1 Interactions between nitrogen nutrition, canopy

2 architecture and photosynthesis in rice, assessed using

3 high resolution 3D reconstruction

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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

78 necessarily improve yield or the crop's tolerance to uncertain climatic conditions (Kropff et al.,

79 1993; Murchie et al., 2009; Peng et al., 2010). Furthermore, studies indicate that at any given soil

80 N content, significantly lower yields were achieved towards the end of the 21st century than the

81 preceding three decades (Cassman, 1999). Application of N fertiliser in excess of that required

82 can even lead to negative effects including mutual shading, lodging and pest damage (Peng et

83 al., 2006; Peng et al., 2010). Thus, understanding the crop response to a change in N levels, and

84 selecting varieties that are capable of outperforming others will be critical to reduce overreliance

85 on fertilisers.

86

87 As a primary constituent of essential proteins and enzymes that are involved in important plant

88 metabolic processes, N is essential in the formation of the plant canopy and increasing

89 photosynthetic leaf area. Photosynthetic components are a significant sink for leaf N:

90 chloroplasts account for up to 80 % of total leaf N with Rubisco being the dominant enzyme

91 (Makino & Osmond, 1991). Leaf photosynthetic capacity and Rubisco content per unit leaf area

92 is highly correlated with leaf N both within and between species (Evans, 1989; Theobald *et al.*,

93 1998). N affects a number of developmental traits including plant height, panicle number, leaf

94 size and spikelet number, all of which contribute to the yield potential of the crop. As a key

95 requirement for cell division and expansion, N is integral for development, growth and final

96 organ size (Wann and Raper, 1979). During the vegetative growth stage, absorbed N primarily

97 promotes early growth and increases the number of tillers (Mae, 1997). For the formation of

98 dense canopies, large concentrations of N are required (Connor *et al.*, 2011). In N deficient

99 conditions, the plant counterbalances the lack of N by producing a lower number of tillers; a

100 compensations step that allows for fewer but fully functional leaves (Chen et al., 2003).

101 Consequently, N deficiency generally reduces leaf area index, intercepted radiation, plant height

102 and canopy photosynthesis rate (Connor *et al.,* 2011). Even a mild N deficiency can make

103 moderate changes to plant structure that will have a large impact on the light distribution and

104 thus productivity of canopies but little is known about the changes in 3D structure (and hence

105 light dynamics) in crop canopies with differing N content.

106

107 Assessing the productivity of crops is confounded by heterogeneous nature of plant and crop

108 canopies; they commonly consist of multiple plants exhibiting different growth and

109 developmental patterns (Kozłowska-Ptaszyńska, 1993; Godin, 2000). Therefore, understanding

110 plant response to changes in N levels requires experimental data combined with high-resolution

111　information on the physiological characteristics associated with a particular canopy architecture.
112　This could be achieved through modelling approaches that can make more accurate predictions
113　of the canopy light environment, and thus the influence of architecture, compared to manual
114　measurements. Monitoring plant growth and estimating canopy photosynthesis rate and
115　efficiency in the field on a large scale is a complex task. Whilst some research has been carried
116　out to study the effects of varying N treatments on crop systems (e.g. Harasim *et al.,* 2016 for
117　wheat) and on isolated rice varieties (Mae, 1997; Herman et al., 2015), few studies exist to
118　investigate how different varieties respond to varying N treatments in terms of changes to their
119　canopy architecture. Using photosynthesis measurements alongside the three-dimensional (3D)
120　modelling of crop canopies, we can explore plant structure and estimate crop productivity at the
121　whole canopy scale, which would not be feasible using manual measurements (Song et al., 2013;
122　Burgess et al., 2017).
123
124　Here we employ such methods (Pound *et al.* 2014) to investigate the effect of N availability on
125　three rice lines. Because N is an integral component in photosynthetic machinery and in forming
126　structural tissue we hypothesise that the differences in both canopy architecture and
127　photosynthesis from alteration of N content will influence not only the vertical light gradient and
128　the spatio-temporal variation in light. Further we hypothesise that specific N-dependent changes
129　in architecture such as leaf angle will influence this canopy light distribution. This work will
130　identify whether the optimal canopy architecture for reduced N conditions differs to that of high
131　N conditions.
132

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.

165 Soil-plant analyses development (SPAD) measurements were taken *in situ* using the Minolta 502

166 Plus Chlorophyll Meter (Spectrum Technologies, USA), to obtain the total available chlorophyll

167 within a specified leaf area. Chlorophyll a and b content were determined spectrophotometrically.

168 Frozen leaf samples of known area were ground in 80% acetone. The samples were then

169 centrifuged for 5 minutes at 300 rpm and the absorbance (at 663 and 645 nm) of the supernatant

170 was measured using a spectrophotometer. Chlorophyll a and b content were calculated using the

171 protocol of Porra *et al.* (1989).

172

173 Leaf thickness was measured at the major and minor veins using leaf sectioning. Sections of the

174 penultimate leaf on the main stem were cut from the widest part of the leaf using a sharp razor

175 blade, for mounting on microscope slides. After mounting, the leaf sections were cleared using

176 85% (w/v) lactic acid saturated with chloral hydrate. The slides were heated in a hot water bath

177 (70°C) for an hour. After clearing, the leaf sections were washed with distilled water and stained

178 using 1% toluidine blue dye in 1% (w/v) disodium tetraborate. A few drops of glycerol were

179 added to the leaf sections to preserve the samples before being viewed under a calibrated light

180 microscope and images captured using a digital camera (Nikon DXM 1200). Stomatal density

181 and length were determined using leaf impressions of both the adaxial and abaxial surfaces on

182 the widest part of the flag leaf. Impressions were made using Coltène® PRESIDENT Plus

183 silicone-based impression putty. Clear nail varnish was then applied to the hardened putty and

184 later peeled and mounted on microscope glass slides for view under a 40x-magnification confocal

185 light microscope. Images of six fields of view were taken for each variety under each treatment

186 for analysis (Hubbart et al., 2012). All images for leaf thickness and stomata were analysed using

187 the analytical software ImageJ.

188

189 **2.3   Leaf Nitrogen**

190 Leaf nitrogen analysis was carried out by Lancrop Laboratories, York, United Kingdom. Three

191 plants per plot were chosen at random for this analysis. For each plant, leaves were clipped at the

192 top portion of the canopy (including the flag leaf, at 10 cm height) to make up 200 grams of fresh

193 plant material. Samples were labeled and couriered to the laboratory on the same day.

194

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 μmol m^{-2} 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 μmol m^{-2} 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, 17th 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).

$$d_i = \max_{j=1,2,3; 1 \leq i \leq n} z_i^j - (z_i^1 + z_i^2 + z_i^3)/3 \tag{1}$$

260

261 *d* was also used as a reference to dived canopies into layers, with all triangles above the midpoint,

262 d_{mid} assigned the upper layer, and those below the lower layer. Two reference points were used

263 for GS5 to split the canopy into three layers: top, middle and bottom.

264

265 We calculated the sum of the areas of all triangles and then divided this sum by ground area. The

266 cumulative LAI as a function of depth through the canopy was calculated using Eq. 2.

267

268 $$cLAI = \frac{\sum_{i=1}^{n} I(d_i \leq d) S_i}{\left(\max_{1 \leq i \leq n} x_i - \min_{1 \leq i \leq n} x_i\right)\left(\max_{1 \leq i \leq n} y_i - \min_{1 \leq i \leq n} y_i\right)},$$ (2)

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

270

271 Leaf angle distributions were calculated for each canopy and averaged at each canopy depth by

272 using the angle of each 2D triangular face relative to horizontal; where an angle of 0 indicates a

273 more horizontal leaf section and an inclination angle of 90 indicates a more vertical leaf section.

274

275 The light extinction coefficient of the canopy was calculated using the 3D structural data and the

276 light distribution obtained from ray tracing. In order to calculate fractional interception, *F*, within

277 a canopy as a function of depth at time *t*, we first identified all triangles lying above depth, *d* (Eq.

278 1). We then calculated their contribution to intercepted light by multiplying PPFD received per

279 unit surface area (ray tracing output) by the area of triangle. The light intercepted was summed

280 for all triangles above the set *d,* and divided by light intercepted by ground area according to Eq.

281 3.

282

283 $$F = \frac{\sum_{i=1}^{n} I(d_i \leq d) S_i L_i(t)}{L_0(t) * ground\ area},$$ (3)

284 where *L_0(t)* is light received on a horizontal surface with a ground area $\left(\max_{1 \leq i \leq n} x_i - \right.$

285 $\left.\min_{1 \leq i \leq n} x_i\right)\left(\max_{1 \leq i \leq n} y_i - \min_{1 \leq i \leq n} y_i\right)$ and $L_i(t)$ is light intercepted by a triangle *i*.

286

287 The light extinction coefficient derived from ray tracing data, k_{rt}, was calculated by fitting (by

288 least squares) the function according to Burgess et al. (2017):

289 $$F(x) = a(1 - e^{-krt\ x})$$ (4)

290   to the set of points $\{cLAI(d), F(d, t)\}$ calculated by varying depth from 0 to the height at total

291   c*LAI* with step $\Delta d = 1$ mm (Supplementary Figure S6.4), where *a* in Eq.(4) is a fitted parameter.

292

293   The response of photosynthesis to light irradiance, *L*, was calculated using a nonrectangular

294   hyperbola given by Eq. 5:

295    $$P = \frac{\phi L + (1 + \alpha)P_{max} - \sqrt{(\phi L + (1 + \alpha)P_{max})^2 - 4\theta\phi L(1 + \alpha)P_{max}}}{2\theta} - \alpha P_{max}$$

296   (5)

297   The nonrectangular hyperbola is defined by four parameters: the quantum use efficiency, ϕ; the

298   convexity, θ; the maximum photosynthetic capacity; *P$_{max}$*, and the rate of dark respiration, *R$_d$*.

299   We assumed that the rate of dark respiration is proportional to the maximum photosynthetic

300   capacity, according to the relationship $R_d = \alpha\ P_{max}$ (Retkute et al., 2015). Values for *P$_{max}$* were

301   determined from leaf gas exchange measurements for the two canopy layers: top and bottom**.** For

302   GS1-4 (prior to flowering), the light response curve data was averaged prior to LRC fitting, as

303   the shorter 7-point curves (see Materials and Methods: Gas Exchange) do not give a good fit. For

304   GS5, all individual curves were fit; the mean ± SEM is presented in Table 6.3. Curve fitting was

305   carried out using the Mathematica command **FindFit** with a minimum constraint on dark

306   respiration at 0.05 and convexity at 0.7.

307

308   As each canopy was divided into two layers, each triangle from the digital plant reconstruction

309   was assigned to a particular layer *m* according to the triangle centre (i.e. with triangle centre

310   between upper and lower limit of a layer depth). Carbon gain per unit leaf area was calculated as

311   daily carbon assimilation over a whole canopy divided by the total surface area of the canopy

312   according to Eq. 6.

313    $$C_l = \frac{\sum_{i=1}^{n} P_i}{\sum_{i=1}^{n} S_i}.$$ (6)

314

315   Carbon gain per unit ground area was calculated as daily carbon assimilation over a whole canopy

316   divided by the area inside the ray tracing boundaries according to Eq. 7.

317    $$C_g = \frac{\sum_{i=1}^{n} P_i}{(\max x_i - \min x_i)(\max y_i - \min y_i)}.$$ (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

350　increased chlorophyll density in the upper layers (below). This is consistent with the leaf angles

351　given in Figure 2 but only for IR64. However, the lack of consistency in angle distributions of

352　leaf material indicates that angle is not substantially influenced by N treatment and that light

353　attenuation is likely to be more influenced by LAI in the upper layers of the canopy.

354

355　Figure 3 shows how the accumulation of plant material along the vertical transect of the canopy

356　(cumulative leaf area index; cLAI) and the corresponding interception of light (fractional

357　interception; F) from modelled data. High N treatment plants accumulated a greater LAI at

358　middle and upper portions of the canopy in GS1-GS3. In GS4 there was a higher accumulation

359　of LAI throughout the canopy in the reduced N treatment but with a lower overall plant height,

360　resulting in a reduced overall LAI. This indicates that reduced N treatment had a delayed

361　progression of canopy development. These differences in leaf accumulation influences the

362　canopy depth at which most light interception occurs, indicated by the steepness of the curve in

363　Figure 3. By GS5, the overall F was equivalent for all treatments but differences in distribution

364　of F between treatment and variety could be seen in intermediate layers.

365

366　Figure 4 show how PPFD is distributed throughout the canopy according to the fraction of total

367　canopy leaf surface area. In this way it becomes possible to visualize the prevalence of different

368　PPFD values. This is analogous to a frequency histogram and was used in a previous study

369　(Burgess et al., 2015) to examine the distribution of levels of radiation that are saturating and sub

370　– saturating for photosynthesis, which is also relevant here. For example, an erect canopy would

371　be expected to have a higher leaf area exposed to a higher PPFD values overall. This is especially

372　important for the lower layers of the canopy which, in erect canopies, have increased light

373　penetration which would allow leaves in these lower layers to increase photosynthesis . Here we

374　cannot see consistent patterns in terms of treatment. We note that reduced N IR64 has a larger

375　proportion of leaf area exposed to PPFDs of 100 μmol m^{-2} s^{-1} or lower, indicating a high level of

376　self-shading consistent with the higher k_{rt} value at GS5 (Table 1) and the particularly low leaf

377　angle in upper layers (Fig 2C) suggesting that in IR64, N treatment results in altered leaf angle

378　and altered light distribution. Optimising light distribution at reduced N could therefore be

379　achieved by either altering depth dependent leaf area accumulation or leaf angle.

380

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 cholorophyll 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_{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_{rt} values, Table 1). It is possible to conclude that the drop in P_{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_{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_{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_{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_{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_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

414 leaf area (Fig 8A) especially in GS4 and GS5. The higher values for reduced N treatment would

415 seem to arise from the improved light distribution as a result of the lower k. However, when

416 expressed per unit ground area there was a consistently higher carbon gain for the HN plants,

417 with IR64 showing the highest values. At GS4 and GS5 there was little difference. This is

418 partially consistent with the accumulation of LAI (Figs. 2 & 3), indicating that despite a slightly

419 lower carbon gain per unit leaf area, the increased biomass compensated for this reduction and

420 improved over carbon gain on a land area basis.

421

422 **3.4   Increased nitrogen increases harvestable biomass of selected lines**

423 Altered N nutrition and corresponding changes in canopy development had opposing effects on

424 biomass production in each of the three varieties at harvest but not throughout development.

425 Biomass (dry weights) (Table 2) between varieties and treatments were not statistically different

426 from GS1 to GS4. In GS5, all varieties in the reduced N treatment had lower biomass than the

427 high N treatments ($p<0.05$). Similarly, harvest biomass was observed to be significantly different

428 between treatments, with MR253 showing substantially lower biomass in reduced N plants

429 relative to high N plants ($p<0.0001$). However high N treatment showed significantly ($P<0.05$)

430 higher harvest DW and seed DW compared to reduced N, except for in IR64 where the effects

431 were minimal suggesting that under these conditions, biomass accumulation in IR64 is not N-

432 dependent. Within the Malaysian lines, the higher LAI, particularly at GS3, may have been

433 critical in driving the increase in biomass at harvest.

434

4   Discussion

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,

468 modifying k values and indeed the ratio between k for light and for nitrogen, something that has
469 been suggested to be the result of management practice affecting cytokinin synthesis although
470 the reasons for these changes may not always be apparent (Gu et al., 2017a). However a recent
471 study showed how leaf angle responds to nutrient deficiencies in rice, mediated by strigolactone
472 (Shindo et al., 2020). As leaf angle infuences light distribution and there is a functional link
473 between N and light profiles, there is clearly a need to further understand how N deficiency might
474 interact with light profiles to determine canopy photosynthesis.

475

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

490

491 A tradeoff between leaf area, N and photosynthesis has been seen previously in field-grown rice
492 and can be viewed as a tendency to prevent 'dilution' of canopy N (Chen et al., 2003). N regulates
493 growth rate, such that N is allocated to a smaller number of leaves, resulting in conserved P_{max}
494 values irrespective of N treatment. This is consistent with results in this study, where minimal
495 effects were seen between P_{max} values under different N treatments. Interestingly, MR219
496 showed slightly more susceptibility to a lowered N status compared to MR253, which could
497 explain why MR253 is suitable for more marginal growing conditions in Malaysia. The residual
498 N levels in this trial were mildly deficient (Rice Knowledge Bank, 2015). This may have
499 contributed to the results witnessed here; namely no change in light saturated photosynthesis but
500 an increase in biomass in Malaysian lines. The similar leaf photosynthetic rates between N

501 treatments mean that the differences in biomass and yield may come from canopy level
502 processes, influenced by structure and development. Canopy photosynthesis rate can be thought
503 of as the 'sum' of photosynthesis in all leaves in the canopy at any given time point. The light
504 within the canopy will fluctuate on wide spatio-temporal scales according tofactors such as solar
505 movement. Hence it is necessary, when considering changes in canopy architecture to use ray
506 tracing and modelling of photosynthesis. A higher canopy photosynthesis rate is seen in high N
507 treated canopies but largely in the early growth stages (GS1- 3) when the differences in LAI
508 between treatments were greatest. It is interesting to note the observed higher carbon gain per
509 unit leaf area in IR64 subject to reduced N, particularly during the mid-growth stages. This could
510 be attributed to a possible growth 'advantage' of having a smaller canopy with less tillers,
511 resulting in leaf tissue being exposed to higher light intensities relative to the same positions
512 under high N. Therefore, we can conclude that the accumulation of leaf area and therefore light
513 capture during canopy development is important in enhancing canopy photosynthesis rates.
514 Second, the supposedly improved leaf angle in the reduced N treated plants was not sufficient to
515 enable these plants to achieve the canopy photosynthesis rates seen in the high N plants.

516

517 Increased LAI also corresponded to greater height and greater dry weight (DW), however whilst
518 the two Malaysian lines exhibit a strong response to N treatment, IR64 is less sensitive. These
519 results are consistent with previous studies on the effect of N application on IR64, which
520 indicated that applications above 90-100 kg N ha^{-1} (using green manure) did not increase the
521 agronomic efficiency of the system (Morris *et al.,* 1989; Diekmann *et al.,* 1996) and on MR219
522 where increases in the N application rate led to concurrent increases in the grain (Nori *et al.,*
523 2008). Similar patterns can be seen for seed dry weight per plant: with IR64 exhibiting similar
524 values under both N treatments but a large increase for both MR219 and MR253. Grain weights
525 were not consistent with modelled canopy photosynthesis rates indicating that there may be other
526 factors such as partitioning during the grain filling period. However, the ranking of DW during
527 GS5 are consistent with ranking of modelled photosynthesis (per unit ground area) during most
528 of the growth stages indicating a general correspondence between modelled canopy
529 photosynthesis and measured biomass up to GS5. The results presented here indicate contrasting
530 N uptake and utilisation responses of the three varieties, with MR219 and MR253 capable of
531 utilising the extra N available in the soil.

532

533 Contrasting strategies can be seen in different crops in relation to N availability. In Potato
534 (*Solanum tuberosum* L.), excess N led to enhanced apical branching and prolonged production
535 of vegetative organs leading to a greater number of leaves per plant (Vos and Biemond, 1992;
536 Biemond and Vos, 1992). Conversely, under N limitation leaf size was reduced (via reduced leaf
537 expansion rates) in order to maintain N concentration per unit leaf area and the photosynthetic
538 capacity of the leaf (Vos and Van der Putten, 1998). In contrast, Maize (*Zea Mays* L.) exhibits a
539 more conservative response to changes in leaf size relative to potato and reduces total leaf area
540 by approximately 30% (Vos *et al.,* 2005). Furthermore, maintaining higher leaf area comes at
541 the expense of decrease N per unit leaf area and a decrease in photosynthetic capacity. This
542 reflects two opposing strategies to N availability: the maintenance of photosynthetic productivity
543 per unit leaf area at the expense of total leaf area or; the maximisation of light interception per
544 unit leaf area at the expense of photosynthetic productivity. It is broadly expected, with some
545 exceptions, that these contrasting strategies represent the dicot versus the Gramineae response
546 (see Vos *et al.,* 2005 and references within). Whilst this study did not use limiting amounts of N
547 availability, results suggest that under excess N conditions, N is used for the production of
548 increased tiller number, a greater leaf area and maintenance of photosynthetic capacity per unit
549 leaf area in rice.
550
551

5   Concluding Remarks

553 High-resolution 3D canopy reconstruction revealed novel observations concerning the effect of
554 N treatment on canopy architecture and light distribution in rice. First, leaf photosynthetic
555 capacity was generally less responsive than leaf area to N treatment meaning that light capture
556 and light distribution were more important in determining canopy photosynthesis rates and dry
557 weights. The reduction in leaf area accumulation during the mild N deficiency occurred in the
558 mid canopy region and was associated with an improved canopy light distribution in the reduced
559 N treatment resulting in a higher carbon gain per unit leaf area compared to high N. We show
560 the improved canopy light distribution in reduced N is more likely to be due to depth- dependent
561 leaf area accumulation or of pigment distribution than leaf angle in the case of the Malaysian
562 lines. We show key differences between architecture in the Philippine variety IR64 and the
563 Malaysian cultivars: IR64 had a less upright leaves in upper canopy regions in high N which
564 negatively affected light distribution. Improvement of light distribution would be more beneficial

565   for the high N treatment and may improve yields even further than those seen here. This indicates
566   a potential for increasing yields by improving the light distribution in high N treated plants.
567
568

569   **Data**
570   Data is made available on Figshare (Figshare.com).
571

572

578

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 Legends

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.

622 Figure 6: Percentage leaf nitrogen (of dry weight) in the top layer of the canopy for the three
623 varieties grown under high- (HN) or reduced- (RN) nitrogen levels obtained from Kjeldahl
624 analysis conducted by Lancrop Laboratories (Pocklington, York). Shown are the means and SEM
625 (n=3). Bars indicate significant difference (p<0.05) following a two-way ANOVA and Tukey's
626 multiple comparisons test.
627
628 Figure 7: Fitted P_{max} values from light response curves at full canopy closure (GS5) of three rice
629 varieties grown under high- (HN) or reduced- (RN) nitrogen levels.
630
631 Figure 8: Modelled predicted carbon gain per unit leaf (A) and ground (B) area for each growth
632 stage of rice grown under high- (HN) or reduced- (RN) nitrogen levels. An empirical model of
633 photosynthesis was employed that calculates carbon gain from ray tracing values, parameterised
634 from measured light response curves. This is integrated over the whole canopy over the course
635 of the day for each growth stage. Fitted P_{max} values used during modelling are given in
636 Supplementary Table S2.
637
638

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).

655 **Tables**

656 **Table 1**

657

	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

666 Table 1: Modelled canopy extinction coefficient (k_{rt}) values calculated from reconstructions and
667 ray tracing in rice grown under high- (HN) or reduced- (RN) nitrogen levels at growth stages 2-
668 5; where GS5 represents full canopy closure.

669   **Table 2**

670

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±3.58^{bc}	18.59±1.98^{b}	14.74±1.67
IR64 HN	2.41±0.41	10.75±1.13	10.60±1.20	27.07±7.68^{a}	19.36±1.86^{b}	14.42±1.40
MR219 RN	2.57±0.42	13.13±3.64	14.94±3.37	16.72±4.33^{c}	15.14±1.50^{bc}	16.45±1.86
MR219 HN	3.27±0.82	8.50±2.82	15.04±0.89	25.75±8.58^{ab}	27.23±3.22^{ab}	22.82±3.04
MR253 RN	2.06±0.49	6.13±1.26	11.44±0.35	9.99±1.50^{d}	17.46±1.85^{b}	14.79±2.18
MR253 HN	2.19±0.69	11.77±0.86	13.63±2.32	18.47±2.98^{c}	29.99±3.44^{a}	27.06±4.34

671

672   Table 2: Plant dry weight measurements throughout development in rice varieties grown under

673   high- (HN) or reduced- (RN) nitrogen levels. Mean± SEM, n=3. $^{a-d}$Means in a column without a

674   common superscript letter differ ($P < 0.05$), as analyzed by two-way ANOVA and Tukey's

675   multiple comparisons test.

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789

	GS1	GS2	GS3	GS4	GS5

IR64 RN

IR64 HN

10 cm

MR219 RN

MR219 HN

MR253 RN

MR253 HN

cLAI   FI

GS1

GS2

GS3

GS4

GS5

A.

B.