

Supplementary Information

Microscope-based light gradient generation for quantitative studies of photosynthetic micro-organisms

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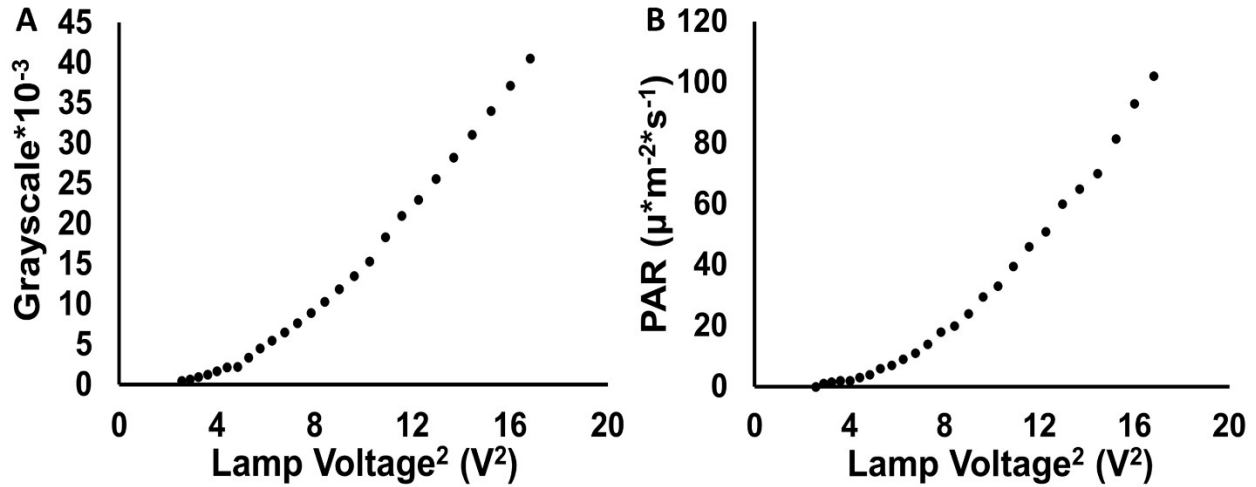


Figure S1 (A) Light source voltage squared versus mean adjusted grayscale value measured by the CCD camera. Grayscale was measured from bright field images in ImageJ before adjustment via subtraction of a 0 V blank, division by exposure time in ms, and multiplication by the ND8 filter ratio (to convert from 1 or 2 ND8 filters to the no filter condition). This adjusted grayscale is plotted on the y-axis. **(B)** Light source voltage squared versus light intensity measured by the PAR meter.

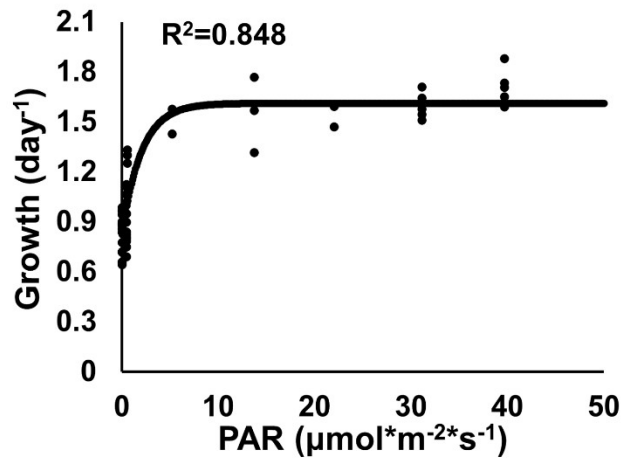


Figure S2 Growth rate of *C. reinhardtii* cells as a function of PAR using an array microhabitat. Dots are experimental values and line is a fit to van Oorschot model (equation 2). The fitted coefficients with 95% confidence bounds are $\mu_0=0.826\pm0.07$, $\mu_{max}'=0.787\pm0.095$ day⁻¹, and $K_S=2.03\pm1.1$ μmol/(m²*s), with an R-square value of 0.848.

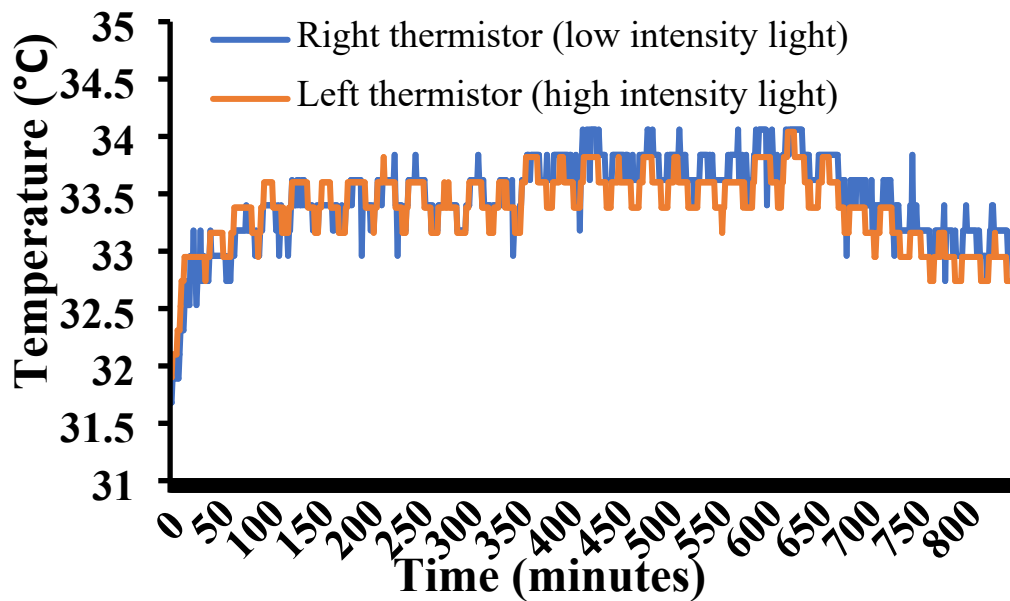


Figure S3 Temperature of wells in 96 well plate at low and high light intensities over time. Two thermistors were positioned at the bottom of the 96-well plate, one on the low light intensity side, the other on the high light intensity side. Temperature was recorded every minute for over 800 minutes. Despite the initial jump, the temperature was stably maintained over time with minor fluctuations within 1°C. There was no significant difference between the temperatures under low versus high light intensities.

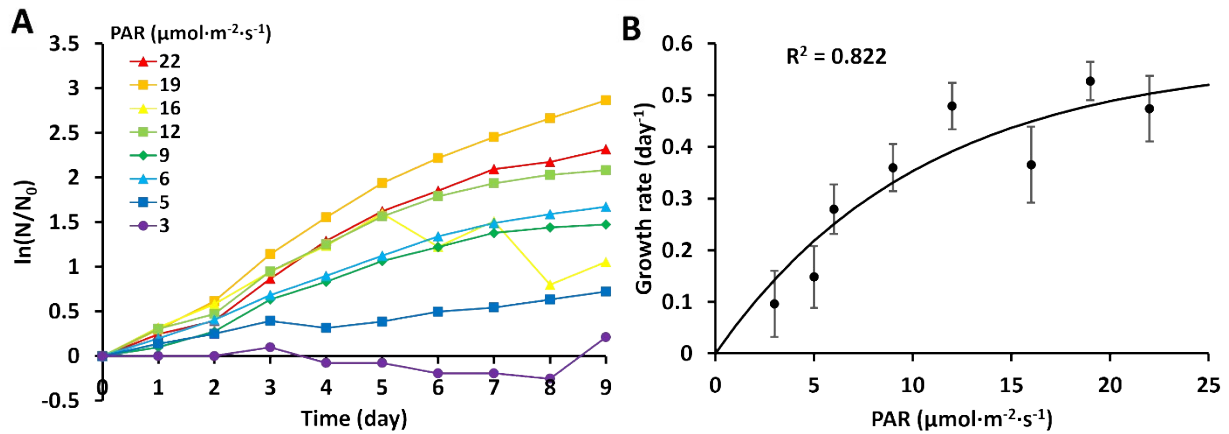


Figure S4 Light intensity regulates the growth rate of *M. aeruginosa* in a 96 well plate at 25°C. (A) The growth curves of *M. aeruginosa* under various light intensities. Average cell number (N) was obtained by measuring absorbance of replicated wells at the same light intensity, as shown in Fig. 3D. (B) The growth rates of *M. aeruginosa* under different light intensities. The growth rates were calculated using the growth curves of individual wells, and the error bars represent standard error of the mean (SEM). The dots contain experimental results, and the solid line is a fit to the van Oorschot model (Equation 2). The fitted coefficients with 95% confidence bounds are $\mu_{max}=0.57\pm0.27 \text{ day}^{-1}$, and $K_S=10.41\pm10.23 \text{ } \mu\text{mol}/(\text{m}^2\cdot\text{s})$, with an R-square value of 0.822.

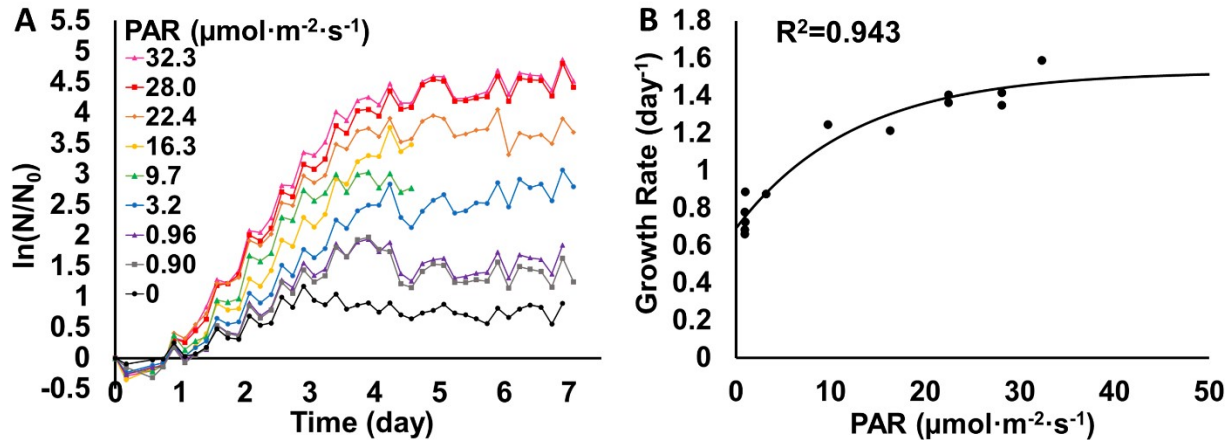


Figure S5 Growth response of *C. reinhardtii* to light intensity gradient in an array microhabitat. (A) Growth curves of *C. reinhardtii* cells at various light intensities. Each curve is an average of growth curves of suitable habitats (at least 1 per column) in the same column. PAR values represent light intensity at each column of habitats, with 0 PAR representing the control habitats. **(B)** Growth rate of *C. reinhardtii* cells as a function of PAR. Dots are experimental values and line is a fit to van Oorschot model (Equation 2). The fitted coefficients with 95% confidence bounds are $\mu_0=0.692\pm 0.11 \text{ day}^{-1}$, $\mu_{max}'=0.836\pm 0.24 \text{ day}^{-1}$, and $K_S=12.5\pm 11.9 \mu\text{mol}/(\text{m}^2\cdot\text{s})$, with an R-squared value of 0.943. This data was collected from 1 of 3 replicates.

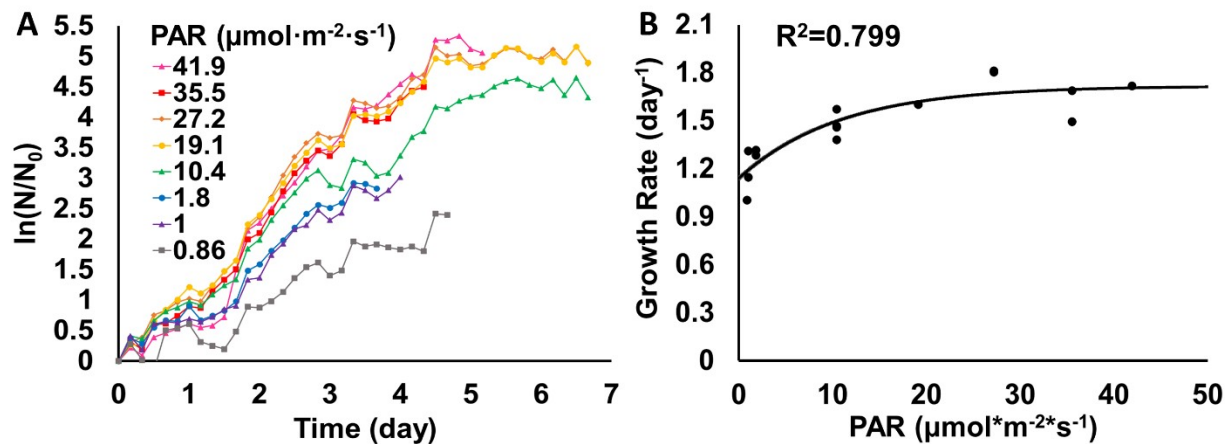


Figure S6 Growth response of *C. reinhardtii* to light intensity gradient in an array microhabitat. (A) Growth curves of *C. reinhardtii* cells at various light intensities. Each curve is an average of growth curves of suitable habitats (at least 1 per column) in the same column. PAR values represent light intensity at each column of habitats, with no control habitats. **(B)** Growth rate of *C. reinhardtii* cells as a function of PAR. Dots are experimental values and line is a fit to van Oorschot model (Equation 2). The fitted coefficients with 95% confidence bounds are $\mu_0=1.14\pm 0.14 \text{ day}^{-1}$, $\mu_{max}'=0.577\pm 0.18 \text{ day}^{-1}$, and $K_S=10.8\pm 10.4 \mu\text{mol}/(\text{m}^2\cdot\text{s})$, with an R-squared value of 0.799. This data was collected from 1 of 3 replicates.

Table S1: μ_{max} and K_S for *M. aeruginosa* and *C. reinhardtii* from experiments using microhabitats and 96 well plates

Experimental Setup	Parameter	<i>C. reinhardtii</i>	<i>M. aeruginosa</i>
Microhabitats	μ_{max} (day ⁻¹)	1.68 ¹	
	K_S ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)	1.9 ¹	
96-well plate	μ_{max} (day ⁻¹)	4.28 ²	0.57 ¹ , 0.77 ²
	K_S ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)	7.97 ²	10.41 ¹ , 7.17 ²
Macro-scale system (cm and above)	μ_{max} (day ⁻¹)	1.4-5.46 ¹	0.211 ³
	K_S ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)	81.4-215 ¹	8.79 ³

All *C. reinhardtii* values were obtained from a Monod model fit. All *M. aeruginosa* values were obtained from a van Oorschot model fit.
 1. Experiments performed at 25 °C. 2. Experiments performed at 31 °C. 3. Experiments performed at 20 °C.