

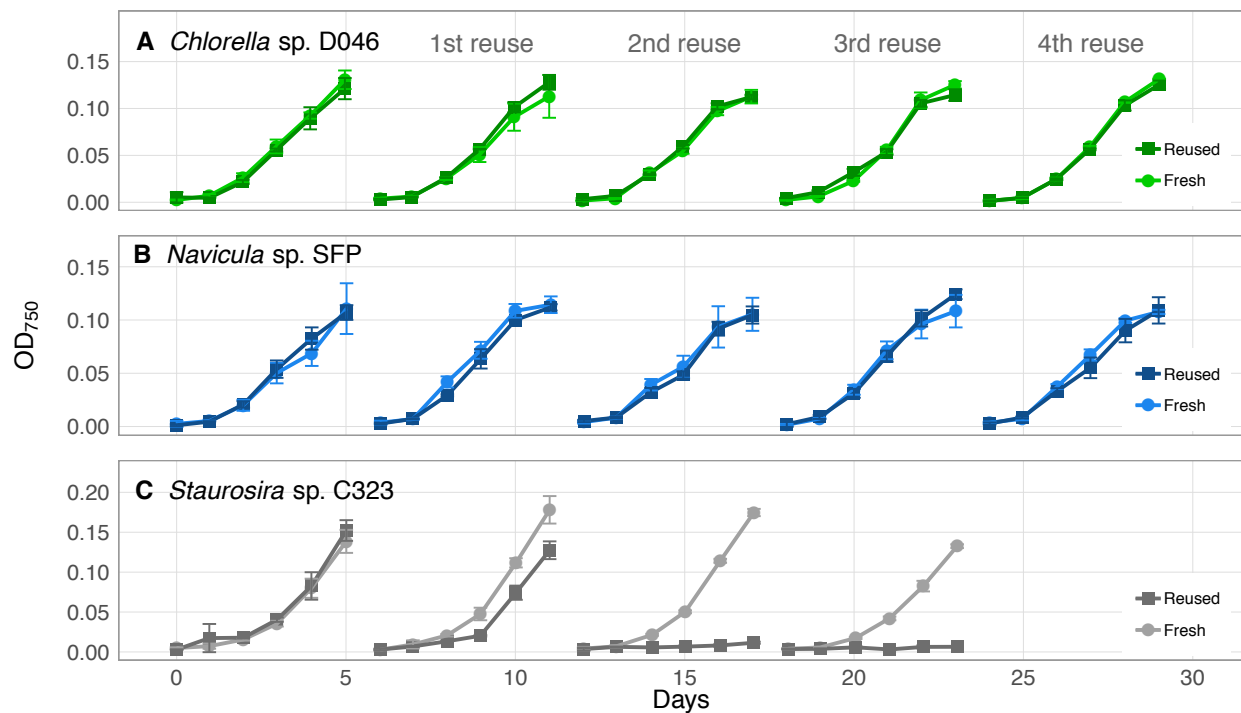
## Supplementary Material

# Reused Cultivation Water Accumulates Dissolved Organic Carbon And Uniquely Influences Different Marine Microalgae

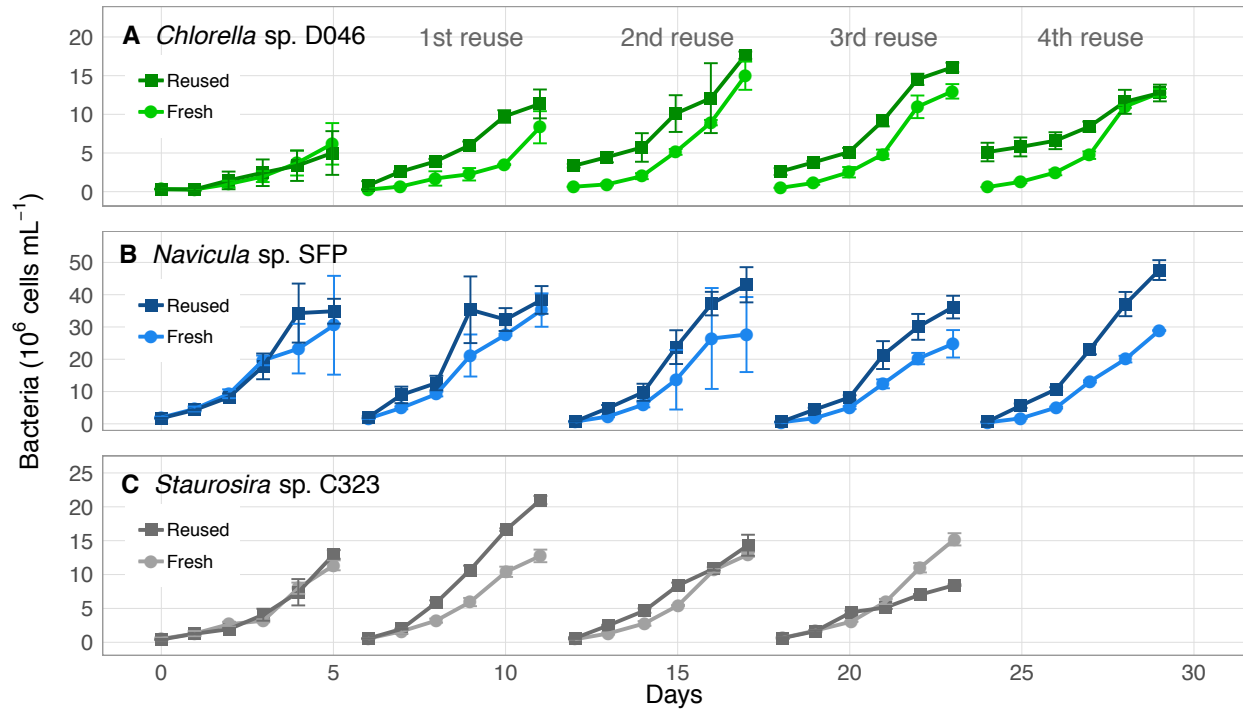
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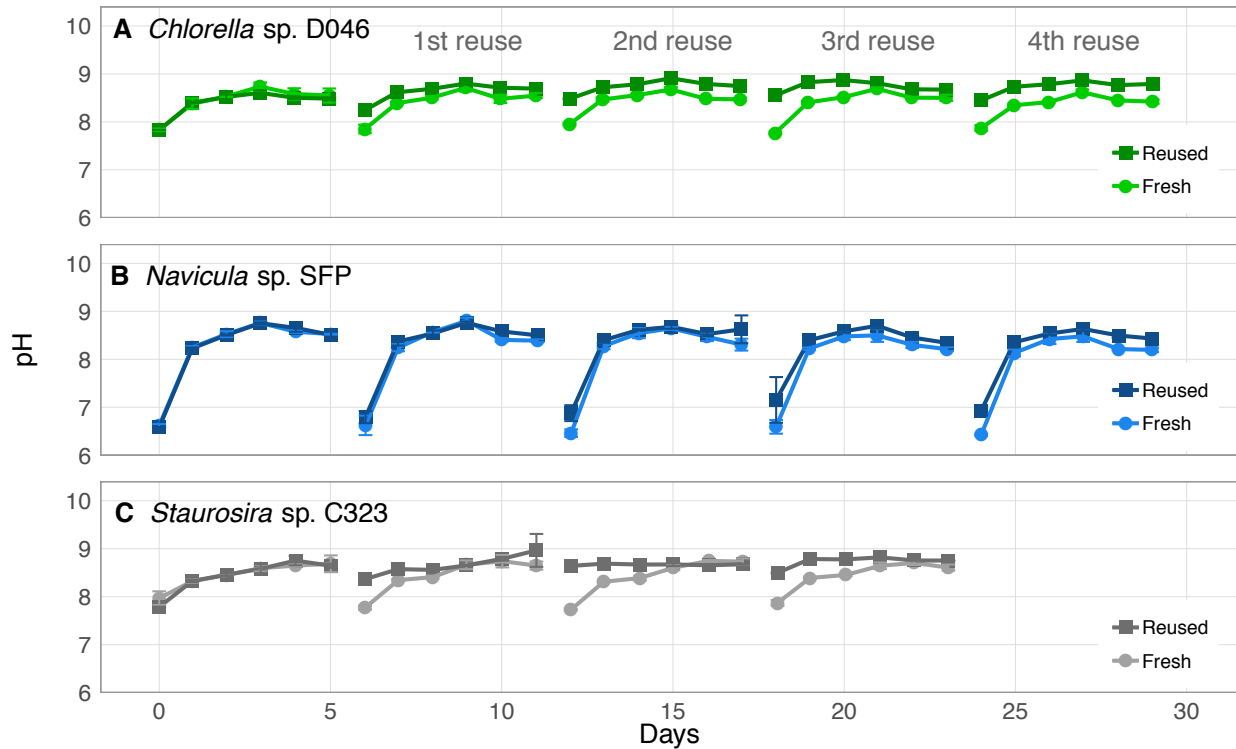
## 1 Supplementary Figures



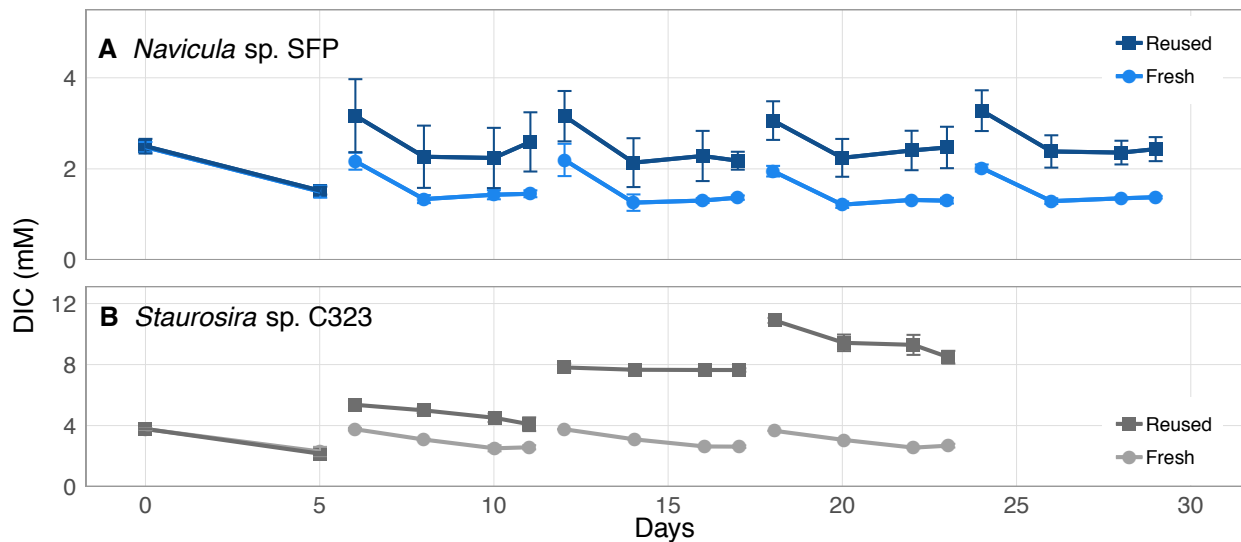
**Figure S1:** Blank-corrected optical density (OD) at 750 nm of the cultures (A) *Chlorella* sp. D046, (B) *Navicula* sp. SFP, and (C) *Staurosira* sp. C323 in fresh and reused growth medium. Error bars represent standard deviation of three replicate cultures.



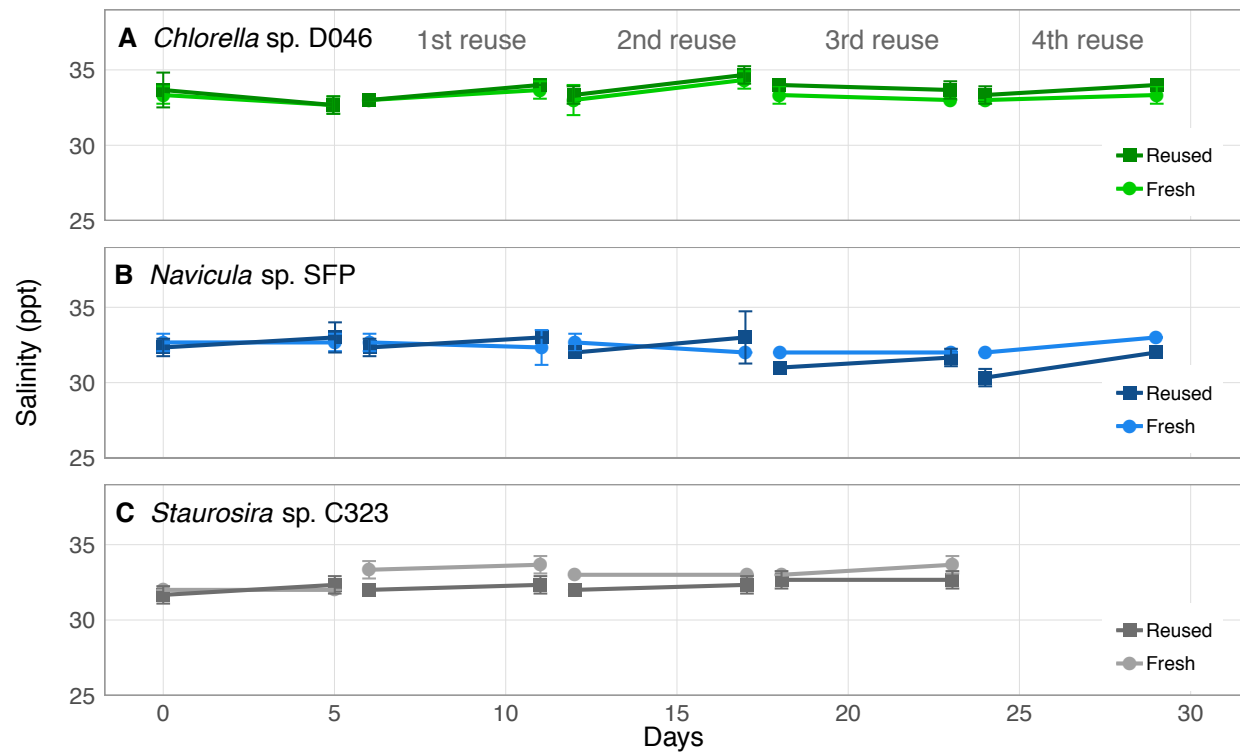
**Figure S2:** Bacteria cell concentrations over time in cultures of (A) *Chlorella* sp. D046, (B) *Navicula* sp. SFP, and (C) *Staurosira* sp. C323 in fresh and reused growth medium. Error bars represent standard deviation of three replicate cultures.



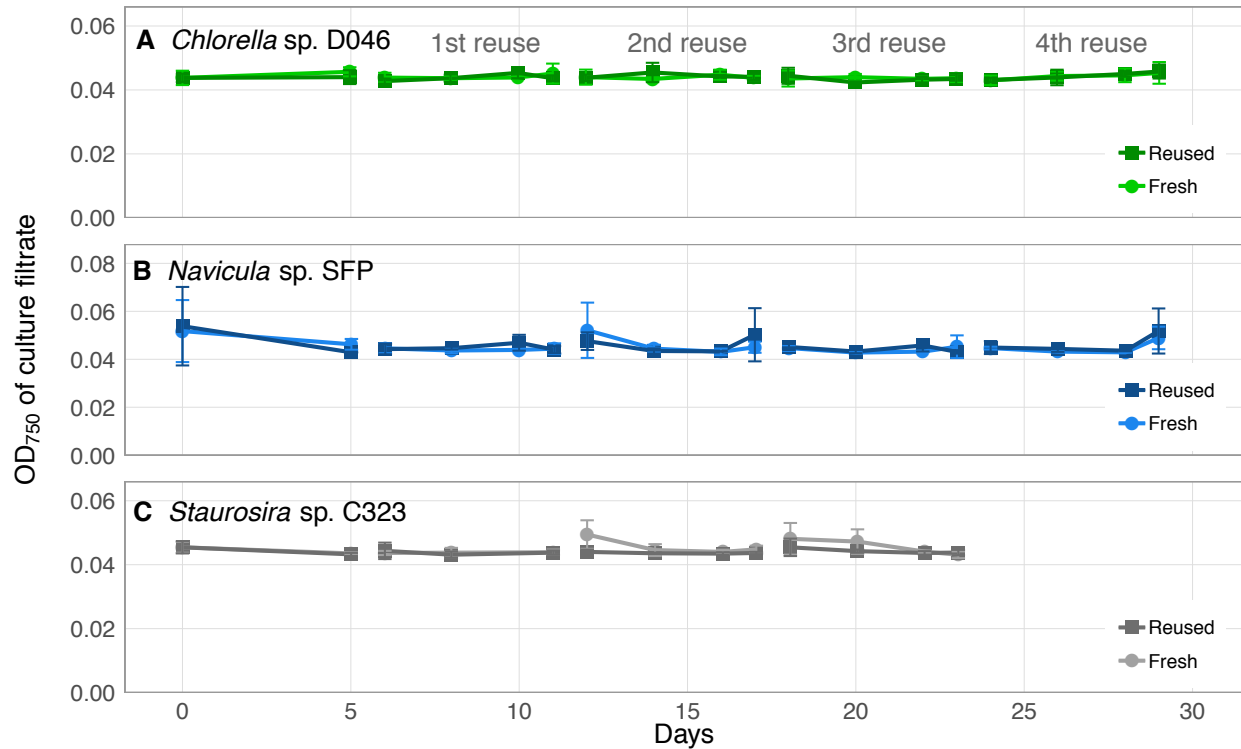
**Figure S3:** pH of cultures of (A) *Chlorella* sp. D046, (B) *Navicula* sp. SFP, and (C) *Staurosira* sp. C323 in fresh and reused growth medium. Error bars represent standard deviation of three replicate cultures.



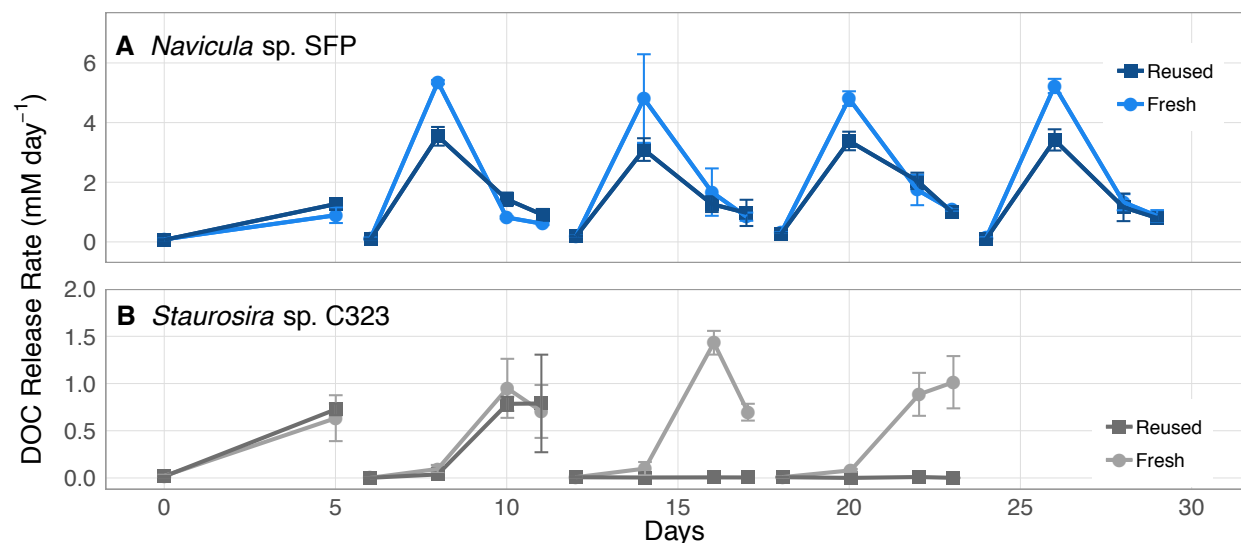
**Figure S4:** Dissolved inorganic carbon (DIC) in cultures of (A) *Navicula* sp. SFP and (B) *Staurosira* sp. C323 over time. DIC samples were unavailable for *Chlorella* sp. D046, except for the final day of the experiment in which samples were measured on a Shimadzu TOC-L Analyzer (Fresh Medium =  $2.84 \pm 0.34$  mM, Reused Medium =  $8.53 \pm 0.29$  mM). Error bars represent standard deviation of three replicate cultures.



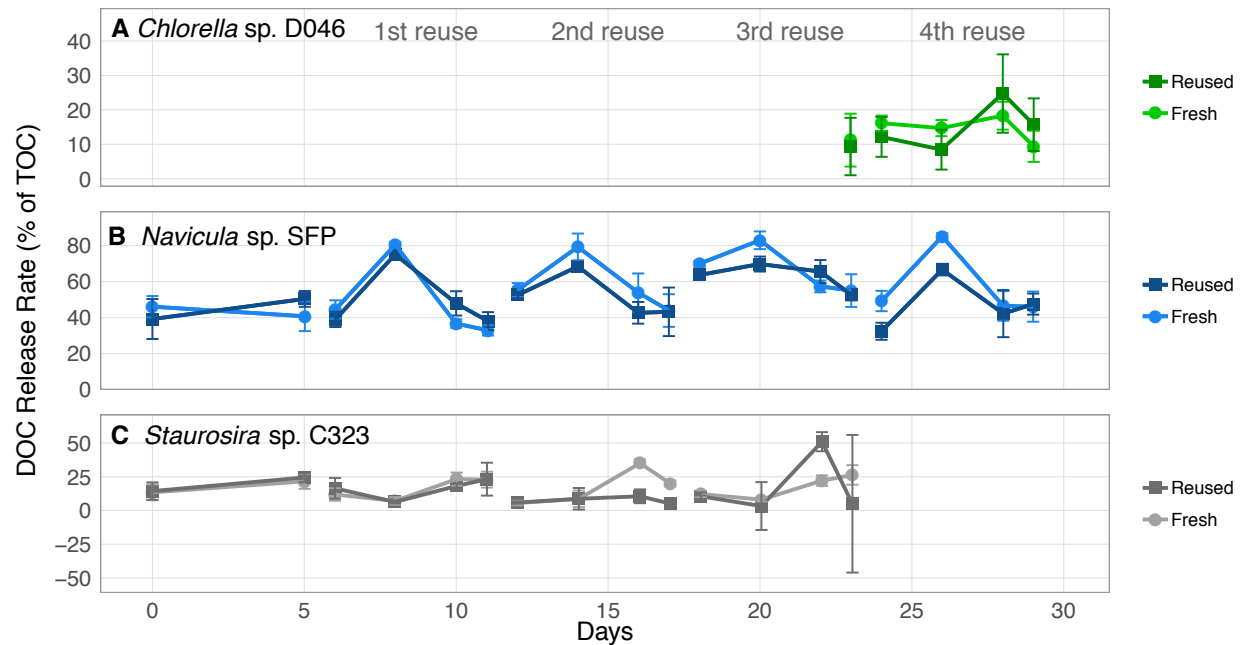
**Figure S5:** Salinity of cultures of (A) *Chlorella* sp. D046, (B) *Navicula* sp. SFP, and (C) *Staurosira* sp. C323 cultures at the start and end of each round in fresh and reused growth medium. Error bars represent standard deviation of three replicate cultures.



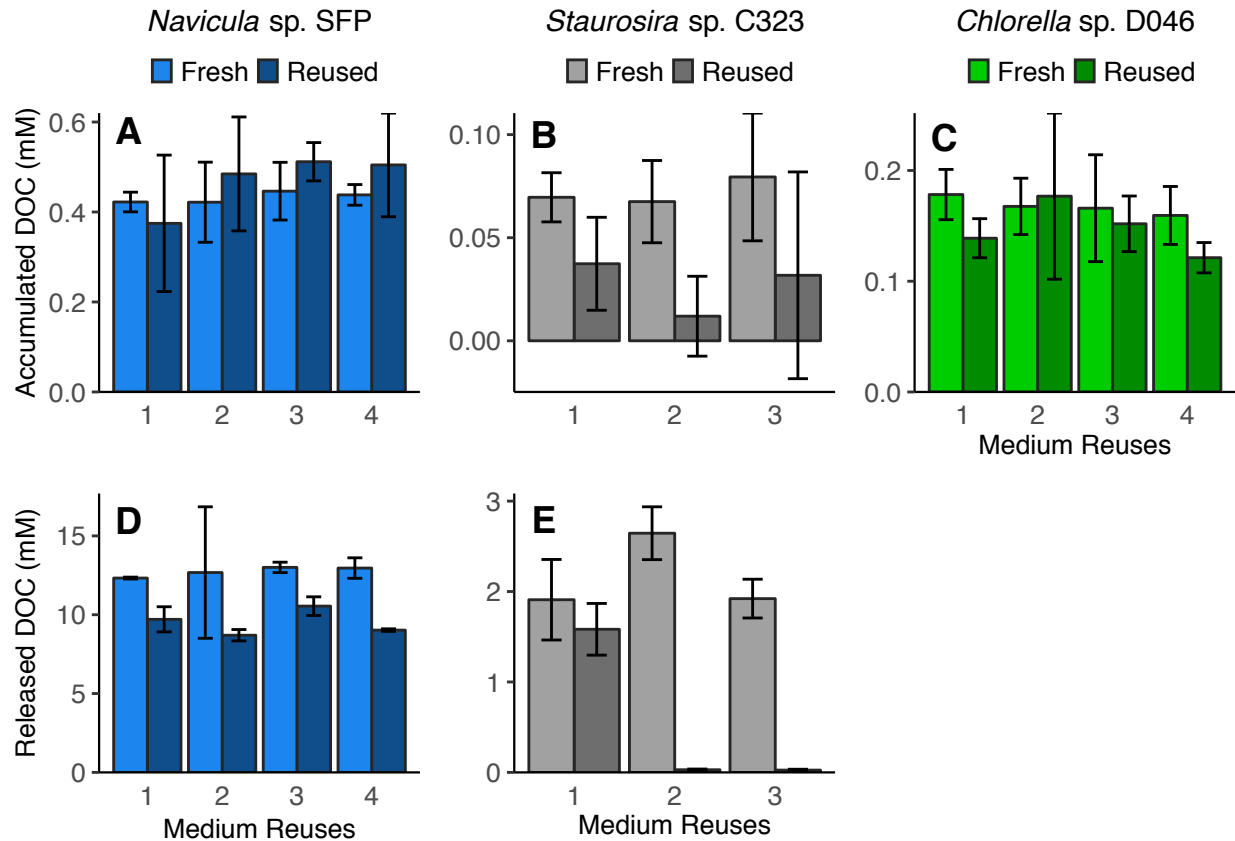
**Figure S6:** OD at 750 nm of 0.2- $\mu$ m culture filtrate of (A) *Chlorella* sp. D046, (B) *Navicula* sp. SFP, and (C) *Staurosira* sp. C323 in fresh and reused medium. Error bars represent standard deviation of three replicate culture filtrates. Average OD of ASW medium, measured daily, was  $0.0432 \pm 0.0009$  ( $n = 84$ ) across all experiments.



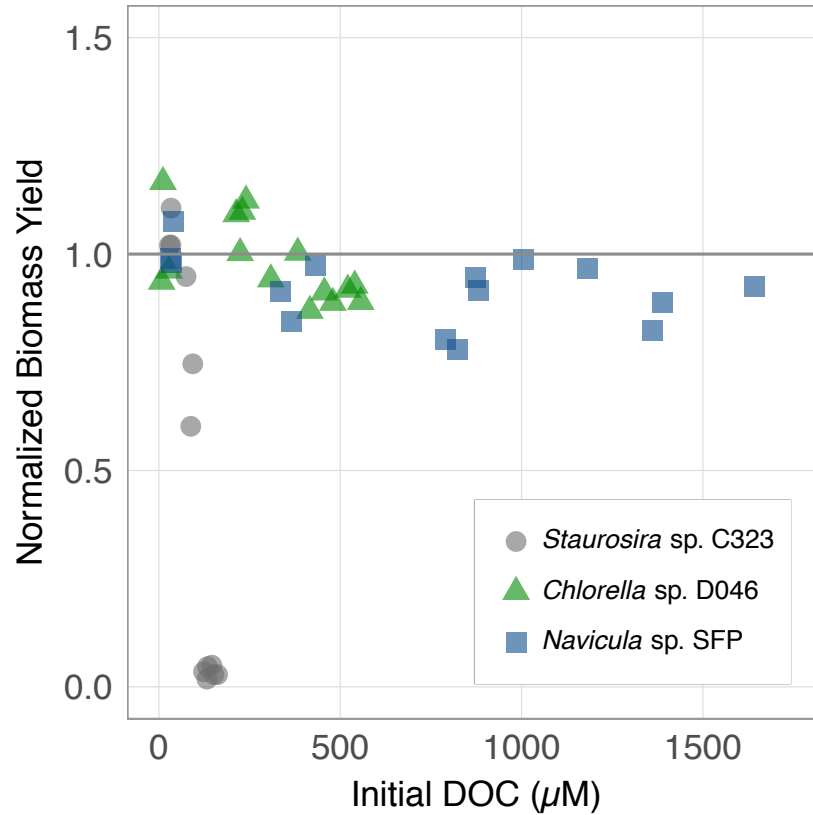
**Figure S7:** DOC release rates ( $\text{mM day}^{-1}$ ) over time for (A) *Navicula* sp. SFP and (B) *Staurosira* sp. C323 in fresh and reused growth medium. Error bars represent standard deviation of three replicate cultures.



**Figure S8:** DOC release rate as a percentage of total organic carbon (TOC) production rate over time for (A) *Chlorella* sp. D046, (B) *Navicula* sp. SFP, and (C) *Staurosira* sp. C323 in fresh and reused growth medium. Error bars represent standard deviation of three replicate cultures.



**Figure S9: (A-C)** Accumulated DOC (difference between Day 0 and Day 5) and **(D, E)** Released DOC (predicted from DOC release rates) per round. Error bars represent standard deviation of three replicate cultures. Note: *Chlorella* sp. D046 Reused Treatment in Reuse 2 is from 2 replicates only. Released DOC data are unavailable for *Chlorella* sp.



**Figure S10:** Biomass yield (change in particulate carbon) in reused medium, normalized by mean biomass yield in fresh medium, versus biologically-derived DOC concentration ( $\mu\text{M}$ ) in reused medium at the start of each round. Bolded horizontal line indicates equal biomass yield in fresh and reused medium.