The following supplement accompanies the article

N-fixation and related O_2 constraints on model marine diazotroph *Pseudomonas stutzeri* BAL361

Ryan W. Paerl*, Tobias N. G. Hansen, Nathalie N. S. E. Henriksen, Asmus K. Olesen, Lasse Riemann

*Corresponding author: rpaerl@ncsu.edu

Aquatic Microbial Ecology 81: 125–136 (2018)

Figure S1. *Pseudomonas stutzeri* BAL361 forms extensive biofilms when grown on marine broth 2216 under still conditions. The arrow points to biofilm pieces that are readily seen when agitating the culture in a sterile 15 mL polypropylene Falcon tube (Corning Life Sciences, Tewksbury, MA, USA).

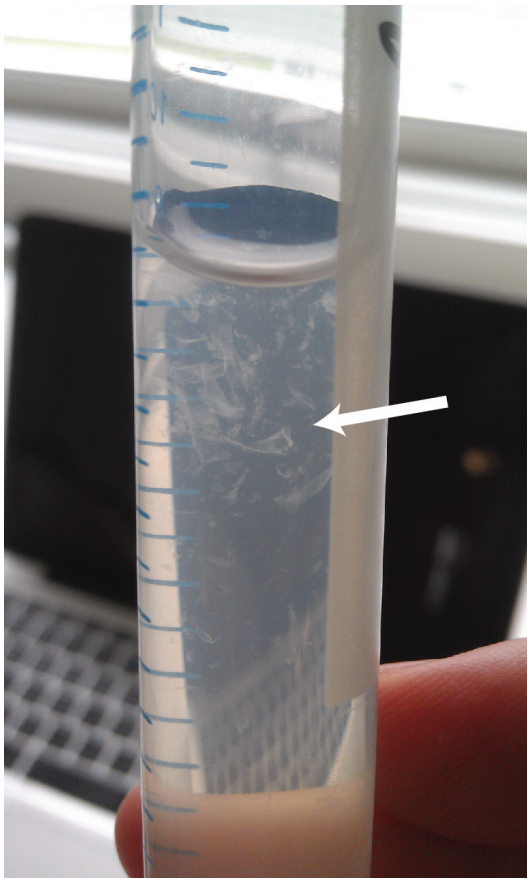


Figure S2. Aerobic cultures of BAL361 fail to exploit different available particles to perform significant N-fixation. (A) Nitrogenase activity (ethylene production) of BAL361 cultures with amended particles was not significantly above that of the particle free, aerobic (Full O_2) cultures. Only 35 µM O_2 cultures exhibited significantly higher nitrogenase activity versus the Full O_2 cultures (noted with asterisks; based on t-tests, $p < 0.05$). Mean (columns) and standard deviation (error bars) values are plotted for triplicate cultures. (B) BAL361 cell abundances in cultures during the particle addition experiment. Acetylene was added for AR assays on Day 1 (marked with a black arrow). The mean (data points) and standard deviation (error bars – some are too small to visualize) for triplicate flasks are plotted. GF/F = glass fiber filter particles; Zos = *Zostera*-derived particles; TEP = Xanthan gum, transparent expolymer particles.

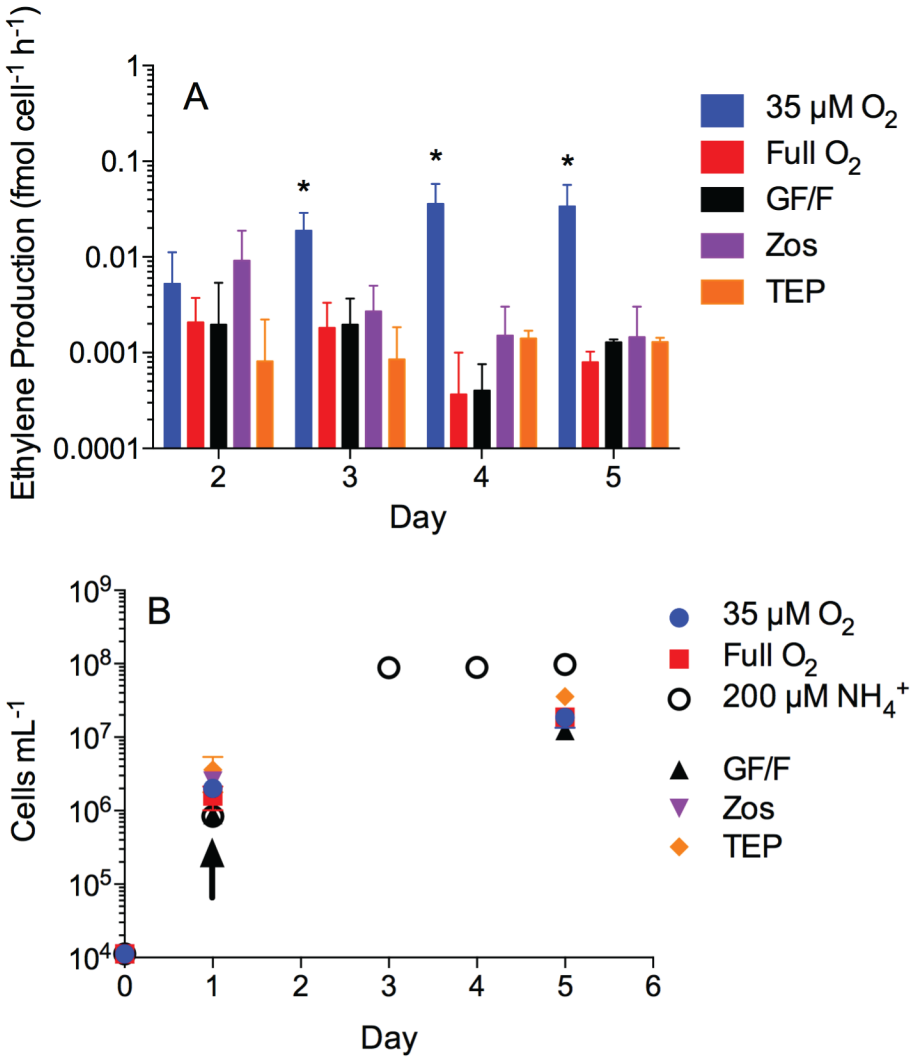


Figure S3. Adding a natural microbial plankton community (>0.2, <0.8 µm in cell diameter; Comm.) with particles did not facilitate N-fixation in aerobic BAL361 cultures. (A) Nitrogenase activity (ethylene production) in cultures; only the 35 µM O_2 cultures exhibited significantly higher nitrogenase activity (marked with an asterisk) versus the Full O_2 cultures (based on two-tailed unpaired t-tests; $p < 0.05$). The means (columns) and standard deviations for triplicate cultures are plotted. (B) Bacterioplankton abundances in cultures during the experiment. A black arrow denotes when acetylene was added into vials. Increased growth in the 200 µM NH_4^+ cultures in (B) confirms that BAL361 was N-limited in the other cultures (receiving 40 µM NH_4^+ at the start). Means (data points) and standard deviations (error bars) for triplicate cultures are plotted. GF/F = glass fiber filter particles; TEP = Xanthan gum, transparent exopolymer particles.

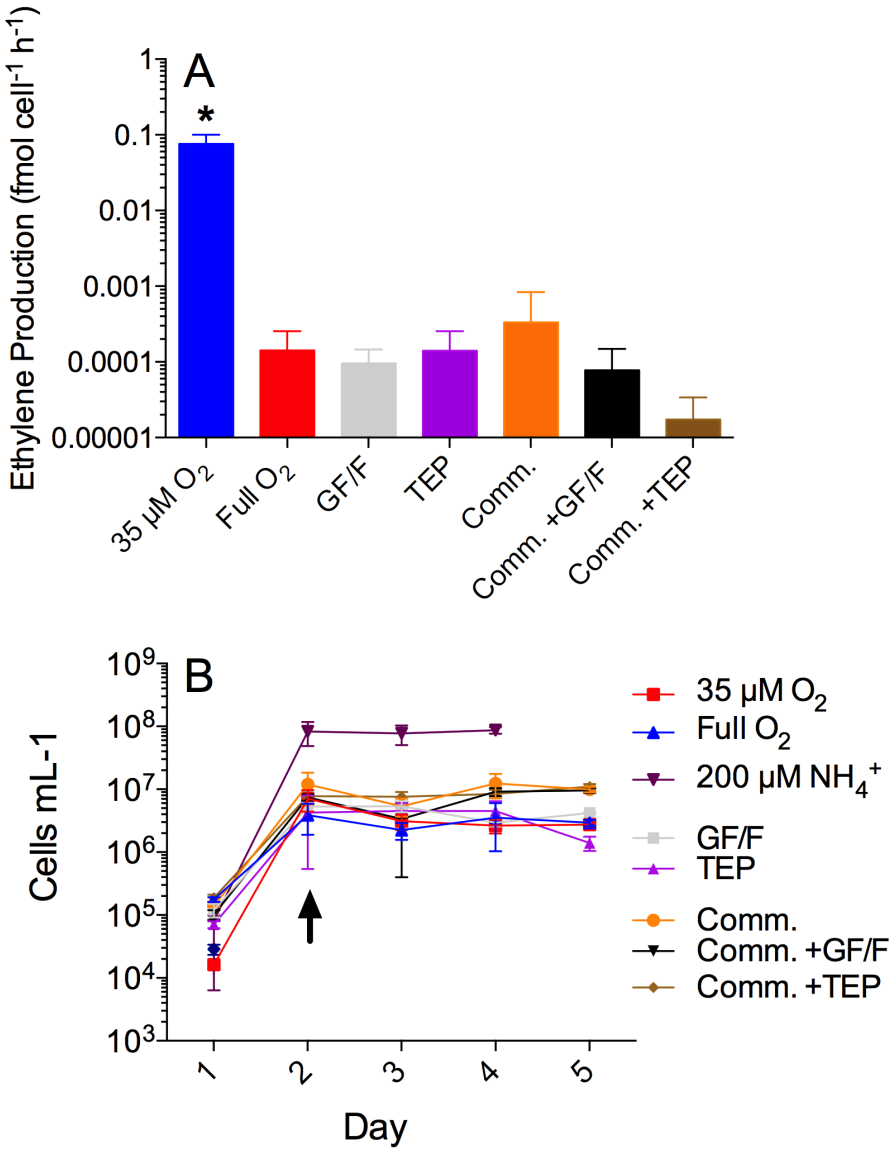


Figure S4. Evidence of BAL361 attachment to various supplied particles in N-limited diazotrophic medium. Arrows point to BAL361 cells attached to (A) a *Zostera* particle, (B) TEP particle, and (C, D) precombusted GF/F particles. Micrographs were taken from different experiments; A-C are from the experiment linked to data in Figure S3, D is from the experiment linked to Figure S2.

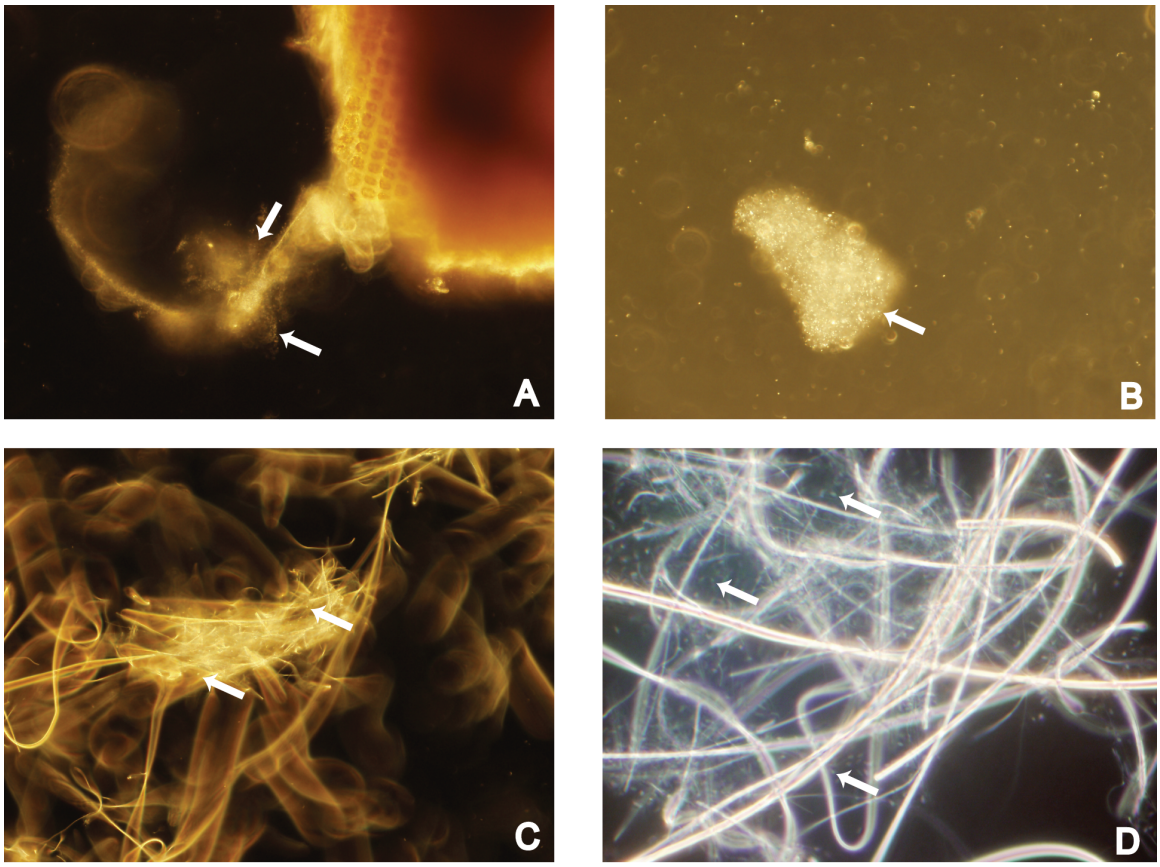


Figure S5. Forcibly concentrating exponentially growing BAL361 cells onto GF/F filters, with or without an agarose covering, induces reduced oxygen concentrations on the surface (Surf) of filters relative to the surrounding culture (Bulk). Measured oxygen concentrations from the primary bulk culture (Bulk Culture), the source of BAL361 cells for concentrating onto filters, are plotted and represent pseudo-replicate measurements. All other plotted data represent measurements from triplicate flasks (means are represented by columns and standard deviations are represented by error bars).

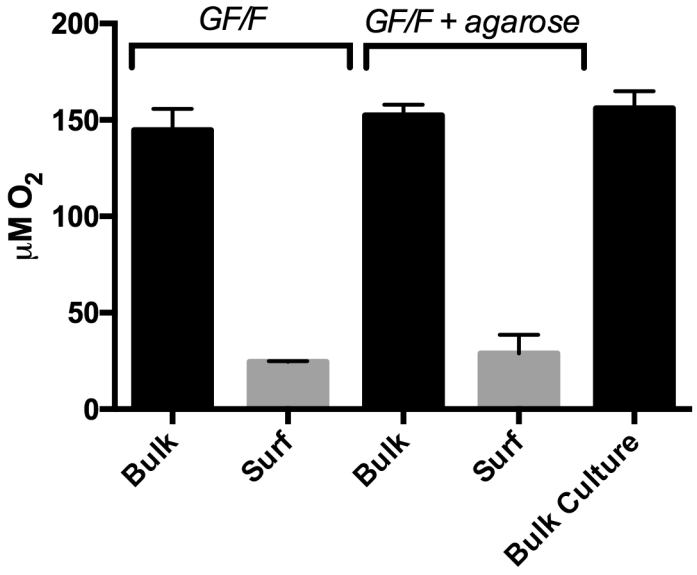


Figure S6. Cell abundances of a primary BAL361 culture sub-sampled for testing nitrogenase activity of BAL361 cells concentrated upon filters (nitrogenase activity data is presented in Figure 5). Arrows mark the days in which the culture was sub-sampled – falling within early and late stationary phases of the growth curve.

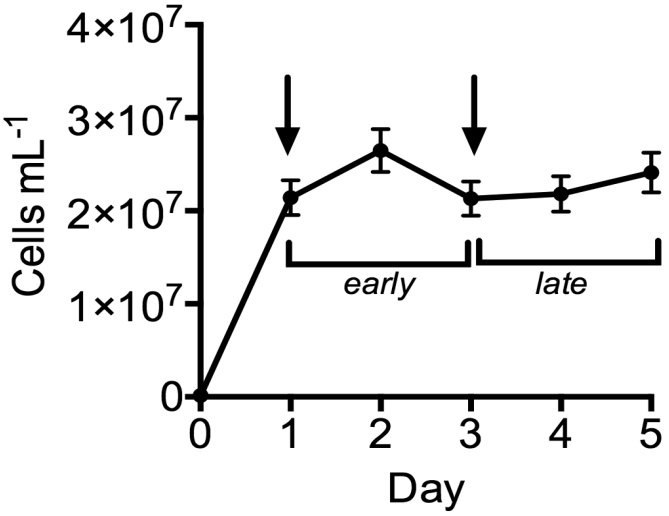
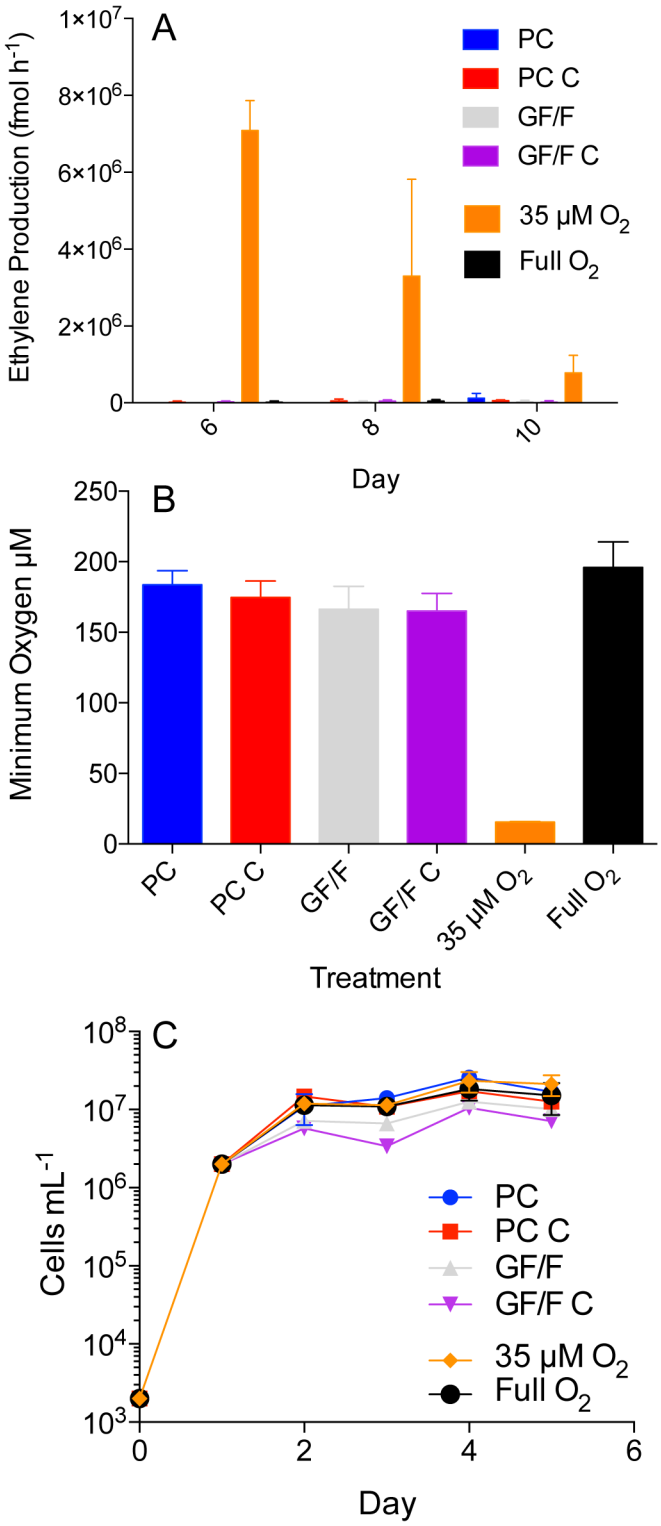


Figure S7. 'Seeding' ~2,000 BAL361 cells via filtration upon filters (polycarbonate (PC C) or glass fiber (GF/F C)) does not lead to notable downstream nitrogenase activity under aerobic conditions. (A) Considerable nitrogenase activity (ethylene production per hour (per vial)) was detected only in cultures with reduced oxygen concentrations (35 μM O_2, lowered on Day 5, and injected with acetylene – see methods). (B) Oxygen concentrations on the surface of filters in treatment cultures (with filters) minimally declined relative to that of fully aerobic (Full O_2) cultures (without filters) during the experiment. (C) Cell abundances of cultures during the experiment. BAL361 grew rapidly in the medium as by Day 1, >1 x 10^6 planktonic BAL361 cells mL^{-1} were detected in all cultures, including those where the only cells concentrated on filters were added at the start of the experiment, an indication of rapid detachment of BAL361 cells initially concentrated on filters. Plotted columns and data points, as well as respective error bars, represent mean and standard deviation values for triplicate cultures.


A
Ethylene Production (fmol h-1)
1×10^7
8×10^6
6×10^6
4×10^6
2×10^6
0
6
8
10
Day
PC
PC C
GF/F
GF/F C
35 µM O2
Full O2
B
Minimum Oxygen µM
250
200
150
100
50
0
PC
PC C
GF/F
GF/F C
35 µM O2
Full O2
Treatment
C
Cells mL-1
10^8
10^7
10^6
10^5
10^4
10^3
0
2
4
6
Day
PC
PC C
GF/F
GF/F C
35 µM O2
Full O2