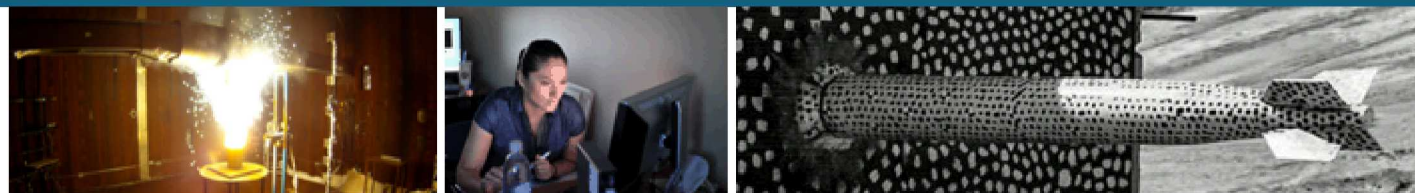


# Turning Over a New Leaf: Cultivation of Multiple Algae Strains for Biofuels and Bioproducts with Plant Waste

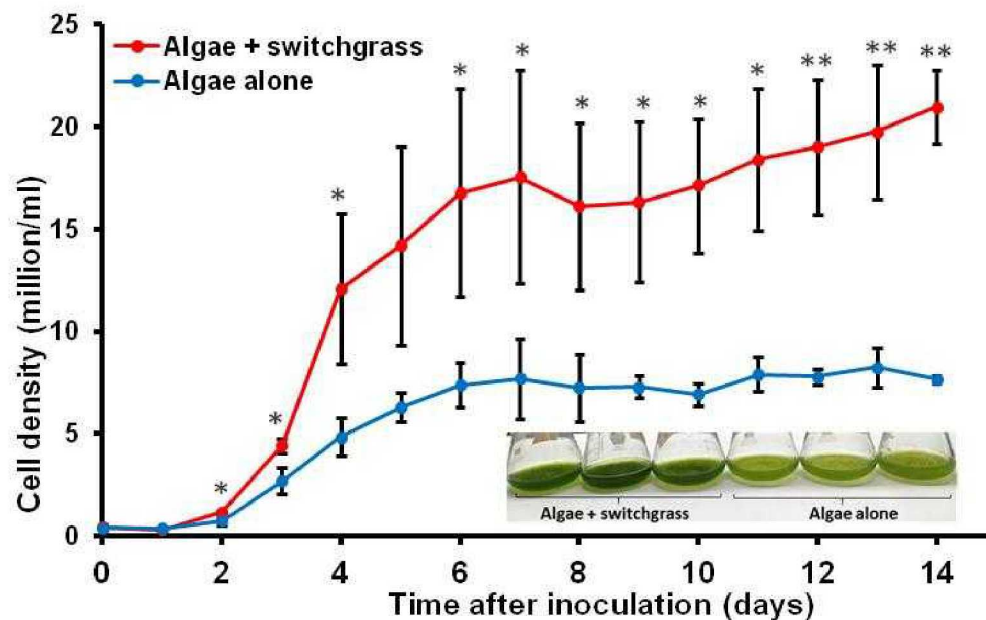


Amanda Barry (Formerly at LANL)

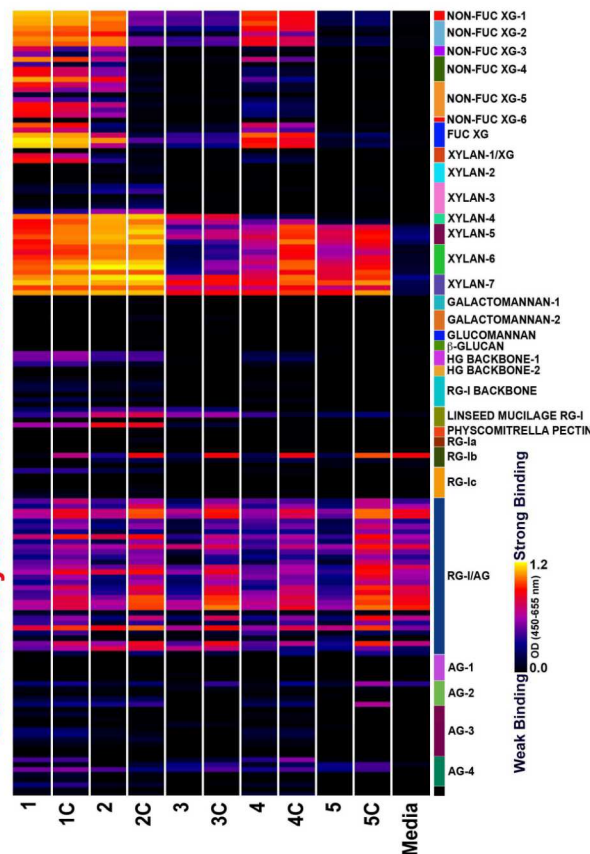


# Characterization of plant carbon substrate utilization by *Auxenochlorella protothecoides*

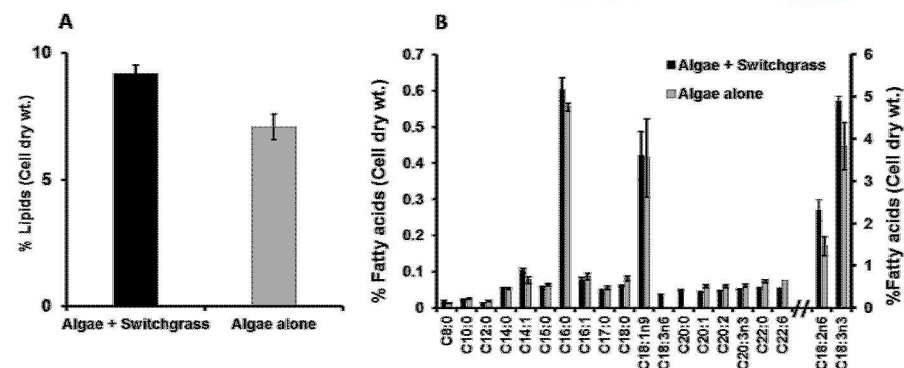
Brian W. Vogler <sup>a, b</sup>, Shawn R. Starkenburg <sup>a</sup>, Nilusha Sudasinghe <sup>a</sup>, Jenna Y. Schambach <sup>a</sup>, Joseph A. Rollin <sup>c</sup>,  
Sivakumar Pattathil <sup>d, 1</sup>, Amanda N. Barry <sup>a</sup>  



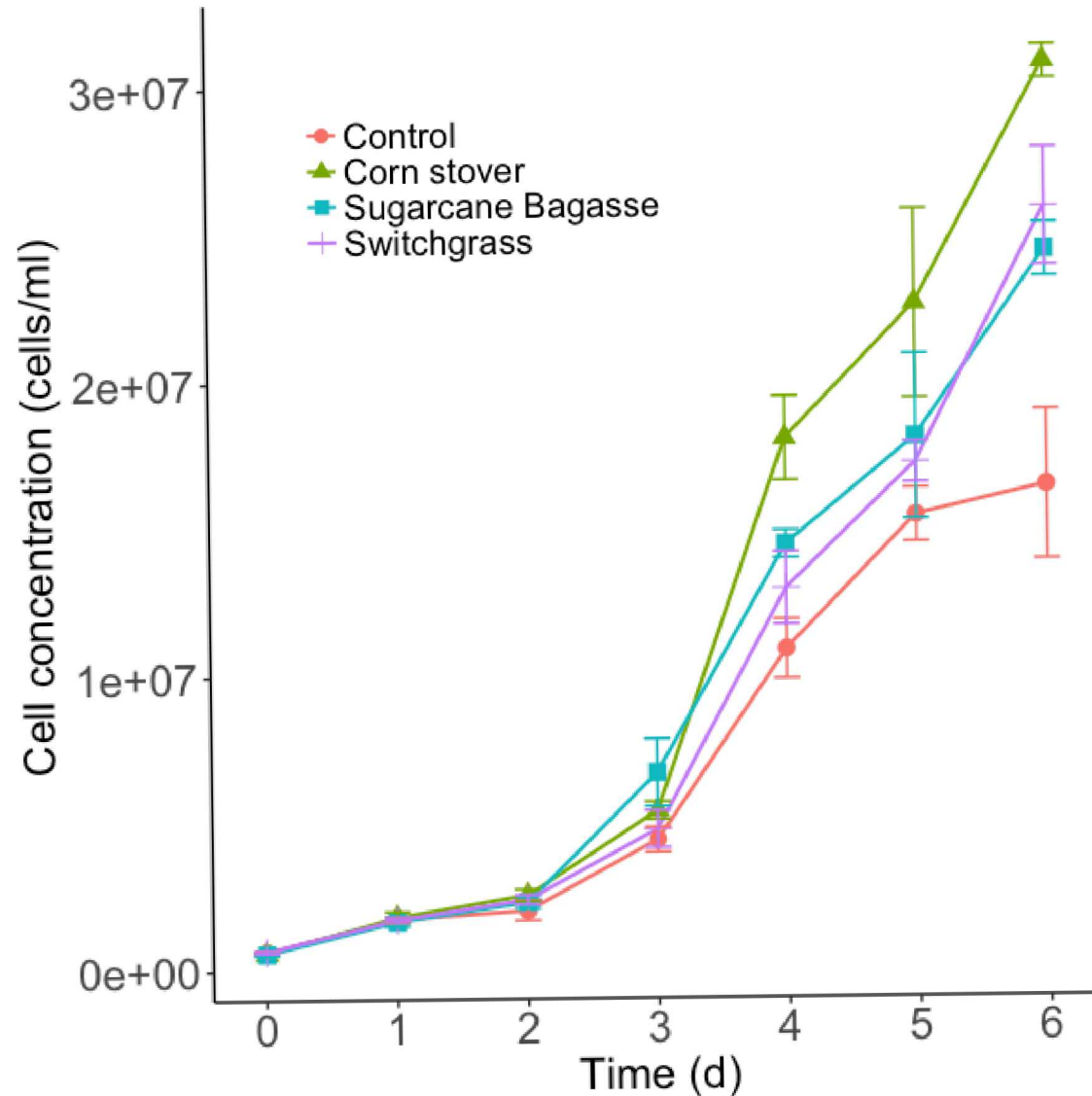
Cell Wall Glycan Directed Monoclonal Antibodies



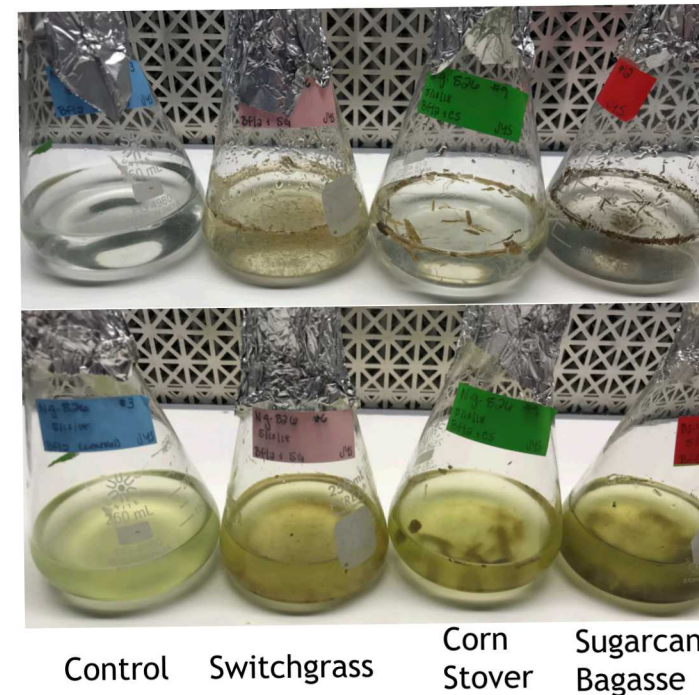
| Gene ID            | sp. 0710   | Family | CDD GH Hit | E-Value | FC       | CMC Dark | CMC Light |
|--------------------|------------|--------|------------|---------|----------|----------|-----------|
| UTEX 25            | sp. 0710   | Family | E-Value    | FC      | p-value  | FC       | p-value   |
| APUTEX2500001730   | KFM23057.1 | GH2    | 0.0E+000   | 1.0     | 7.84E-01 | 0.9      | 4.40E-01  |
| APUTEX2500000235   | KFM29315.1 | GH5    | 1.9E-020   | 0.5     | 1.59E-05 | 1.1      | 5.56E-01  |
| APUTEX2500000742   | KFM23002.1 | GH5    | 7.1E-020   | 1.2     | 2.31E-01 | 1.3      | 9.65E-02  |
| APUTEX2500000844   | KFM25716.1 | GH5    | 1.3E-022   | 1.3     | 6.66E-02 | 1.2      | 2.06E-01  |
| APUTEX2500000985   | KFM28016.1 | GH5    | 3.5E-017   | 0.2     | 8.26E-10 | 1.2      | 6.12E-01  |
| APUTEX2500002156   | KFM22632.1 | GH5    | 4.0E-025   | 1.0     | 8.95E-01 | 1.1      | 4.56E-01  |
| APUTEX2500002671   | KFM29130.1 | GH5    | 2.7E-021   | 1.2     | 2.55E-01 | 1.1      | 6.37E-01  |
| APUTEX2500003890   | KFM28332.1 | GH5    | 7.4E-013   | 0.9     | 7.76E-01 | 1.0      | 7.96E-01  |
| APUTEX2500004827   | KFM23138.1 | GH5    | 8.2E-021   | 1.6     | 7.48E-03 | 0.9      | 7.69E-01  |
| APUTEX2500000468   | KFM25061.1 | GH9    | 2.9E-084   | 1.1     | 6.18E-01 | 1.0      | 7.91E-01  |
| APUTEX2500000981   | KFM28012.1 | GH9    | 3.4E-050   | 1.4     | 2.17E-02 | 0.9      | 6.95E-01  |
| APUTEX2500003119   | KFM24581.1 | GH9    | 4.6E-098   | 1.0     | 9.80E-01 | 1.2      | 2.00E-01  |
| APUTEX2500005369-A | KFM24183.1 | GH9    | 1.1E-113   | 1.8     | 2.09E-03 | 1.3      | 1.70E-01  |
| APUTEX2500005369-B | KFM24184.1 | GH9    | 9.2E-104   | 0.9     | 6.35E-01 | 0.9      | 5.24E-01  |
| APUTEX2500005369-C | KFM24182.1 | GH9    | 2.8E-100   | 1.4     | 3.50E-02 | 0.9      | 4.54E-01  |
| APUTEX2500005777   | KFM24064.1 | GH9    | 3.6E-067   | 1.4     | 1.07E-02 | 1.1      | 4.89E-01  |
| APUTEX2500000143   | KFM24770.1 | GH16   | 3.0E-065   | 1.2     | 3.73E-01 | 1.2      | 3.66E-01  |
| APUTEX2500002026   | KFM25245.1 | GH18   | 6.8E-009   | 1.5     | 9.56E-03 | 0.9      | 7.15E-01  |
| APUTEX2500002706   | KFM22671.1 | GH20   | 2.9E-104   | 1.5     | 1.73E-01 | 1.0      | 1.00E+00  |
| APUTEX2500005097   | KFM28226.1 | GH27   | 8.8E-128   | 0.7     | 5.57E-02 | 1.2      | 2.21E-01  |
| APUTEX2500001996   | KFM25289.1 | GH31   | 0.0E+000   | 0.7     | 5.00E-02 | 1.0      | 1.00E+00  |
| APUTEX2500003474   | KFM25547.1 | GH31   | 0.0E+000   | 0.5     | 4.20E-04 | 1.0      | 9.63E-01  |
| APUTEX2500000041   | KFM23987.1 | GH32   | 1.7E-067   | 1.6     | 2.85E-02 | 1.1      | 5.19E-01  |
| APUTEX2500000101   | KFM28270.1 | GH38   | 6.6E-065   | 0.9     | 6.64E-01 | 1.0      | 8.29E-01  |
| APUTEX2500005781   | KFM24068.1 | GH43   | 3.9E-015   | 1.1     | 5.89E-01 | 0.7      | 1.02E-01  |
| APUTEX2500000370   | KFM24944.1 | GH43   | 2.3E-005   | 1.1     | 4.15E-01 | 1.0      | 8.85E-01  |
| APUTEX2500000577   | KFM26117.1 | GH47   | 3.7E-172   | 0.9     | 6.71E-01 | 1.2      | 2.64E-01  |
| APUTEX2500002068   | KFM28922.1 | GH47   | 6.1E-111   | 1.0     | 7.84E-01 | 1.0      | 8.47E-01  |
| APUTEX2500004355   | KFM22865.1 | GH47   | 0.0E+000   | 1.1     | 4.19E-01 | 1.1      | 4.14E-01  |
| APUTEX2500001195   | KFM26337.1 | GH63   | 0.0E+000   | 1.4     | 1.45E-02 | 1.1      | 6.12E-01  |



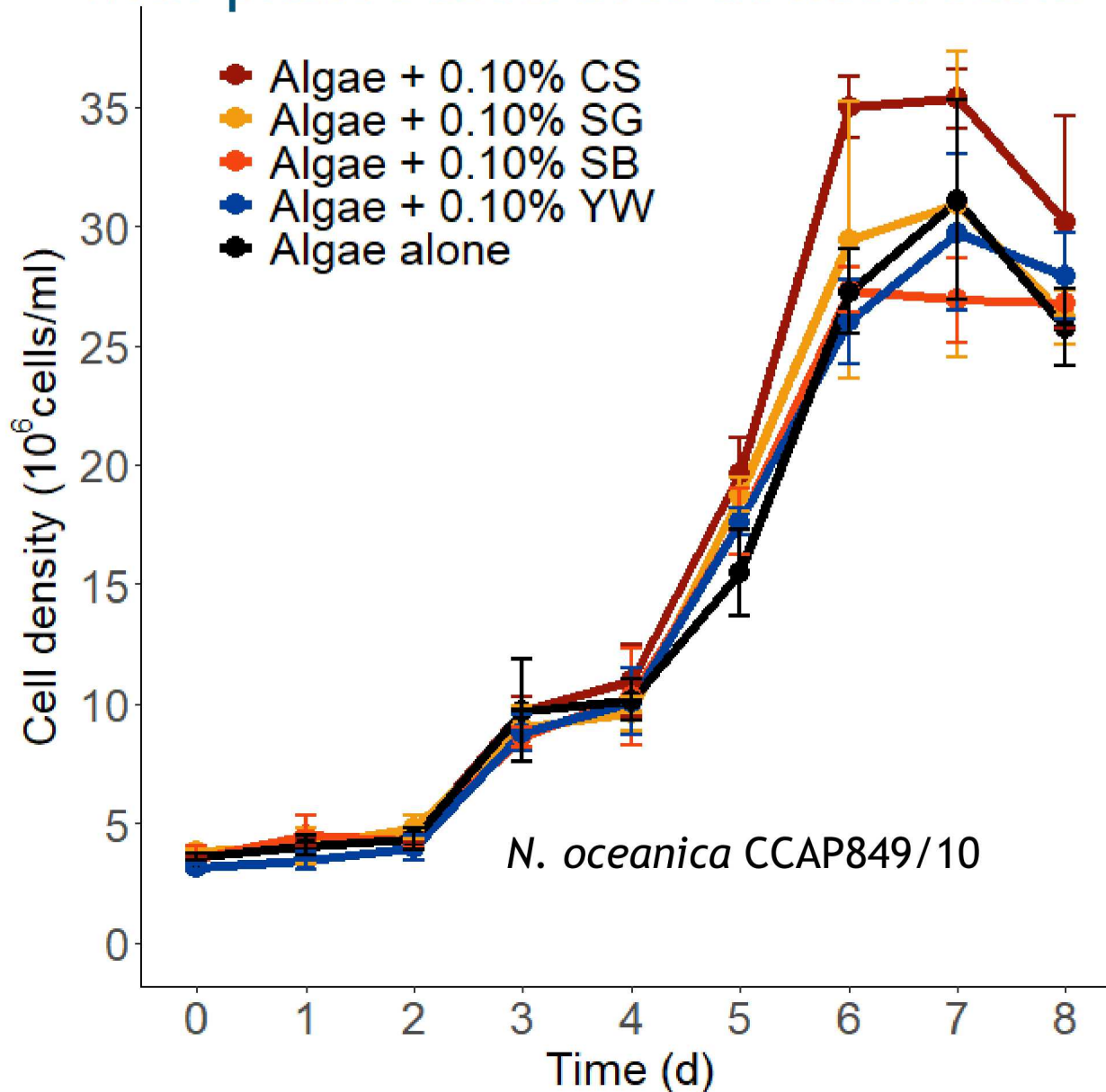
# *Nannochloropsis gaditana* demonstrates improved cell growth with plant substrates at flask scale



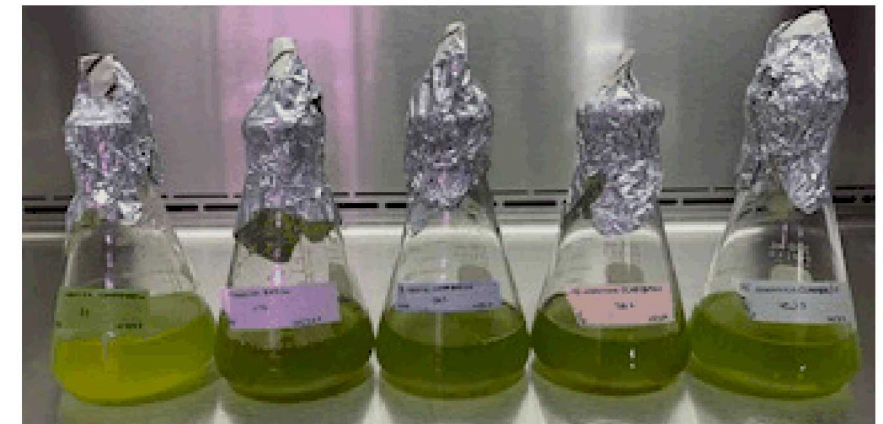
| Treatment         | Specific Growth Rate ( $\mu$ ) |
|-------------------|--------------------------------|
| Control           | $0.53 \pm 0.03$                |
| Corn Stover       | $0.64 \pm 0.03$                |
| Sugarcane Bagasse | $0.62 \pm 0.01$                |
| Switchgrass       | $0.61 \pm 0.02$                |



# *Nannochloropsis oceanica* demonstrates improved cell growth with plant substrates at flask scale



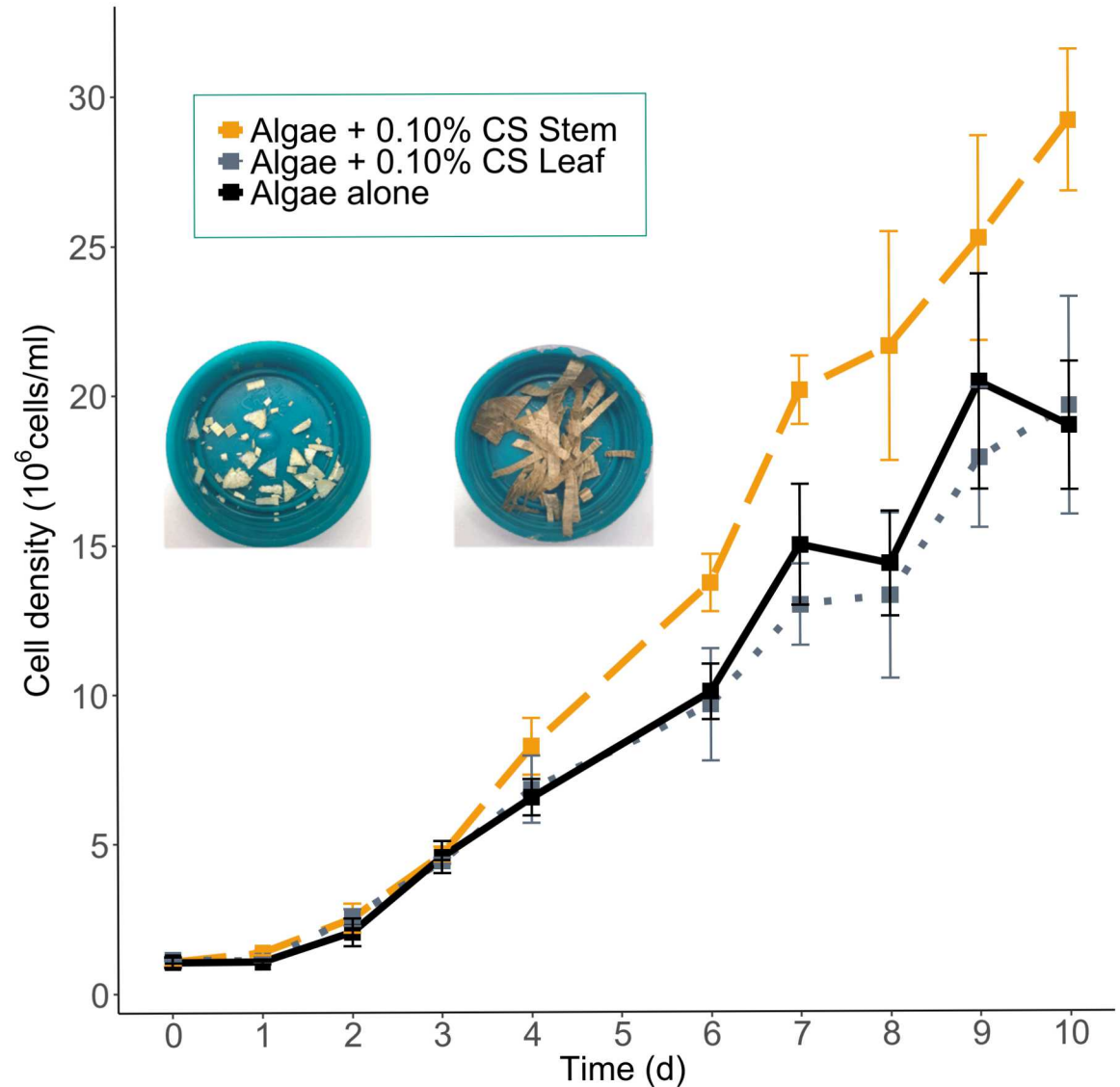
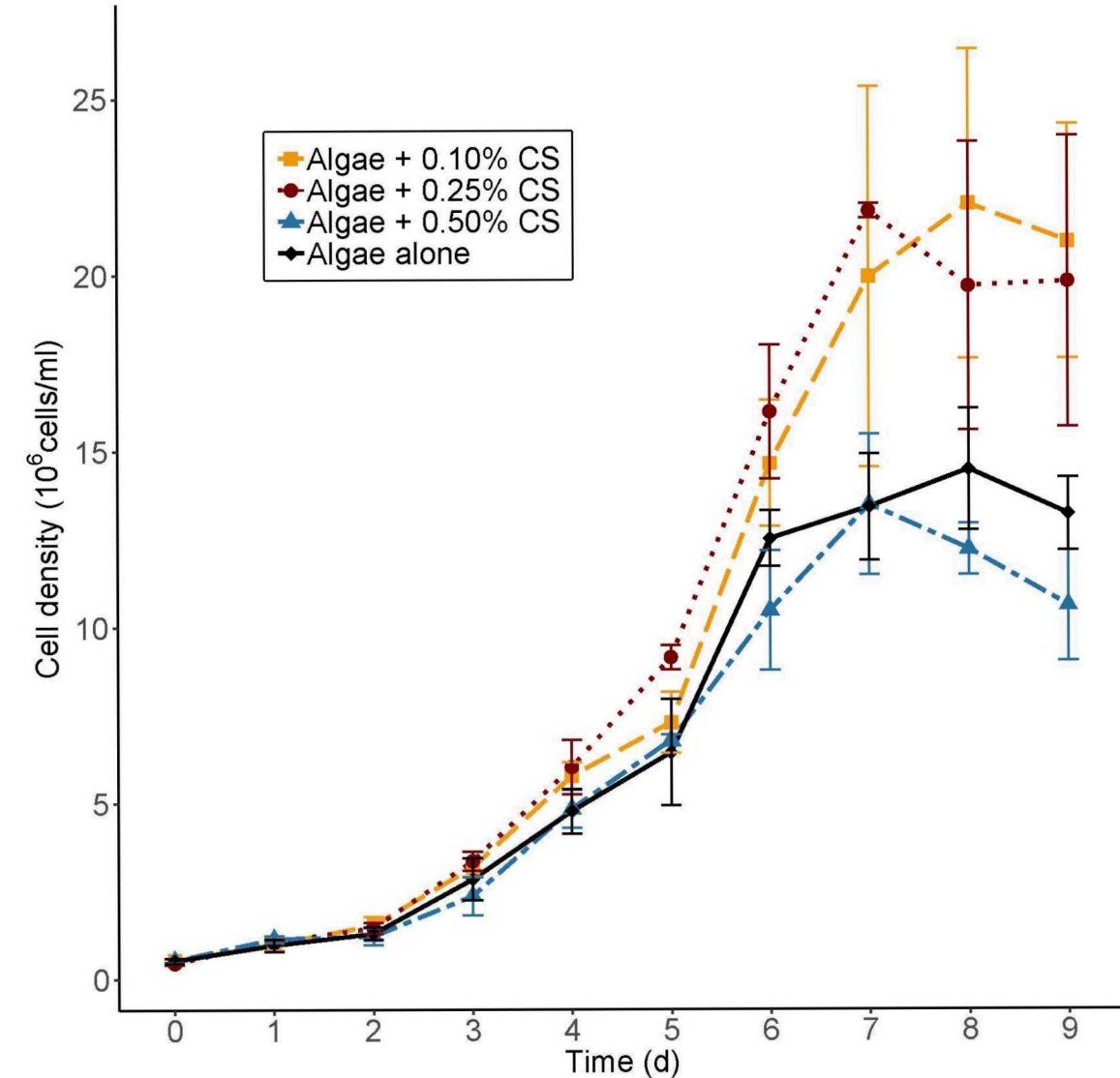
| Treatment         | Specific Growth Rate ( $\mu$ ) |
|-------------------|--------------------------------|
| Control           | $0.49 \pm 0.03$                |
| Corn Stover       | $0.58 \pm 0.03$                |
| Sugarcane Bagasse | $0.50 \pm 0.01$                |
| Switchgrass       | $0.56 \pm 0.02$                |



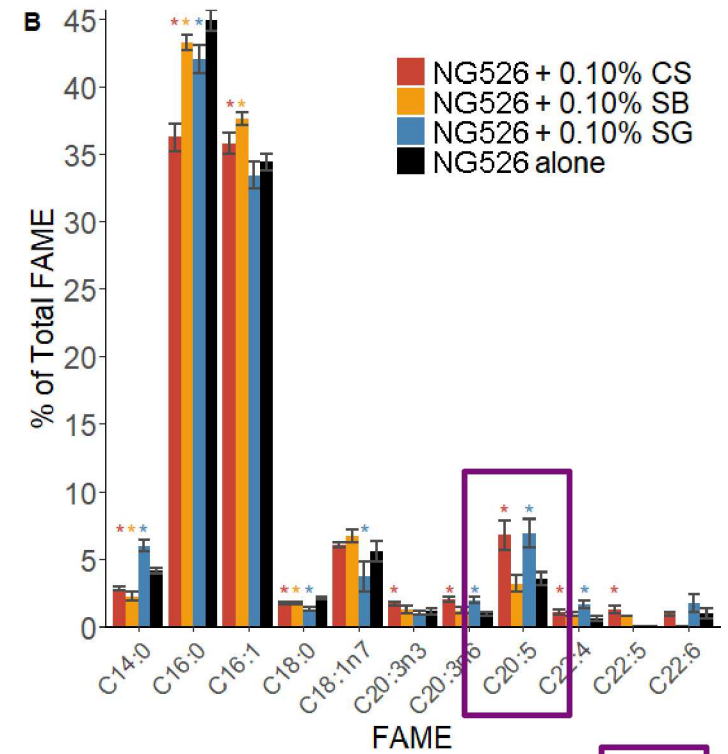
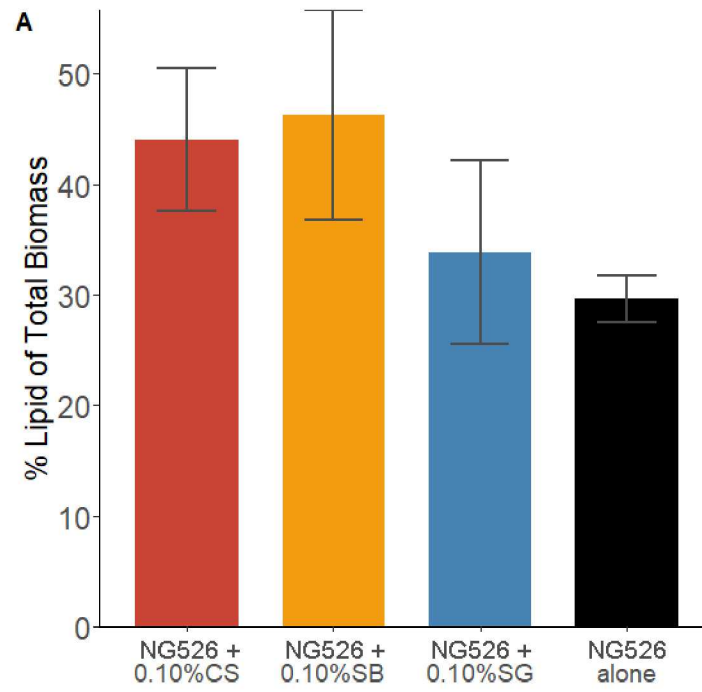
Control CS SG SB YW



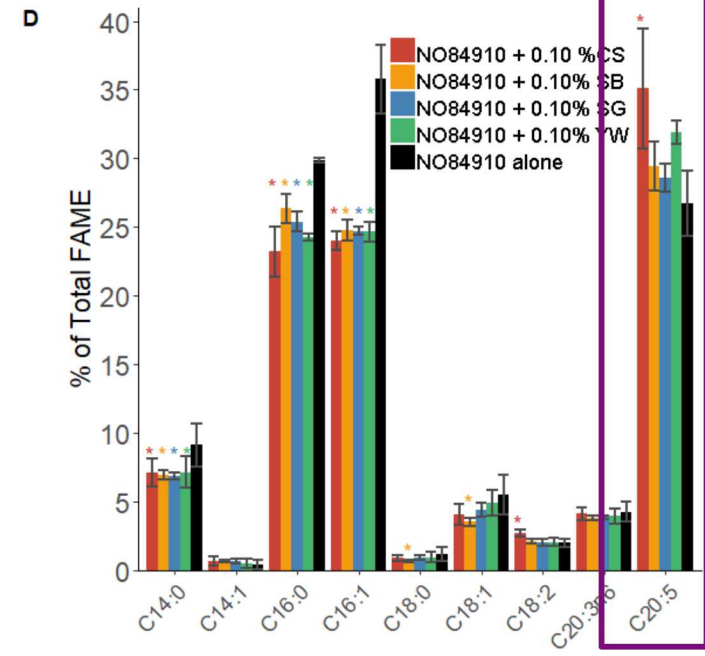
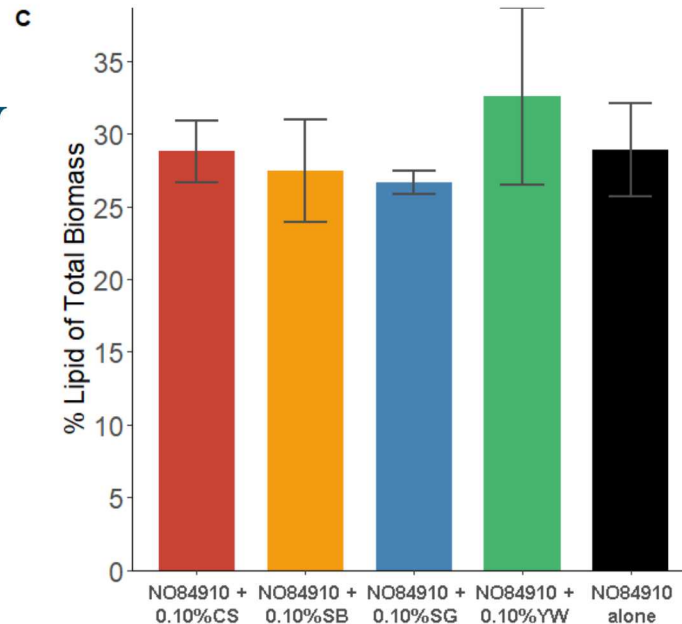
# Plant substrate concentration and physiology matter

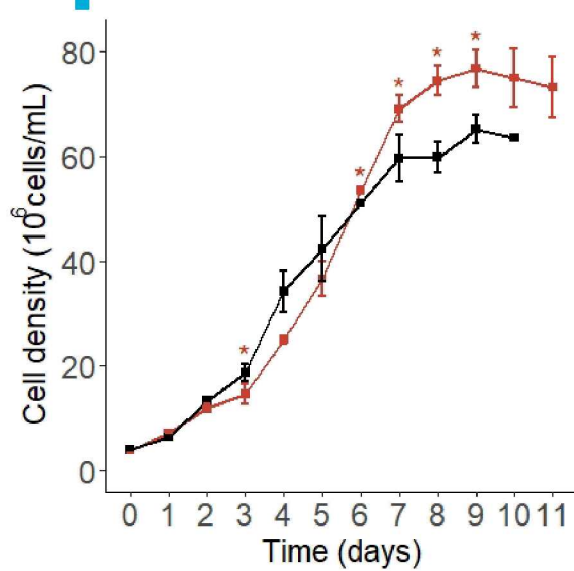


*N. gaditana* has increased total lipid content and changes in the lipid profile

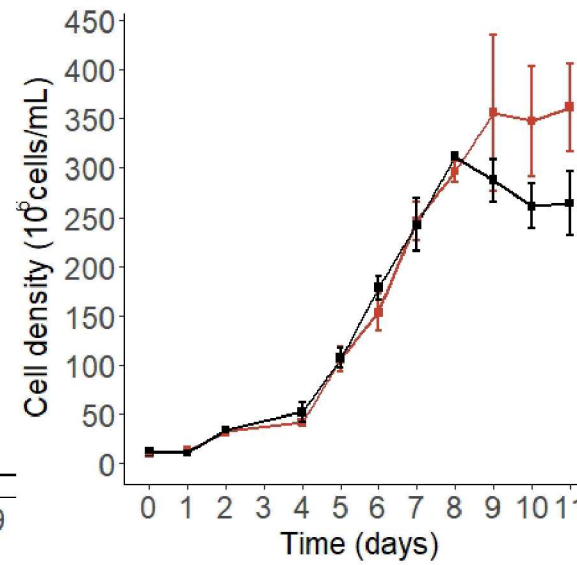
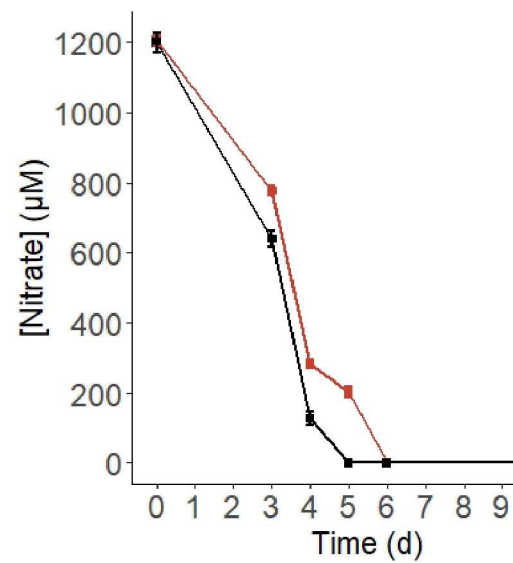


*N. oceanica* does not show a difference in total lipid content, but there is a shift in the lipid profile

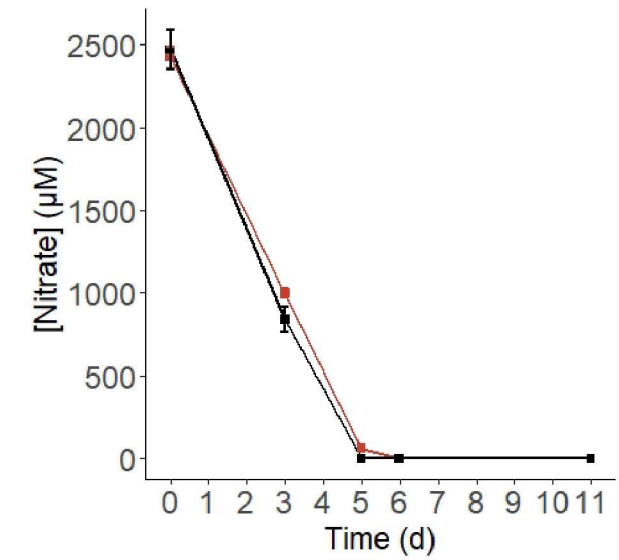




—■— NG526 + 0.25% CS —●— NG526 alone



—■— NO84910 + 0.10% CS —●— NO84910 alone

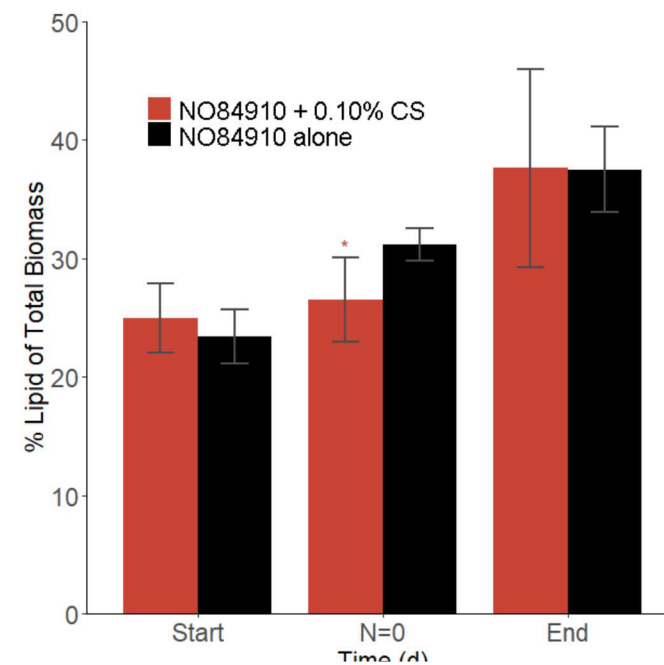
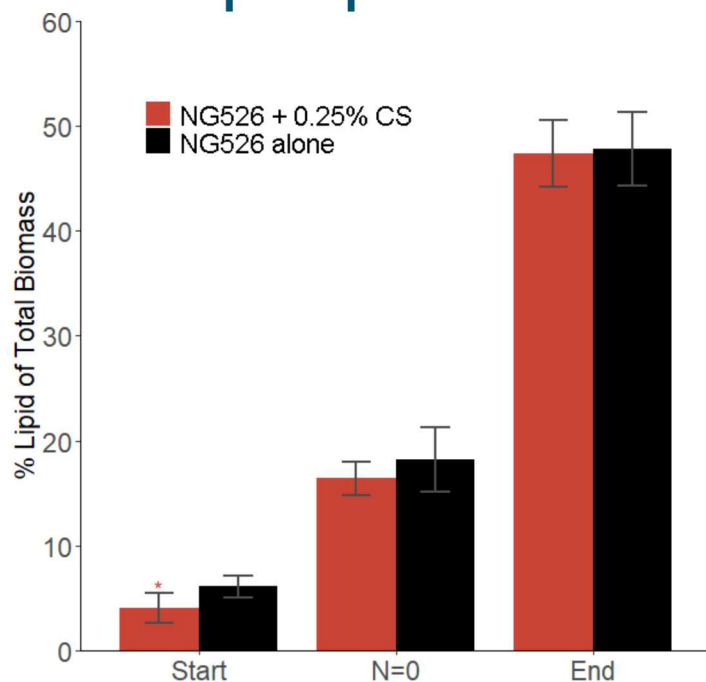


Mini raceway outdoor pond experiments demonstrate improved growth for *N. gaditana* with plant substrates with a delay in N starvation

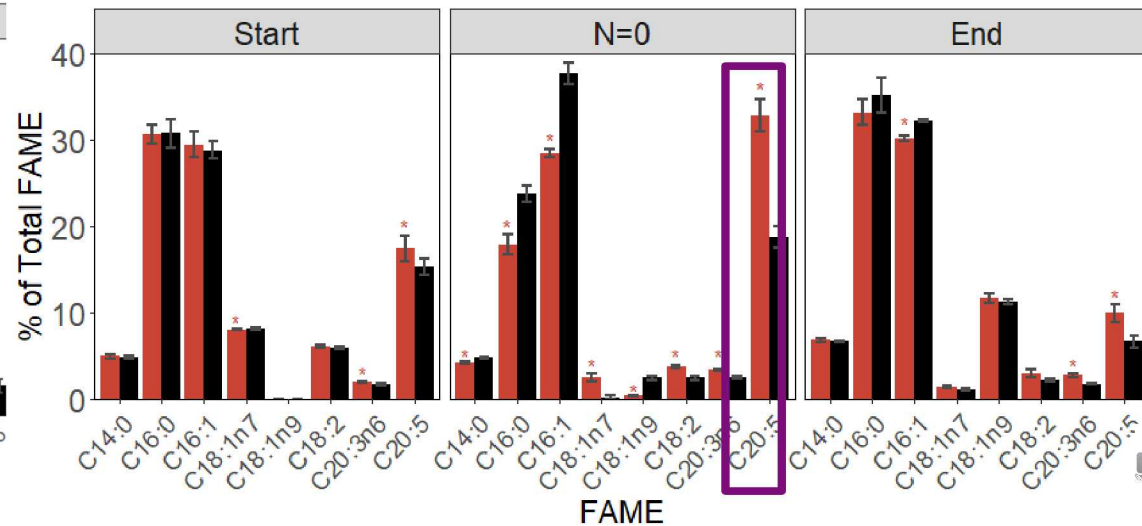
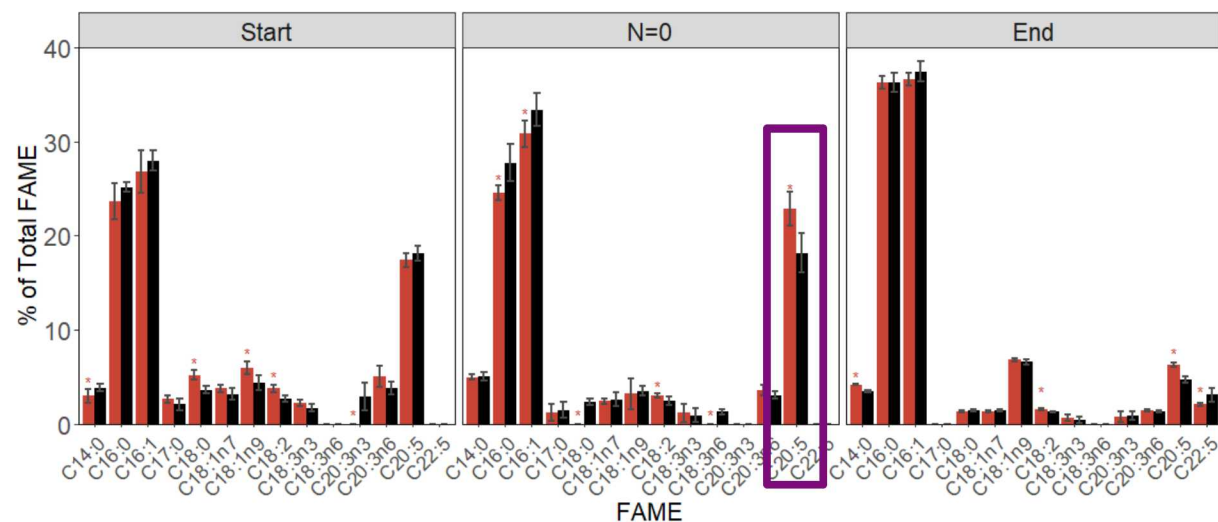
*N. oceanica* shows no difference with plant substrates at mini raceway pond scale



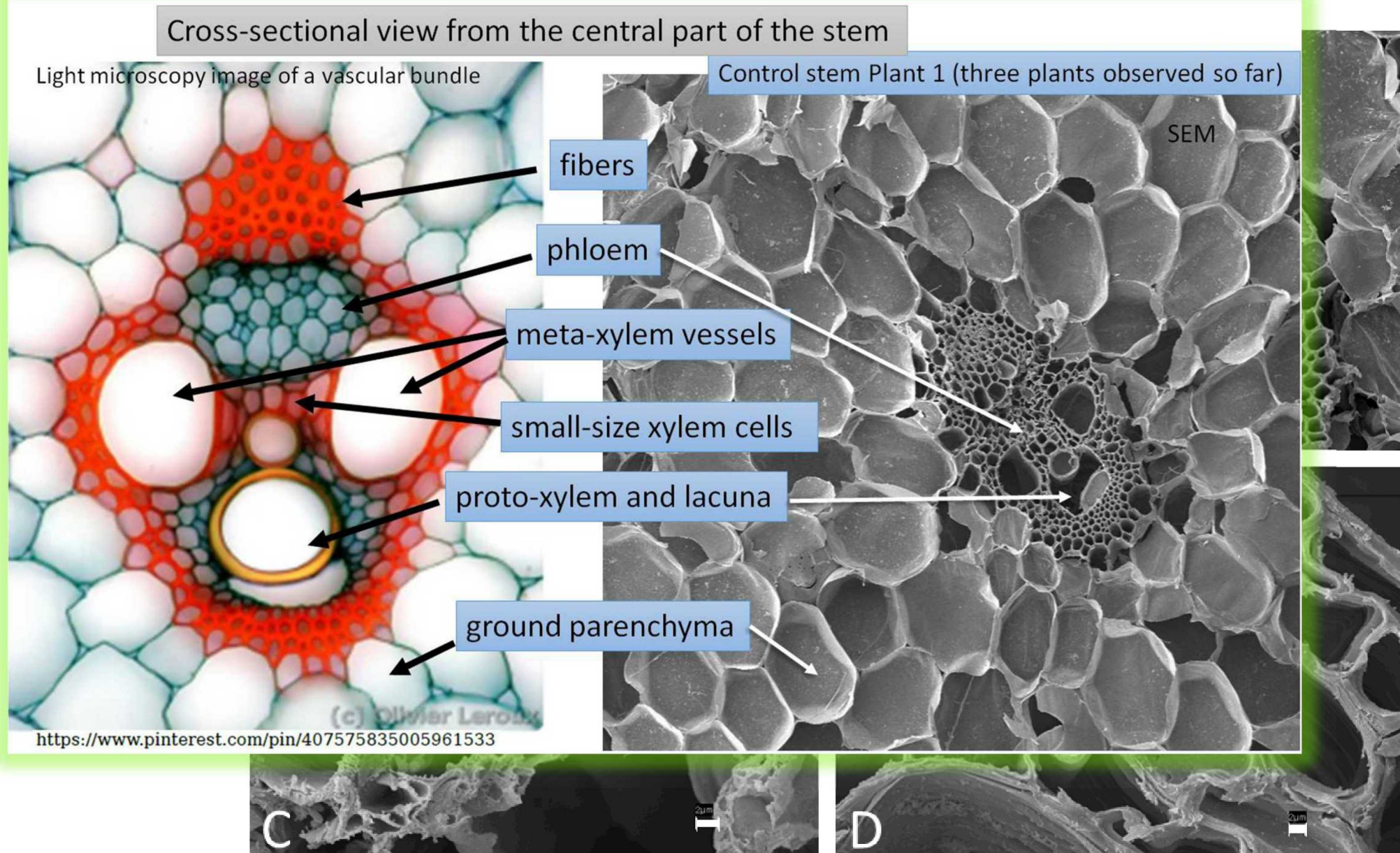
# Mini pond experiments show no difference in total lipids but a shift in the lipid profile at N starvation



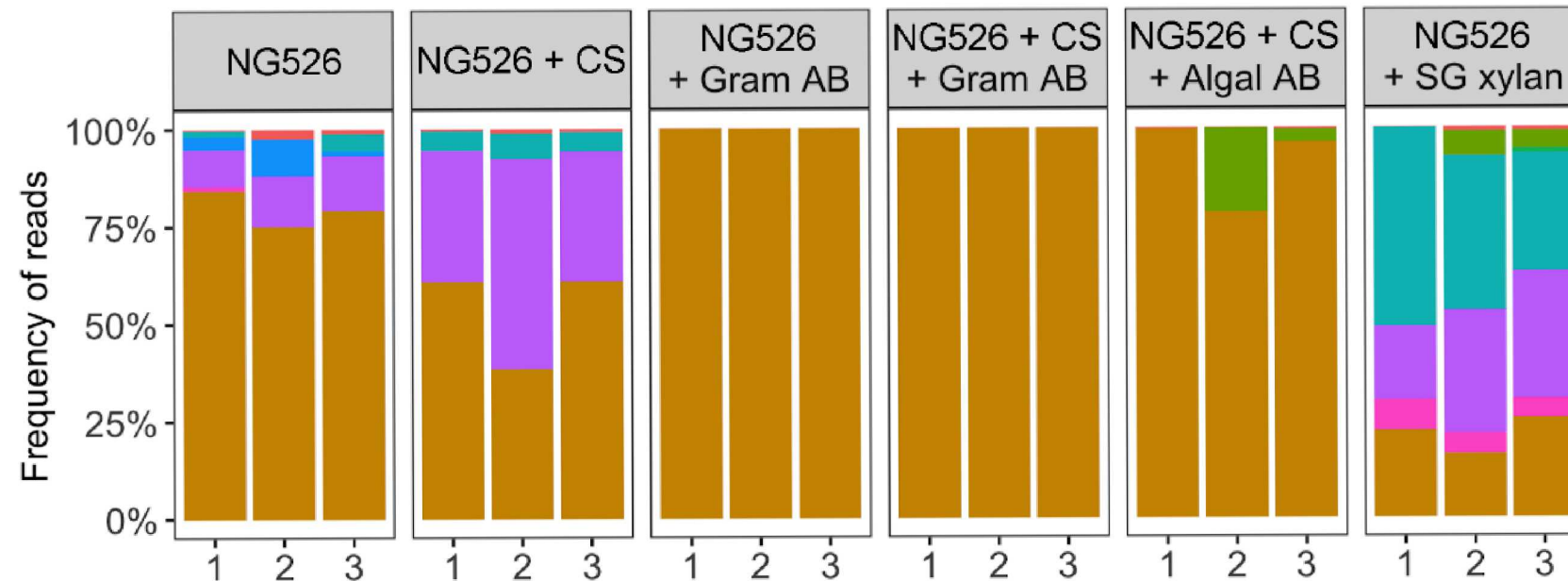
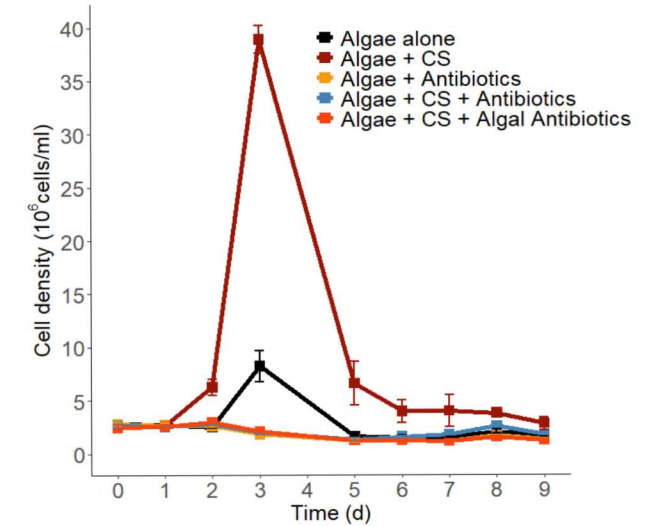
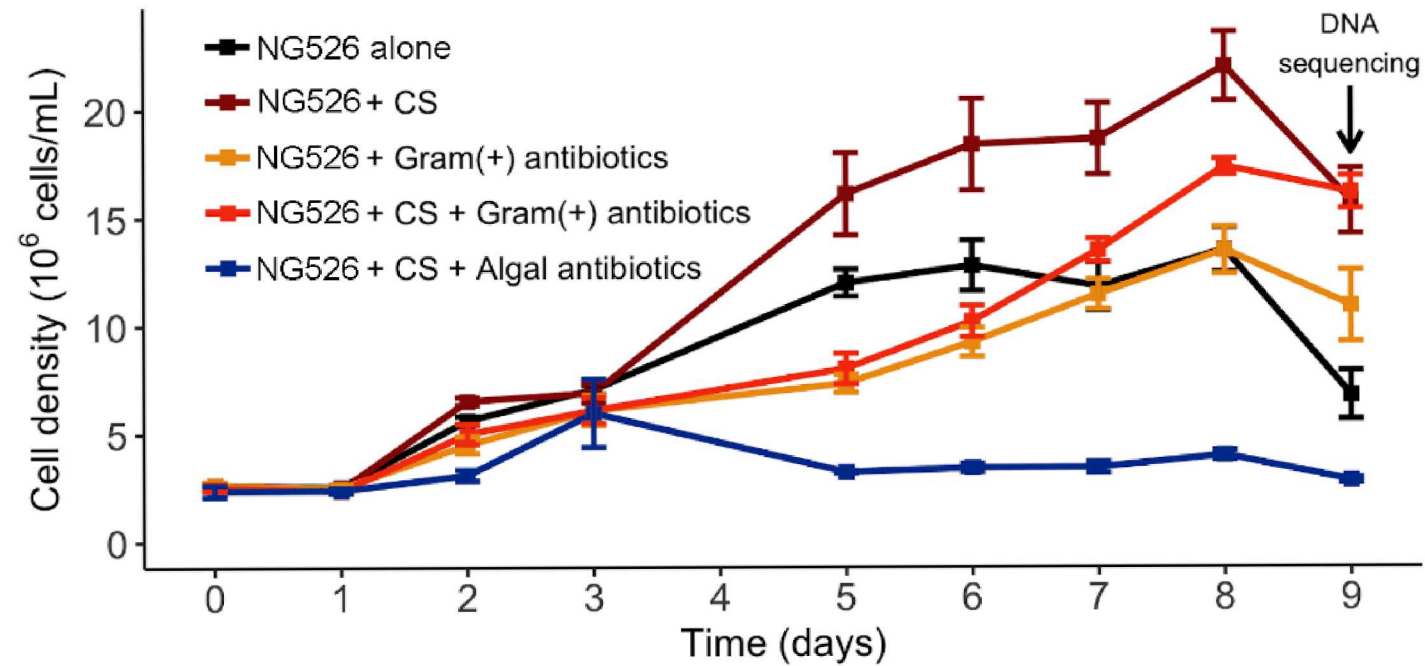
B



# Plant substrate structure changes with algae growth



# What about the microbiome?





Brian Vogler  
(Now at Conagen)



Jenna Schambach



Anna Finck

### LEAF Project Collaborators

Nick Nagle, NREL

Phil Pienkos, NREL

Christopher Hunt, USFS

Peter Kitin, UW-Madison

Shawn Starkenburg, LANL

Erik Hanschen, LANL



LANL LDRD Early Career Award FY17-FY19  
3-year AOP LEAF Project started FY20



|            | Ash       | Klason Lignin | Arabinan  | Galactan  | Glucan     | Xylan      | Mannan | Tot Carb   | Yield     |
|------------|-----------|---------------|-----------|-----------|------------|------------|--------|------------|-----------|
| CS         | 0.9 (0.9) | 13.4 (1.8)    | 3.2 (0.8) | 0.6 (1.2) | 33.1 (3.4) | 18.6 (1.9) | 0      | 55.4 (4.4) | 69.8(7.0) |
| NG526 + CS | 0.6 (1.1) | 14.1 (1.1)    | 3.5 (1.0) | 0         | 33.6 (2.1) | 18.5 (1.9) | 0      | 55.7(4.7)  | 70.4(5.5) |

|             | Arabinan |          | Galactan |          | Glucan |          | Xylan |          | Mannan |          |
|-------------|----------|----------|----------|----------|--------|----------|-------|----------|--------|----------|
|             | Avg      | 95% C.I. | Avg      | 95% C.I. | Avg    | 95% C.I. | Avg   | 95% C.I. | Avg    | 95% C.I. |
| NG526 alone | 0.0      | N/A      | 54.6     | 18.6     | 49.1   | 37.3     | 9.0   | 8.6      | 16.0   | 11.4     |
| CS alone    | 30.9     | 19.9     | 55.3     | 43.8     | 109.5  | 30.1     | 59.2  | 30.1     | 0.0    | N/A      |
| Sum         | 31.0     | 19.9     | 110.0    | 37.5     | 159.0  | 31.4     | 68.0  | 27.4     | 16.0   | 11.4     |
| NG526 + CS  | 29.5     | 16.9     | 100.9    | 64.7     | 78.5   | 11.5     | 44.0  | 18.3     | 20.2   | 43.8     |
| p value     | 0.797    |          | 0.656    |          | 0.002  |          | 0.051 |          | 0.716  |          |