$ (Eq. 1).

$$d_i = \max_{j=1,2,3;1 \leq s \leq n} z_i^j - (z_i^1 + z_i^2 + z_i^3)/3$$
was also used as a reference to dived canopies into layers, with all triangles above the midpoint, \textit{d}_{mid} assigned the upper layer, and those below the lower layer. Two reference points were used for GS5 to split the canopy into three layers: top, middle and bottom.

We calculated the sum of the areas of all triangles and then divided this sum by ground area. The cumulative LAI as a function of depth through the canopy was calculated using Eq. 2.

\[
cLAI = \frac{\sum_{i=1}^{n} I(d < d_i) S_i}{(\max x_i - \min x_i)(\max y_i - \min y_i)},
\]

where \( I(A) = 1 \) if condition \( A \) is satisfied and \( S_i \) is the area of a triangle \( i \).

Leaf angle distributions were calculated for each canopy and averaged at each canopy depth by using the angle of each 2D triangular face relative to horizontal; where an angle of 0 indicates a more horizontal leaf section and an inclination angle of 90 indicates a more vertical leaf section.

The light extinction coefficient of the canopy was calculated using the 3D structural data and the light distribution obtained from ray tracing. In order to calculate fractional interception, \( F \), within a canopy as a function of depth at time \( t \), we first identified all triangles lying above depth, \( d \) (Eq. 1). We then calculated their contribution to intercepted light by multiplying PPFD received per unit surface area (ray tracing output) by the area of triangle. The light intercepted was summed for all triangles above the set \( d \), and divided by light intercepted by ground area according to Eq. 3.

\[
F = \frac{\sum_{i=1}^{n} I(d_i < d) S_i L_i(t)}{L_0(t) \text{ground area}},
\]

where \( L_0(t) \) is light received on a horizontal surface with a ground area \( (\max x_i - \min x_i)(\max y_i - \min y_i) \) and \( L_i(t) \) is light intercepted by a triangle \( i \).

The light extinction coefficient derived from ray tracing data, \( k_{rt} \), was calculated by fitting (by least squares) the function according to Burgess et al. (2017):

\[
F(x) = a(1 - e^{-k_{rt}x})
\]
to the set of points \( \{cLAI(d), F(d, t)\} \) calculated by varying depth from 0 to the height at total 
cLAI with step \( \Delta d = 1 \) mm (Supplementary Figure S6.4), where \( a \) in Eq.(4) is a fitted parameter.

The response of photosynthesis to light irradiance, \( L \), was calculated using a nonrectangular 
hyperbola given by Eq. 5:

\[
p = \frac{\phi L + (1 + a)P_{\text{max}}}{\sqrt{(\phi L + (1 + a)P_{\text{max}})^2 - 4\theta \phi L(1 + a)P_{\text{max}}} - \alpha P_{\text{max}}} 
\]

(5)

The nonrectangular hyperbola is defined by four parameters: the quantum use efficiency, \( \phi \); the 
convexity, \( \theta \); the maximum photosynthetic capacity; \( P_{\text{max}} \), and the rate of dark respiration, \( R_d \).

We assumed that the rate of dark respiration is proportional to the maximum photosynthetic 
capacity, according to the relationship \( R_d = \alpha P_{\text{max}} \) (Retkute et al., 2015). Values for \( P_{\text{max}} \) were 
determined from leaf gas exchange measurements for the two canopy layers: top and bottom. For 
GS1-4 (prior to flowering), the light response curve data was averaged prior to LRC fitting, as 
the shorter 7-point curves (see Materials and Methods: Gas Exchange) do not give a good fit. For 
GS5, all individual curves were fit; the mean \( \pm \) SEM is presented in Table 6.3. Curve fitting was 
carried out using the Mathematica command **FindFit** with a minimum constraint on dark 
respiration at 0.05 and convexity at 0.7.

As each canopy was divided into two layers, each triangle from the digital plant reconstruction 
was assigned to a particular layer \( m \) according to the triangle centre (i.e. with triangle centre 
between upper and lower limit of a layer depth). Carbon gain per unit leaf area was calculated as 
daily carbon assimilation over a whole canopy divided by the total surface area of the canopy 
according to Eq. 6.

\[
C_l = \frac{\sum_{i=1}^{n} P_i}{\sum_{i=1}^{n} S_i} \quad \text{(6)}
\]

Carbon gain per unit ground area was calculated as daily carbon assimilation over a whole canopy 
divided by the area inside the ray tracing boundaries according to Eq. 7.

\[
C_g = \frac{\sum_{i=1}^{n} P_i}{(\max x_i-\min x_i)(\max y_i-\min y_i)} \quad \text{(7)}
\]
3 Results

3.1 Canopy architecture and the light environment under different N treatments

The canopy reconstructions for each treatment for each of the five growth stages during development are provided as a visual representation in Figure 1, where GS5 indicates full canopy closure and GS1-4 represent vegetative stages two weeks apart starting 18 DAT. Visual differences can be discerned between the lines and between treatments e.g. all lines show a greater amount of plant material under the high N treatment relative to the low N treatment and this is apparent at all stages. Similarly, differences are seen in plant height between treatments (Figure 2A). Generally, IR64 plants were observed to be significantly shorter than the Malaysian varieties, in both high N and low N plants. In both Malaysian varieties, significant differences were observed between high N and low N plants ($p<0.05$), where low N plants were at least 25% shorter than high N plants. However, no significant differences were found between the two treatments for IR64 ($p>0.05$). There were both varietal and treatment differences in the number of tillers (Supplementary Figure S1). IR64 HN plants produced the highest number of tillers ($p<0.0001$) relative to the rest of the varieties and treatments.

Previous publications validated 3D reconstruction as a means of measuring canopy leaf area and leaf angle (Pound et al. 2014; Burgess, Retkute, Pound, Preston, et al. 2015). Figure 2B shows modelled whole-canopy LAI throughout development. It is clear that high N accumulated a greater total LAI after day 40, consistent with Figure 1, indicating that greater soil N availability stimulated greater growth. Whilst LAI values are high, particularly at GS4-5, we note published values of 8 - 14 at high N (e.g. Zhong et al. 2002; Fagade et al. 1971). Figure 2C shows distribution of the angle of plant material according to height above ground, where a higher inclination angle indicating a more upright posture. The variation between lines and treatments is greater towards the top of the canopy (i.e. 60 cm and above), reflecting differences between the lines and treatments in terms of upright versus curled leaf material in the top portion of the canopy (Figure 1). There does not seem to be a consistent response to N treatment, however IR64 low N and MR253 high N showed a less vertical posture. Extinction coefficients are greatly influenced by leaf angle (Murchie and Reynolds, 2012). The modelled light extinction coefficient ($k_{rt}$) values in Table 1 show variety-dependent responses. In IR64 and MR219 but not MR253, $k_{rt}$ values for high N were consistently lower than reduced N indicating a steeper curve for light extinction in the latter. This could be caused by the higher LAI in the upper layers or by an
increased chlorophyll density in the upper layers (below). This is consistent with the leaf angles
given in Figure 2 but only for IR64. However, the lack of consistency in angle distributions of
leaf material indicates that angle is not substantially influenced by N treatment and that light
attenuation is likely to be more influenced by LAI in the upper layers of the canopy.

Figure 3 shows how the accumulation of plant material along the vertical transect of the canopy
(cumulative leaf area index; cLAI) and the corresponding interception of light (fractional
interception; F) from modelled data. High N treatment plants accumulated a greater LAI at
middle and upper portions of the canopy in GS1-GS3. In GS4 there was a higher accumulation
of LAI throughout the canopy in the reduced N treatment but with a lower overall plant height,
resulting in a reduced overall LAI. This indicates that reduced N treatment had a delayed
progression of canopy development. These differences in leaf accumulation influences the
canopy depth at which most light interception occurs, indicated by the steepness of the curve in
Figure 3. By GS5, the overall F was equivalent for all treatments but differences in distribution
of F between treatment and variety could be seen in intermediate layers.

Figure 4 show how PPFD is distributed throughout the canopy according to the fraction of total
canopy leaf surface area. In this way it becomes possible to visualize the prevalence of different
PPFD values. This is analogous to a frequency histogram and was used in a previous study
(Burgess et al., 2015) to examine the distribution of levels of radiation that are saturating and sub
– saturating for photosynthesis, which is also relevant here. For example, an erect canopy would
be expected to have a higher leaf area exposed to a higher PPFD values overall. This is especially
important for the lower layers of the canopy which, in erect canopies, have increased light
penetration which would allow leaves in these lower layers to increase photosynthesis. Here we
cannot see consistent patterns in terms of treatment. We note that reduced N IR64 has a larger
proportion of leaf area exposed to PPFDs of 100 µmol m\(^{-2}\) s\(^{-1}\) or lower, indicating a high level of
self-shading consistent with the higher \(k_{rt}\) value at GS5 (Table 1) and the particularly low leaf
angle in upper layers (Fig 2C) suggesting that in IR64, N treatment results in altered leaf angle
and altered light distribution. Optimising light distribution at reduced N could therefore be
achieved by either altering depth dependent leaf area accumulation or leaf angle.
3.2 Nitrogen had limited effects on leaf composition

Chlorophyll content in upper leaves was analysed using a hand-held chlorophyll meter (Figure 5). This revealed similar patterns in chlorophyll content in both high N and reduced N plants – greenness fell sharply at 44 and 79 days after transplanting in a majority of the plants. Significant differences between high N and reduced N plants were observed in all three varieties (p<0.05), and while this was more obvious later in development, lower N MR219 plants were consistently lower in greenness as compared to high N plants. Reduced N MR253 plants contained significantly higher percentage leaf N in the top layer of the canopy than reduced N MR219 and IR64 plants (Figure 6). No varietal differences were observed in high N plants. Within varieties, no differences in percentage leaf nitrogen were seen between treatments. At reduced N IR64 and MR253 had higher chlorophyll contents than MR219 indicating that the former were able to retain chlorophyll at reduced N but there is no indication that this is linked to protein or N content of leaves.

3.3 Nitrogen enhances carbon gain per unit ground area but not leaf photosynthetic rate

All lines showed a significant reduction in P\textsubscript{max} from top to the base of the canopy (P<0.05: see Figure 7 for GS5), however it was not clear whether the differences in attenuation according to N treatment consistently matched the differences in light attenuation (k\textsubscript{rt} values, Table 1). It is possible to conclude that the drop in P\textsubscript{max} in IR64 between top and middle canopy layers was substantially higher than the other two varieties, consistent with the less erect leaf stature and greater light attenuation; observed under both N treatments. There were no significant differences between cultivars for P\textsubscript{max} at middle and bottom layers but in the top layer IR64 had significantly higher values than MR253 (P<0.05). Based on previous studies, this increased P\textsubscript{max} is likely due to varietal differences as opposed to differential response to N (Herman et al., 2015). N treatment did not have a significant effect on P\textsubscript{max} at any layer (P<0.05). A-Ci analysis of the top layer of leaves reveals a reduction in the maximum carboxylation rate of Rubisco (V\textsubscript{cmax}) and electron transport rate (J) in reduced N MR253 plants compared to high N plants at GS3, but this was not evident in the other varieties (Supplementary Table S1), again indicating no significant effect of N availability on photosynthetic response to altered CO\textsubscript{2} concentrations.

Carbon gain per unit leaf area and carbon gain per unit ground area is presented in Figure 8. There were few consistent differences between cultivars or N treatment when expressed per unit
leaf area (Fig 8A) especially in GS4 and GS5. The higher values for reduced N treatment would seem to arise from the improved light distribution as a result of the lower k. However, when expressed per unit ground area there was a consistently higher carbon gain for the HN plants, with IR64 showing the highest values. At GS4 and GS5 there was little difference. This is partially consistent with the accumulation of LAI (Figs. 2 & 3), indicating that despite a slightly lower carbon gain per unit leaf area, the increased biomass compensated for this reduction and improved over carbon gain on a land area basis.

3.4 Increased nitrogen increases harvestable biomass of selected lines

Altered N nutrition and corresponding changes in canopy development had opposing effects on biomass production in each of the three varieties at harvest but not throughout development. Biomass (dry weights) (Table 2) between varieties and treatments were not statistically different from GS1 to GS4. In GS5, all varieties in the reduced N treatment had lower biomass than the high N treatments (p<0.05). Similarly, harvest biomass was observed to be significantly different between treatments, with MR253 showing substantially lower biomass in reduced N plants relative to high N plants (p<0.0001). However high N treatment showed significantly (P<0.05) higher harvest DW and seed DW compared to reduced N, except for in IR64 where the effects were minimal suggesting that under these conditions, biomass accumulation in IR64 is not N-dependent. Within the Malaysian lines, the higher LAI, particularly at GS3, may have been critical in driving the increase in biomass at harvest.
The influence of architecture on productivity of crops depends on a number of factors including the structure of both the individual plants and the combined or emergent properties of the whole canopy. Previous studies indicate that relatively small changes in canopy architecture can have substantial effects on light dynamics and canopy carbon gain (Zheng et al., 2008; Burgess et al., 2015; Burgess et al., 2017; Rahman et al., 2018). As an essential component, N is critical in determining plant growth and structure, hence the light environment characteristics within a canopy, however this has not previously been addressed using high resolution 3D reconstruction and ray tracing. Here we used such methods for assessing the relationship between different soil N treatments and whole canopy photosynthetic rate. The architectures of three diverse rice cultivars at five different growth stages were captured.

The effect of soil N on the accumulation of leaf area and the distribution of light was strongly dependent on both the position in the canopy and the growth stage measured, with differences between treatments diminishing at the highest LAI values. As roots extend to deeper soil regions, more N may have become available to the reduced N treatment, resulting in the convergence of LAI towards the later stages of growth. The modelled canopy extinction coefficient, $k_{rt}$, was lower for the high N treatment but not convincingly related to leaf angle, implying that leaf area in the upper regions of the canopy were of greater importance. Lower values of $k$ are thought to be advantageous for productivity under high N because they permit a more efficient light penetration and accumulation of a higher LAI. However, under reduced levels of N the priorities may be different. Previous work has shown the advantages of maintaining a low $k$ value (Verhoeven et al., 1997; Chen et al., 2003; Burgess et al., 2015). Leaf inclination angle is critical in determining the flux of solar radiation per unit leaf area (Ehleringer and Werk, 1986; Ezcurra et al., 1991; Falster and Westoby, 2003). Steep leaf inclination angles lead to a decreased light capture when the sun is directly overhead (i.e. during midday hours or during summer) but increases light capture at lower solar angles (i.e. start/ end of the day or during seasonal changes in the higher latitude regions). This feature has a number of practical applications including the decrease in susceptibility to photoinhibition (Ryel et al., 1993; Murchie et al., 1999; Valladares and Pugnaire, 1999; Werner et al., 2001; Burgess et al., 2015); reduced risk of overheating due to reduction in mid-day heat loads (King, 1997); and minimised water-use relative to carbon gain (Cowan et al., 1982). Nitrogen application can influence both light and nitrogen profiles,
modifying $k$ values and indeed the ratio between $k$ for light and for nitrogen, something that has been suggested to be the result of management practice affecting cytokinin synthesis although the reasons for these changes may not always be apparent (Gu et al., 2017a). However a recent study showed how leaf angle responds to nutrient deficiencies in rice, mediated by strigolactone (Shindo et al., 2020). As leaf angle influences light distribution and there is a functional link between N and light profiles, there is clearly a need to further understand how N deficiency might interact with light profiles to determine canopy photosynthesis.

One of the recommendations for this work therefore is that the posture of plants in mild N deficiency should be more upright to enhance photosynthesis. It is also possible that IR64 may benefit from further genetic alteration to improve posture under all N treatments. At most of the growth stages, IR64 showed a consistently higher $k_{rt}$ in comparison to the Malaysian varieties indicating a less upright canopy or a greater accumulation of leaf area in upper canopy layers. Differences in $k$ can also occur due to the pigment content in the upper layers, where high N induces a higher chlorophyll content (Bojović and Marković, 2009; Gu et al., 2017b). This is not necessarily a problem for canopy photosynthetic rate since reduced canopy chlorophyll may enhance light penetration and, as long as it does not affect $P_{\text{max}}$ or light harvesting in lower layers, it should actually increase canopy carbon gain (Song et al., 2017; Walker et al., 2018). The small effect on $P_{\text{max}}$ here suggests that as long as lowered chlorophyll does not substantially impact Rubisco content then it should not adversely affect yield. However, in the context of the current study we are assuming that lowered chlorophyll may be associated with lowered photosynthesis capacity even if this was not convincing under our treatment conditions.

A tradeoff between leaf area, N and photosynthesis has been seen previously in field-grown rice and can be viewed as a tendency to prevent ‘dilution’ of canopy N (Chen et al., 2003). N regulates growth rate, such that N is allocated to a smaller number of leaves, resulting in conserved $P_{\text{max}}$ values irrespective of N treatment. This is consistent with results in this study, where minimal effects were seen between $P_{\text{max}}$ values under different N treatments. Interestingly, MR219 showed slightly more susceptibility to a lowered N status compared to MR253, which could explain why MR253 is suitable for more marginal growing conditions in Malaysia. The residual N levels in this trial were mildly deficient (Rice Knowledge Bank, 2015). This may have contributed to the results witnessed here; namely no change in light saturated photosynthesis but an increase in biomass in Malaysian lines. The similar leaf photosynthetic rates between N
treatments mean that the differences in biomass and yield may come from canopy level processes, influenced by structure and development. Canopy photosynthesis rate can be thought of as the ‘sum’ of photosynthesis in all leaves in the canopy at any given time point. The light within the canopy will fluctuate on wide spatio-temporal scales according to factors such as solar movement. Hence it is necessary, when considering changes in canopy architecture to use ray tracing and modelling of photosynthesis. A higher canopy photosynthesis rate is seen in high N treated canopies but largely in the early growth stages (GS1-3) when the differences in LAI between treatments were greatest. It is interesting to note the observed higher carbon gain per unit leaf area in IR64 subject to reduced N, particularly during the mid-growth stages. This could be attributed to a possible growth ‘advantage’ of having a smaller canopy with less tillers, resulting in leaf tissue being exposed to higher light intensities relative to the same positions under high N. Therefore, we can conclude that the accumulation of leaf area and therefore light capture during canopy development is important in enhancing canopy photosynthesis rates.

Second, the supposedly improved leaf angle in the reduced N treated plants was not sufficient to enable these plants to achieve the canopy photosynthesis rates seen in the high N plants.

Increased LAI also corresponded to greater height and greater dry weight (DW), however whilst the two Malaysian lines exhibit a strong response to N treatment, IR64 is less sensitive. These results are consistent with previous studies on the effect of N application on IR64, which indicated that applications above 90-100 kg N ha\(^{-1}\) (using green manure) did not increase the agronomic efficiency of the system (Morris et al., 1989; Diekmann et al., 1996) and on MR219 where increases in the N application rate led to concurrent increases in the grain (Nori et al., 2008). Similar patterns can be seen for seed dry weight per plant: with IR64 exhibiting similar values under both N treatments but a large increase for both MR219 and MR253. Grain weights were not consistent with modelled canopy photosynthesis rates indicating that there may be other factors such as partitioning during the grain filling period. However, the ranking of DW during GS5 are consistent with ranking of modelled photosynthesis (per unit ground area) during most of the growth stages indicating a general correspondence between modelled canopy photosynthesis and measured biomass up to GS5. The results presented here indicate contrasting N uptake and utilisation responses of the three varieties, with MR219 and MR253 capable of utilising the extra N available in the soil.
Contrasting strategies can be seen in different crops in relation to N availability. In Potato
(Solanum tuberosum L.), excess N led to enhanced apical branching and prolonged production
of vegetative organs leading to a greater number of leaves per plant (Vos and Biemond, 1992;
Biemond and Vos, 1992). Conversely, under N limitation leaf size was reduced (via reduced leaf
expansion rates) in order to maintain N concentration per unit leaf area and the photosynthetic
capacity of the leaf (Vos and Van der Putten, 1998). In contrast, Maize (Zea Mays L.) exhibits a
more conservative response to changes in leaf size relative to potato and reduces total leaf area
by approximately 30% (Vos et al., 2005). Furthermore, maintaining higher leaf area comes at
the expense of decrease N per unit leaf area and a decrease in photosynthetic capacity. This
reflects two opposing strategies to N availability: the maintenance of photosynthetic productivity
per unit leaf area at the expense of total leaf area or; the maximisation of light interception per
unit leaf area at the expense of photosynthetic productivity. It is broadly expected, with some
exceptions, that these contrasting strategies represent the dicot versus the Gramineae response
(see Vos et al., 2005 and references within). Whilst this study did not use limiting amounts of N
availability, results suggest that under excess N conditions, N is used for the production of
increased tiller number, a greater leaf area and maintenance of photosynthetic capacity per unit
leaf area in rice.

5  Concluding Remarks

High-resolution 3D canopy reconstruction revealed novel observations concerning the effect of
N treatment on canopy architecture and light distribution in rice. First, leaf photosynthetic
capacity was generally less responsive than leaf area to N treatment meaning that light capture
and light distribution were more important in determining canopy photosynthesis rates and dry
weights. The reduction in leaf area accumulation during the mild N deficiency occurred in the
mid canopy region and was associated with an improved canopy light distribution in the reduced
N treatment resulting in a higher carbon gain per unit leaf area compared to high N. We show
the improved canopy light distribution in reduced N is more likely to be due to depth- dependent
leaf area accumulation or of pigment distribution than leaf angle in the case of the Malaysian
lines. We show key differences between architecture in the Philippine variety IR64 and the
Malaysian cultivars: IR64 had a less upright leaves in upper canopy regions in high N which
negatively affected light distribution. Improvement of light distribution would be more beneficial
for the high N treatment and may improve yields even further than those seen here. This indicates
a potential for increasing yields by improving the light distribution in high N treated plants.

Data

Data is made available on Figshare (Figshare.com).

Acknowledgement

The authors acknowledge the financial and technical support by Universiti Putra Malaysia and
the Ministry of Education Malaysia for Long-Term Research Grant Scheme (LRGS)-Food
Security. Erik Murchie receives funding from the Biotechnology and Biological Sciences
Research Council [grant number BB/R004633/1]. There are no conflicts of interest to declare.
**Table Legends**

Table 1: Modelled canopy extinction coefficient ($k_{rt}$) values calculated from reconstructions and ray tracing in rice grown under high- (HN) or reduced- (RN) nitrogen levels at growth stages 2-5; where GS5 represents full canopy closure.

Table 2: Plant dry weight measurements throughout development in rice varieties grown under high- (HN) or reduced- (RN) nitrogen levels. Mean± SEM, n=3. "a-d" Means in a column without a common superscript letter differ (P < 0.05), as analyzed by two-way ANOVA and Tukey's multiple comparisons test.
Figure 1: Final canopy reconstructions for three rice varieties grown under high- (HN) or reduced- (RN) nitrogen levels at five growth stages (GS). Three plants per treatment per growth stage were removed from the glasshouse beds and imaged according to the protocol of Pound et al (2014). Reconstructed plants were duplicated, rotated and rearranged in a 3 x 3 canopy grid to replicate growth conditions.

Figure 2: Physiological Measurements of three rice varieties grown under high- (HN) or reduced- (RN) nitrogen levels. (A) Measured plant height over time, (B) Modelled LAI over time. LAI was calculated as total mesh area inside the designated ray tracing boundaries (see Materials and Methods). (C) Modelled average plant material angle distribution as a function of height in the canopy. The average triangle inclination angle throughout the horizontal subsection was calculated with respect to vertical, where a leaf inclination angle towards 0 indicates more horizontal plant material and an inclination angle of 90 indicates more vertical plant material. Results shown for full canopy development (GS5).

Figure 3: Depth distributions of leaf material and light interception for all growth stages of rice grown under high- (HN) or reduced- (RN) nitrogen levels. Left Panel: Cumulative Leaf Area Index (cLAI; leaf area per unit ground area as a function of depth). Right Panel: Fractional interception (FI) as a function of depth.

Figure 4: Modelled averaged light intensity received according to the fraction of leaf [surface] area in the top third of each canopy of three rice varieties grown under high- (HN) or reduced- (RN) nitrogen levels at 1200 h during full canopy development (GS5). The average irradiance received by each line in this canopy layer is indicated by the arrow.

Figure 5: Greenness (SPAD values) of the top of the canopy of three rice varieties grown under high- (HN) or reduced- (RN) nitrogen levels. Shown are the means (n=15) and SEM. Bars indicate significant difference between the comparisons, (p<0.05), following a two-way ANOVA and Tukey’s multiple comparisons test.
Figure 6: Percentage leaf nitrogen (of dry weight) in the top layer of the canopy for the three varieties grown under high- (HN) or reduced- (RN) nitrogen levels obtained from Kjeldahl analysis conducted by Lancrop Laboratories (Pocklington, York). Shown are the means and SEM (n=3). Bars indicate significant difference (p<0.05) following a two-way ANOVA and Tukey’s multiple comparisons test.

Figure 7: Fitted $P_{\text{max}}$ values from light response curves at full canopy closure (GS5) of three rice varieties grown under high- (HN) or reduced- (RN) nitrogen levels.

Figure 8: Modelled predicted carbon gain per unit leaf (A) and ground (B) area for each growth stage of rice grown under high- (HN) or reduced- (RN) nitrogen levels. An empirical model of photosynthesis was employed that calculates carbon gain from ray tracing values, parameterised from measured light response curves. This is integrated over the whole canopy over the course of the day for each growth stage. Fitted $P_{\text{max}}$ values used during modelling are given in Supplementary Table S2.
Supplementary Material Legends

Supplementary Figure S1: Number of tillers for three rice varieties grown under high- (HN) or reduced- (RN) nitrogen levels over time. Data was fitted using a sigmoidal dose-response (variable slope). Shown are the means (n=5) and SEM.

Supplementary Table S1: Maximum carboxylation rate of Rubisco (Vcmax), RuBP regeneration rate (J) and triosphosphate utilization (TPU) for three rice varieties grown under high- (HN) or reduced- (RN) nitrogen levels at 25°C. Measurements were made on the youngest fully extended leaf at GS2 and 3. Values were calculated using the curve-fitting tool by Sharkey et al., (2007). *a-b* Means in a column without a common superscript letter differ (P < 0.05), as analyzed by two-way ANOVA and Tukey's multiple comparison's test.

Supplementary Table S2: P_{max} values taken from fitted light response curves for three rice varieties grown under high- (HN) or reduced- (RN) nitrogen levels, used to calculate canopy carbon gain (Figure 6).
### Table 1

| Genotype | GS2  | GS3  | GS4  | GS5  |
|----------|------|------|------|------|
| IR64 RN  | 0.730| 0.477| 0.417| 0.422|
| IR64 HN  | 0.559| 0.423| 0.322| 0.333|
| MR219 RN | 0.633| 0.512| 0.415| 0.337|
| MR219 HN | 0.560| 0.418| 0.434| 0.311|
| MR253 RN | 0.679| 0.419| 0.437| 0.329|
| MR253 HN | 0.625| 0.419| 0.394| 0.388|

Table 1: Modelled canopy extinction coefficient ($k_{rt}$) values calculated from reconstructions and ray tracing in rice grown under high- (HN) or reduced- (RN) nitrogen levels at growth stages 2-5; where GS5 represents full canopy closure.
Table 2: Plant dry weight measurements throughout development in rice varieties grown under high- (HN) or reduced- (RN) nitrogen levels. Mean± SEM, n=3. "abcd"Means in a column without a common superscript letter differ (P < 0.05), as analyzed by two-way ANOVA and Tukey's multiple comparisons test.

| Treatment | GS2 (g)     | GS3 (g)     | GS4 (g)     | GS5 (g)     | Harvest (g) | Seed Dry Weight (g plant⁻¹) |
|-----------|-------------|-------------|-------------|-------------|-------------|----------------------------|
| IR64 RN   | 2.40±0.35   | 8.94±2.53   | 16.35±7.18  | 18.64±3.58bc| 18.59±1.98b | 14.74±1.67                 |
| IR64 HN   | 2.41±0.41   | 10.75±1.13  | 10.60±1.20  | 27.07±7.68a | 19.36±1.86a | 14.42±1.40                 |
| MR219 RN  | 2.57±0.42   | 13.13±3.64  | 14.94±3.37  | 16.72±4.33c| 15.14±1.50bc| 16.45±1.86                 |
| MR219 HN  | 3.27±0.82   | 8.50±2.82   | 15.04±0.89  | 25.75±8.58ab| 27.23±3.22ab| 22.82±3.04                 |
| MR253 RN  | 2.06±0.49   | 6.13±1.26   | 11.44±0.35  | 9.99±1.50d  | 17.46±1.85b | 14.79±2.18                 |
| MR253 HN  | 2.19±0.69   | 11.77±0.86  | 13.63±2.32  | 18.47±2.98c | 29.99±3.44a | 27.06±4.34                 |
References

Bojović B, Marković A (2009) Correlation between nitrogen and chlorophyll content in wheat (Triticum aestivum L.). Kragujev J Sci 31: 69–74

Burgess AJ, Retkute R, Herman T, Murchie EH (2017) Exploring relationships between canopy architecture, light distribution, and photosynthesis in contrasting rice genotypes using 3D canopy reconstruction. Front Plant Sci. doi: 10.3389/fpls.2017.00734

Burgess AJ, Retkute R, Pound MP, Foulkes J, Preston SP, Jensen OE, Pridmore TP, Murchie EH (2015) High-resolution three-dimensional structural data quantify the impact of photo inhibition on long-term carbon gain in wheat canopies in the field. Plant Physiol 169: 1192–1204

Cassman KG (1999) Ecological intensification of cereal production systems: Yield potential, soil quality, and precision agriculture. Proc Natl Acad Sci U S A 96: 5952–5959

Challinor AJ, Watson J, Lobell DB, Howden SM, Smith DR, Chhetri N (2014) A meta-analysis of crop yield under climate change and adaptation. Nat Clim Chang 4: 287–291

Chen YZ, Murchie EH, Hubbart S, Horton P, Peng SB (2003) Effects of season-dependent irradiance levels and nitrogen-deficiency on photosynthesis and photo inhibition in field-grown rice (Oryza sativa). Physiol Plant 117: 343–351

Cowan IR, Lange OL, Nobel PS, Osmond CB, Ziegler H (1982) Regulation of water use in relation to carbon gain in higher plants. Physiol. plant Ecol. 589–613; 3:

Ehleringer J, Werk K (1986) Modifications of solar-radiation absorption patterns and implications for carbon gain at the leaf level. In T Givnish, ed, Econ. Plant Form Funct. Proc. Sixth Maria Moors Cabot Symp. “Evolutionary constraints Prim. Product. Adapt. patterns energy capture plants,” Harvard For. August 1983. Cambridge University Press, Cambridge, London, New York, New Rochelle, Melbourne, Sydney, pp 57–82

Ezcurra E, Montana C, Arizaga S (1991) Architecture, light interception, and distribution of Larrea species in the Monte Desert, Argentina. Ecology 72: 23–34

Falster D, Westoby M (2003) Leaf size and angle vary widely across species: What consequences for light interception? New Phytol 158: 509–525

Furukawa Y, Ponce J (2010) Accurate, Dense, and Robust Multiview Stereopsis. IEEE Trans Pattern Anal Mach Intell 32: 1362–1376

Godin C (2000) Representing and encoding plant architecture: A review. Ann For Sci 57: 413–438

Gu J, Chen Y, Zhang H, Li Z, Zhou Q, Yu C, Kong X, Liu L, Wang Z, Yang J (2017a)
Canopy light and nitrogen distributions are related to grain yield and nitrogen use efficiency in rice. F Crop Res 206: 74–85

Gu J, Zhou Z, Li Z, Chen Y, Wang Z, Zhang H (2017b) Rice (Oryza sativa L.) with reduced chlorophyll content exhibit higher photosynthetic rate and efficiency, improved canopy light distribution, and greater yields than normally pigmented plants. F Crop Res 200: 58–70

Harasim E, Wesolowski M, Kwiatowski C, Cierpiala R (2016) The effect of retardants and nitrogen fertilization on winter wheat canopy structure. Rom. Agric. Res.

Herman T, Murchie E, Ali A (2015) Rice Production and Climate Change: A Case Study of Malaysian Rice. Pertanika J Trop Agric Sci 38: 321–328

Hubbart S, Ajigboye OO, Horton P, Murchie EH (2012) The photoprotective protein PsbS exerts control over CO2 assimilation rate in fluctuating light in rice. Plant J 71: 402–412

Hubbart S, Smillie I, Heatley M, Swarup R, Foo C, Zhao L, Murchie E (2018) Enhanced thylakoid photoprotection can increase yield and canopy radiation use efficiency in rice. Commun Biol 1: 22

King D (1997) The Functional Significance of Leaf Angle in Eucalyptus. Aust J Bot 45: 619

Kozłowska-Ptaszyńska Z (1993) Changes in the structure and architecture of plant canopy of two- and six-rowed spring barley cultivars as influence by seeding date. Pam Pul 102: 53–63

Kropff MJ, Cassman KG, Van Laar HH, Peng S (1993) Nitrogen and yield potential of irrigated rice. Plant Soil 155–156: 391–394

Mae T (1997) Physiological nitrogen efficiency in rice: Nitrogen utilization, photosynthesis, and yield potential. Plant Soil 196: 201–210

Murchie E, Reynolds M (2012) Crop radiation capture and use efficiency. In R Meyers, ed, Encycl. Sustain. Sci. Technol. Springer, pp 2615–2638

Murchie EH, Chen YZ, Hubbart S, Peng SB, Horton P (1999) Interactions between senescence and leaf orientation determine situ patterns of photosynthesis and photoinhibition in field-grown rice. Plant Physiol 119: 553–563

Murchie EH, Pinto M, Horton P (2009) Agriculture and the new challenges for photosynthesis research. New Phytol 181: 532–552

Peng S, Buresh RJ, Huang J, Zhong X, Zou Y, Yang J, Wang G, Liu Y, Hu R, Tang Q, et al (2010) Improving nitrogen fertilization in rice by sitespecific N management. A review. Agron Sustain Dev 30: 649–656
Peng SB, Buresh RJ, Huang JL, Yang JC, Zou YB, Zhong XH, Wang GH, Zhang FS (2006) Strategies for overcoming low agronomic nitrogen use efficiency in irrigated rice systems in China. F Crop Res 96: 37–47

Porra RJ, Thompson WA, Kriedemann PE (1989) Determination of Accurate Extinction Coefficients and Simultaneous-Equations for Assaying Chlorophyll-a and Chlorophyll-B Extracted with 4 Different Solvents - Verification of the Concentration of Chlorophyll Standards by Atomic-Absorption Spectroscopy. Biochim Biophys Acta 975: 384–394

Pound M, French A, Murchie E, Pridmore T (2014) Automated recovery of 3D models of plant shoots from multiple colour images. Plant Physiol 144: 1688–1698

Rahman S, Duursma R, Muktadir M, Roberts T, Atwell B (2018) Leaf canopy architecture determines light interception and carbon gain in wild and domesticated Oryza species. Environ Exp Bot 155: 672–680

Ray D, Ramankutty N, Mueller N, West P, Foley J (2012) Recent patterns of crop yield growth and stagnation. Nat Commun 3: 1–7

Redfern S, Azzu N, Binamira J (2012) Rice in Southeast Asia: facing risks and vulnerabilities to respond to climate change. Build Resil. Adapt Acclim. Chang. Agri Sect. 23:

Retkute R, Smith-Unna SE, Smith RW, Burgess AJ, Jensen OE, Johnson GN, Preston SP, Murchie EH (2015) Exploiting heterogeneous environments: Does photosynthetic acclimation optimize carbon gain in fluctuating light? J Exp Bot. doi: 10.1093/jxb/erv055

Ryel RJ, Beyschlag W, Caldwell M (1993) Foliage Orientation and Carbon Gain in Two Tussock Grasses as Assessed With a New Whole- Plant Gas-Exchange Model. Funct Ecol 7: 115–124

Shindo M, Yamamoto S, Shimomura K, Umehara M (2020) Strigolactones Decrease Leaf Angle in Response to Nutrient Deficiencies in Rice. Front Plant Sci 11: 135

Song Q, Wang Y, Qu M, Ort DR, Zhu XG (2017) The impact of modifying photosystem antenna size on canopy photosynthetic efficiency—Development of a new canopy photosynthesis model scaling from metabolism to canopy level processes. Plant Cell Environ. doi: 10.1111/pce.13041

Song Q, Zhang G, Zhu X-G (2013) Optimal crop canopy architecture to maximise canopy photosynthetic CO2 uptake under elevated CO2 – a theoretical study using a mechanistic model of canopy photosynthesis. Funct Plant Biol 40: 109–124

Valladares F, Pugnaire F (1999) Tradeoffs Between Irradiance Capture and Avoidance in Semi-arid Environments Assessed with a Crown Architecture Model. Ann Bot 83: 459–469
Verhoeven AS, DemmigAdams B, Adams WW (1997) Enhanced employment of the xanthophyll cycle and thermal energy dissipation in spinach exposed to high light and N stress. Plant Physiol 113: 817–824

Walker B, Drewry D, Slattery R, VanLoocke A, Cho Y, Ort D (2018) Chlorophyll Can Be Reduced in Crop Canopies with Little Penalty to Photosynthesis. Plant Physiol 176: 1215 LP – 1232

Wann M, Raper C (1979) A Dynamic Model for Plant Growth: Adaptation for Vegetative Growth of Soybeans 1. Crop Sci 19: 461–467

Werner C, Ryel RJ, Correia O, Beyschlag W (2001) Effects of photoinhibition on whole-plant carbon gain assessed with a photosynthesis model. Plant Cell Environ 24: 27–40

Wu C (2011) Visual SFM: A visual structure from motion system. http://ccwu.me/vsfm,

Zheng B, Shi L, Ma Y, Deng Q, Li B, Guo Y (2008) Comparison of architecture among different cultivars of hybrid rice using a spatial light model based on 3-D digitising. Funct Plant Biol 35: 900–910
|     | GS1 | GS2 | GS3 | GS4 | GS5 |
|-----|-----|-----|-----|-----|-----|
| IR64 RN | ![Image] | ![Image] | ![Image] | ![Image] | ![Image] |
| IR64 HN | ![Image] | ![Image] | ![Image] | ![Image] | ![Image] |
| MR219 RN | ![Image] | ![Image] | ![Image] | ![Image] | ![Image] |
| MR219 HN | ![Image] | ![Image] | ![Image] | ![Image] | ![Image] |
| MR253 RN | ![Image] | ![Image] | ![Image] | ![Image] | ![Image] |
| MR253 HN | ![Image] | ![Image] | ![Image] | ![Image] | ![Image] |

10 cm
