

1 **List of Tables**

2 **Table 1.** Statistical analysis of the different 2D models indicating the fraction of pixels (non-
 3 void) for the cyanobacteria (red band), EPS (green band) and chlorophyll-a (blue band) with
 4 respect to the total number, and the two structures observed in the corresponding indicator
 5 variograms. Analysis was performed on the 10X images. The range of the first structure
 6 defines the average size of the small-aggregate, while the range of the second structure
 7 defines the average size of the large-aggregate. See also the figure in the Methodology
 8 section.

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	10D			10L			20D			20L		
	R	G	B	R	G	B	R	G	B	R	G	B
non-void pixels (p) x10 ⁻²	0.33	0.034	0.22	2.37	2.82	3.31	1.72	1.05	0.89	6.37	0.88	4.55
Total variance (p(L-p)) x10 ⁻²	0.33	0.034	0.22	2.32	2.75	3.2	1.69	1.04	0.89	5.96	0.87	4.34
Variogram 1st structure												
Sill [-]	0.003	0.0003	0.0019	0.019	0.016	0.021	0.0125	0.007	0.007	0.052	0.0067	0.034
	91%	91%	85%	83%	59%	68%	74%	72%	79%	87%	74%	77%
Range (in pixels)	3.6	2.4	1.6	2	3.6	2.8	3.5	4.2	3	2.5	4	3.2
Model	Exp	Exp	Exp	Exp	Exp	Exp	Exp	Exp	Exp	Exp	Exp	Exp
Variogram 2nd structure												
Sill [-]	0.0003	0.00004	0.00033	0.004	0.011	0.01	0.0045	0.0027	0.0019	0.008	0.0024	0.01
Range (in pixels)	10	4	12	17	18	18	15**	20	20	18	30	40
Model	Exp	Exp	Exp	Exp	Exp	Exp	Sph	Exp	Exp	Exp	Exp	Exp
Total sill x10 ⁻²	0.33	0.034	0.223	2.3	2.7	3.1	1.7	0.97	0.89	6	0.91	4.4

Commented [SR1]: NOTE: I kept the values as they are since Xavi told me they were correct BUT By looking at the images it seems that the graphs of Anna was correct and there was a mislabeling in the table of Xavi. To check I have imported the different channel for the 20D and 10L in imaJ and it looks like the 10L and 20D were switched. Please double check this information/

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Table 2. Statistical analysis of the different models indicating the fraction of pixels occupied
 by bacteria (white band), and the parameters corresponding to the variographic study.
 Analysis was performed on the 40X images.

	10D	10L	20D	20L
non-void pixels (x10 ⁻²)	0.58	2.94	18.8	24.5
Range (in pixels)	<1	12	34	41
Total sill	-	0.028	0.156	0.185

Commented [xs2]: Renumber all tables
ALL CAREFUL HERE These pixels are not the same size as those of table 1. In 10X it was 0.73 microns, so now, in 40X I guess they are 0.18 um, but please DOUBLE CHECK. Values of ranges in both tables should be converted from pixels to microns.

Commented [SR3]: Please Anna F. check the conversion pixel microm. I have in my notes that 3pixels= 1microm

17 **Table 3.** Spatially average respiration rates for the mature (30 days old) biofilm and the one
18 week biofilms growing at different treatments of light and temperature (10D,20D,10L,20L)
19 shown in Figure 3. The coefficient *a* and *b* represent the parameters of exponential regression
20 and R^2 are shown. Values are coefficients and standard error (in parenthesis).

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	a	b (min⁻¹)	R²
10D	99.16 (0.555)	0.0011(0.001)	0.954
10L	67.970(0.646)	0.020(0.001)	0.949
20D	92.913(0.530)	0.026(0.000)	0.988
20L	113.452(2.509)	0.052(0.002)	0.935
Mature Biofilm	275.541(5.546)	0.149(0.003)	0.99

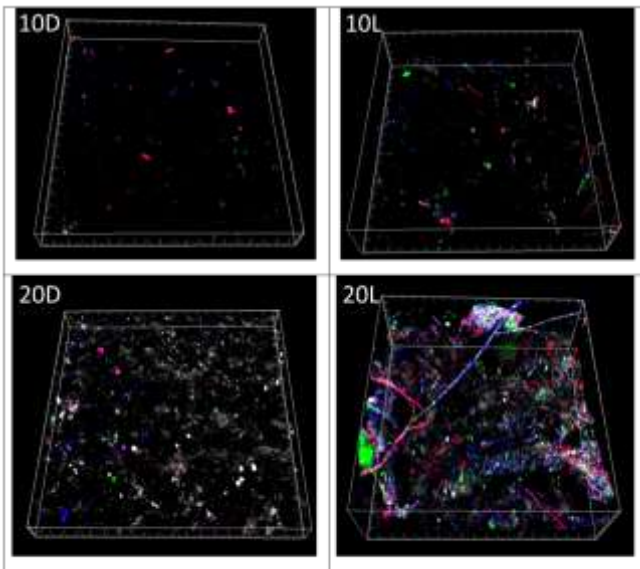
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Commented [SR4]: Anna how you calculate the standard error for a and b?

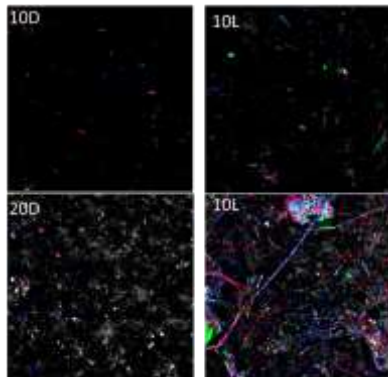
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25 **List of Figures**

26 **Figure 1.** Top: the tree-dimensional CLSM images (40X) of biofilm growth over 7 days for
27 the four the following environmental conditions of light and temperature: 20 °C light, 20 °C
28 dark, 10 °C light and 10 °C dark, indicated as 10D, 20D, 10L and 20L. Bottom: the
29 corresponding two-dimensional projections. In the image 0.76 pixels= 1µm.



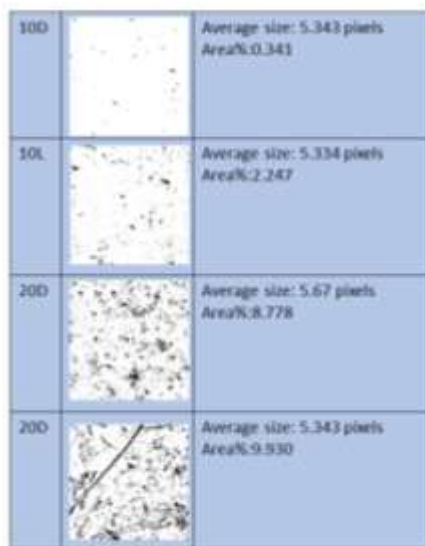
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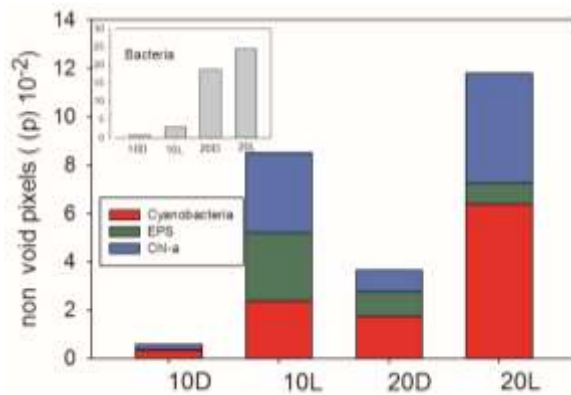
32 **Figure 2** a) Particle analysis for the 2D projection of the confocal images considering all the
 33 channels. Analyses have be done in imaJ, b) Amount of Chlorophyll, EPS and Cyanobacteria
 34 in the 1 week biofilm. Data has been computed by using the multivariate analyses as
 35 described in the methodology section/,

36 a)



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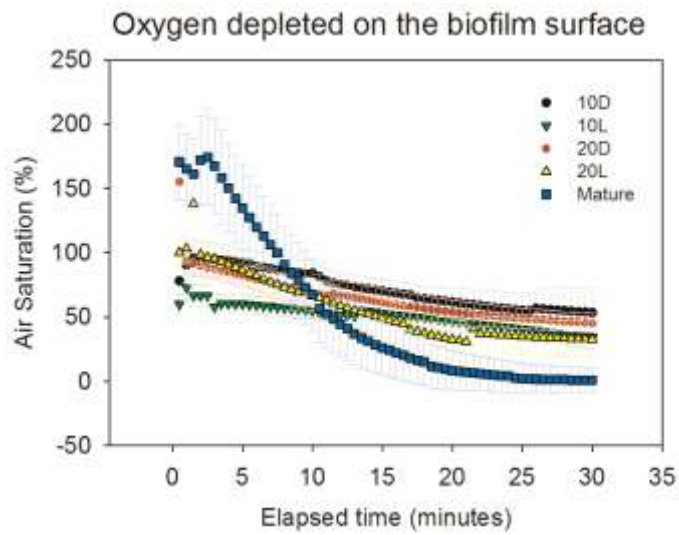
38 b)



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40 **Figure 3.** Spatial average oxygen values [% air saturation] of the one week old biofilm grown
41 under different environmental conditions. Bars represent the standard deviation. The specific
42 rates calculated for each decay curve are summarized in Table 3.

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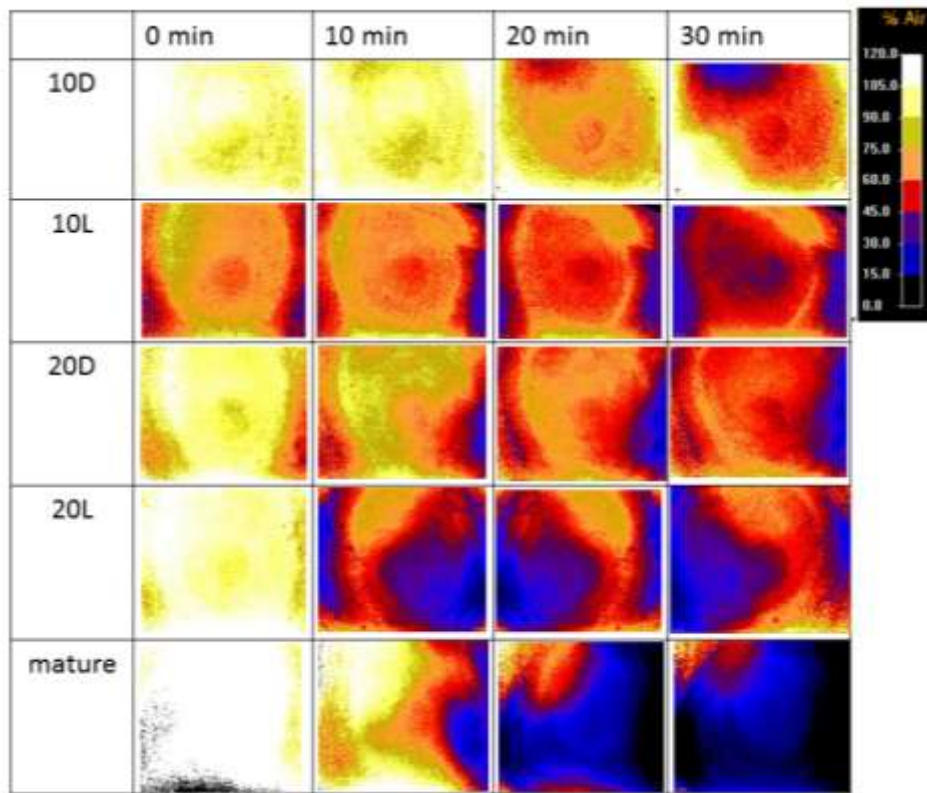
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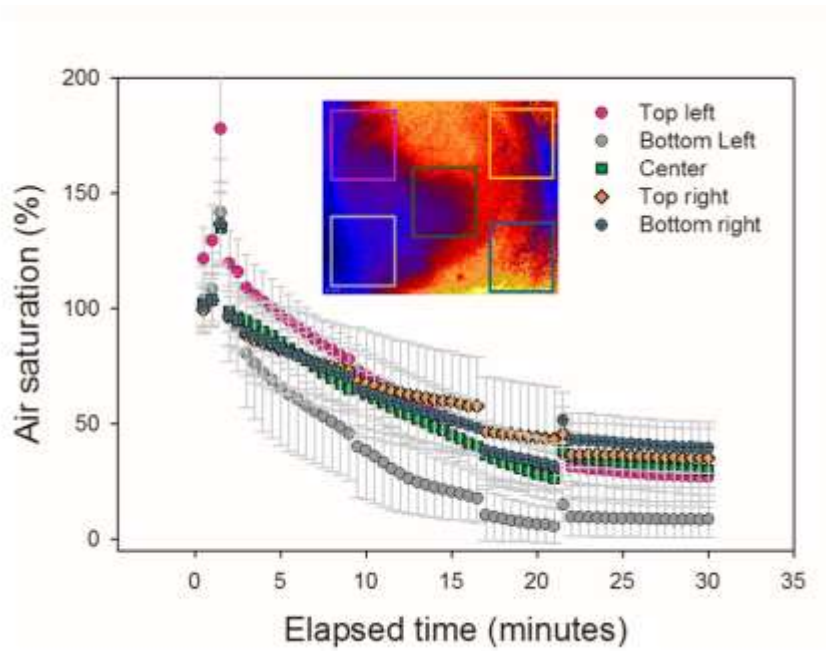
56 **Figure 4.** Maps of oxygen concentration at selected time (initial time 0 and after 10-20 and
57 30 min), highlighting the formation of hotspot and the high variability in space and time on
58 one week old biofilms at each T and light conditions, and mature biofilm (28 days old).
59 The insert show the presence of steep gradient of oxygen within the biofilm surface and the
60 associated heterogeneity in the associated respiration rates for different area of the biofilm.

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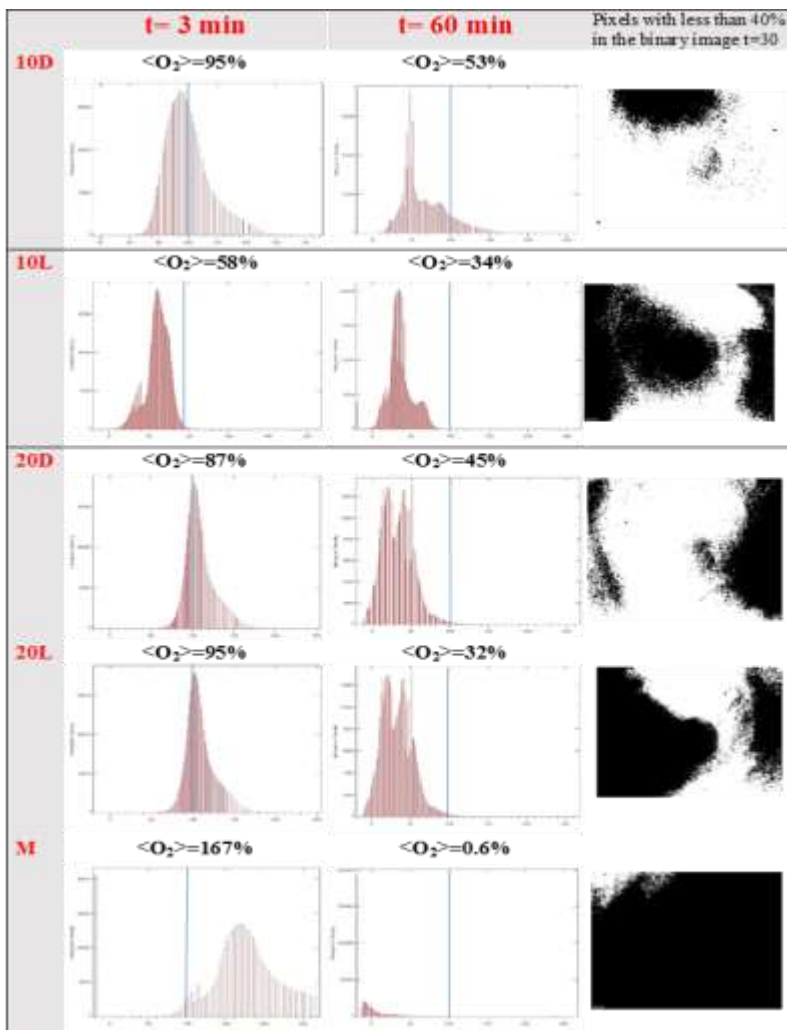
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66 **Figure 5.** Maps of oxygen concentration at selected time (initial time 0 and after 10-20 and
67 30 min), highlighting the formation of hotspot and the high variability in space and time on
68 one week old biofilms at each T and light conditions, and mature biofilm (28 days old). The
69 insert show the presence of steep gradient of oxygen within the biofilm surface and the
70 associated heterogeneity in the associated respiration rates for different area of the biofilm.
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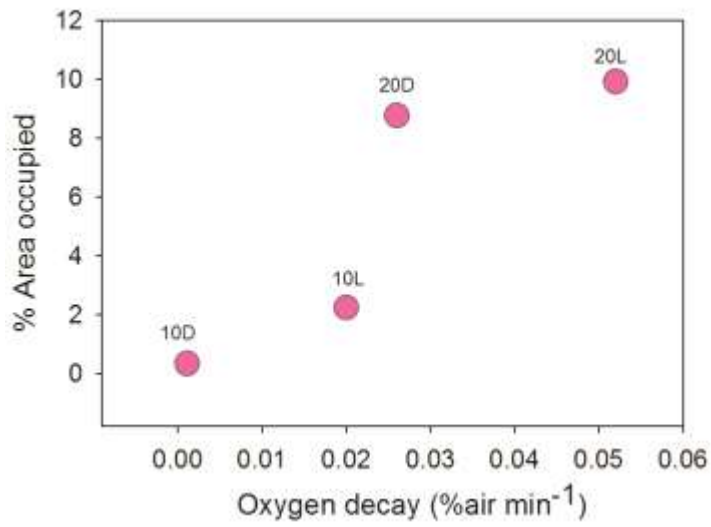
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78 **Figure 6.** On the left: Histograms of the oxygen distribution at $t=0$ (light blue lines) and
 79 $t=30$ minutes (dark blue lines) for biofilms at 10D,10L,20D,20L and the mature biofilm. On
 80 the right: are with low oxygen content (i.e. pixel with oxygen less than 20%) a $t=30$ min. The
 81 light blue lines indicates the 100% air saturation



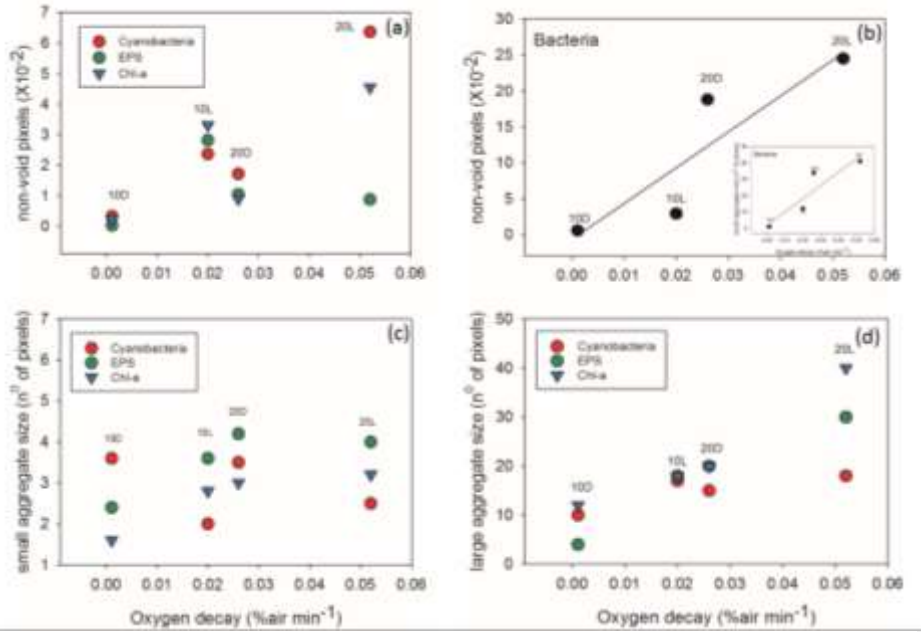
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83 **Figure 7** Relation between respiration rate (given by b in Table 3) and different statistics
84 (range of the nested variograms and proportion of active cells –bottom plot)
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111 **Figure 8** Relation between respiration rate (given by b in Table 3) and different statistics
 112 (range of the nested variograms and proportion of active cells –bottom plot)
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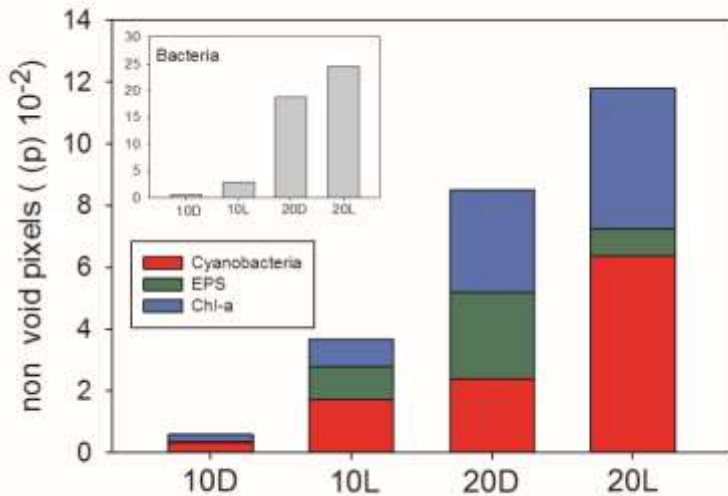
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131 IN CASE THE 10L AND 20D ARE INVERTED THE FIGURES BECOME:

	10D			10L			20D			20L		
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135 If this is the case figure 8 need to be redone changing 10L and 20D. I am attaching the
 136 SigmaPlot graph.

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143 **Supplementary material**

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145 **Images**

146 **FigureS1.** Experimental setup showing the well-plate used to conduct the analyses, the
147 positioning of the sensor and the overlap of the images. During the experiment the sample
148 and the instrument were prevented from light.

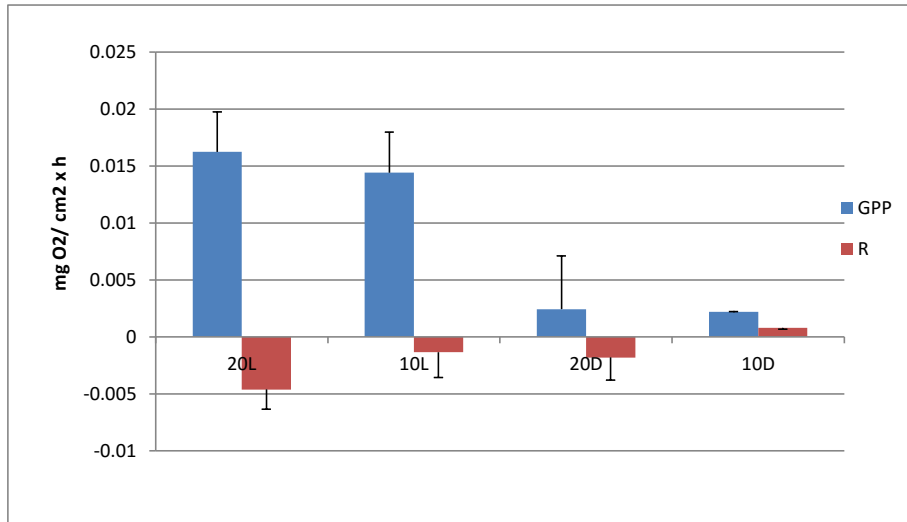


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151 **Figure S2 Gross primary production and respiration**

Commented [SR5]: Not sure you want to keep this one

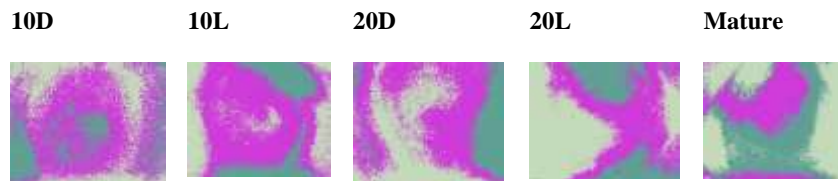


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154 **Figure S3 Cluster analyses.** Cluster analysis performed with MATHEMATICA. The cluster
 155 refer to the oxygen map recorded at t=30 minutes. It possible to notice that for each case 3
 156 areas with different pattern have been isolated.

Commented [SR6]: I am not sure if this one ell much. It jus tell the zones with different ranges at t=30 min



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162 **Videos**

163 In the following we include the videos for the one old week biofilm:

164 1. Video of oxygen evolution in the biofilm grown at 10°C dark conditions file:

165 V1_10D

166 2. Video of oxygen evolution in the biofilm grown at 10°C light conditions file:

167 V2_10L

168 3. Video of oxygen evolution in the biofilm grown at 20°C dark conditions file:

169 V3_20D

170 4. Video of oxygen evolution in the biofilm grown at 20°C light conditions file:

171 V4_20L.

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