

The role of inorganic nitrogen in successful formation of granular biofilms for wastewater treatment that support cyanobacteria and bacteria

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Supporting Information:

This Supporting information contains expansion of data referenced in the text of manuscript. These figures and tables that include: Primer information (Table S1), Phosphate (Figure S1), soluble COD (Figure S2), Suspended Solids (TSS/VSS) (Figure S3) trends referenced in this manuscript. Additionally, data supporting the microbial ecology and gene abundance is presented as a heat map of the top 20 OTUs for the successful and unsuccessful communities at the genus level for times 0 days and 42 days (Figure S4), the absolute gene copy numbers for Cyanobacterial 16S rRNA (*CYAN*), *AmoA*, and *narG* (Figure S5), and the log₂ fold change of the target genes *CYAN*, *amoA*, and *narG* using 16S rDNA as a reference for the successful community and compared to the unsuccessful community for each time point (Figure S6).

Table 1.

Table S1. Primers used in this study for PCR and qPCR analysis.

Target Gene Name or Shorthand	Target Description	Primer Name	Sequence (5' – 3')	Anneal (°C)	Length (bp)	References
<i>amoA</i>	<i>Betaproteobacteria</i> ammonia monooxygenase	amoA-1F amoA-2R	GGGGHTTYTACTGGTGGT CCCCTCKGSAAAGCCTTCTTC	58	491	(Rotthauwe, Witzel, & Liesack, 1997; Segawa et al., 2014)
<i>narG</i>	Nitrate reductase	narG-F narG-R	TCGCCSATYCCGGCSATGTC GAGTTGTACCAGTCRGC SGAYTCSG	60	173	(Bru, Sarr, & Philippot, 2007)
Cyanobacteria 16S rRNA (CYAN)	Cyanobacteria 16S rRNA gene	CYAN 108F CYAN 377R	ACGGGTGAGTAACRCGTRA CCATGGCGGAAAATTCCC	52	269	(Martins & Vasconcelos, 2011; Urbach, Robertson, & Chisholm, 1992)
16S rRNA gene	Universal 16S	1114 1275	CGGCAACGAGCGCAACCC CCATTGTAGCACGTGTGTAGCC	60	161	(Denman & McSweeney, 2006)

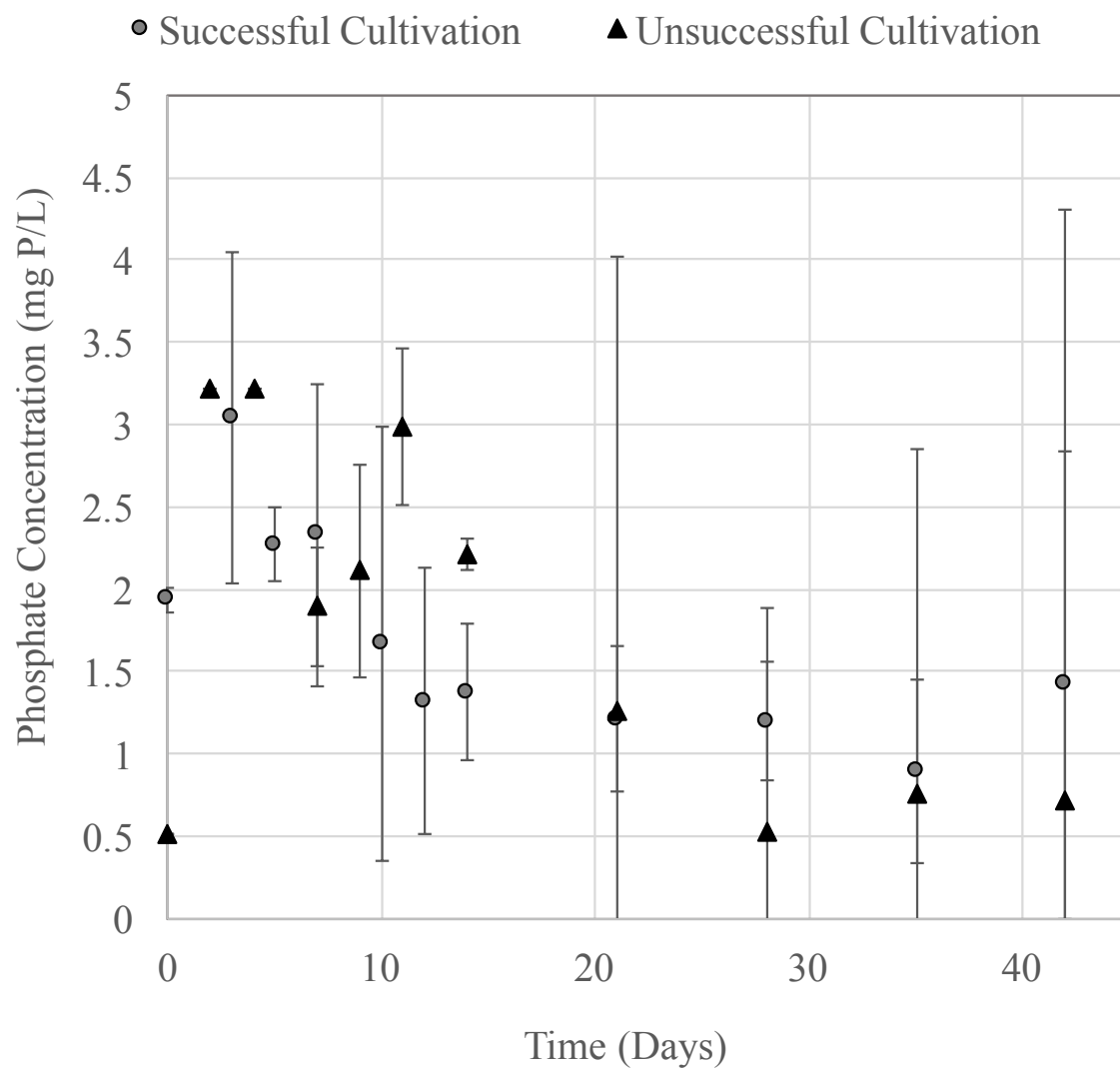


Figure S1. Phosphate concentration for successful and unsuccessful cultivations

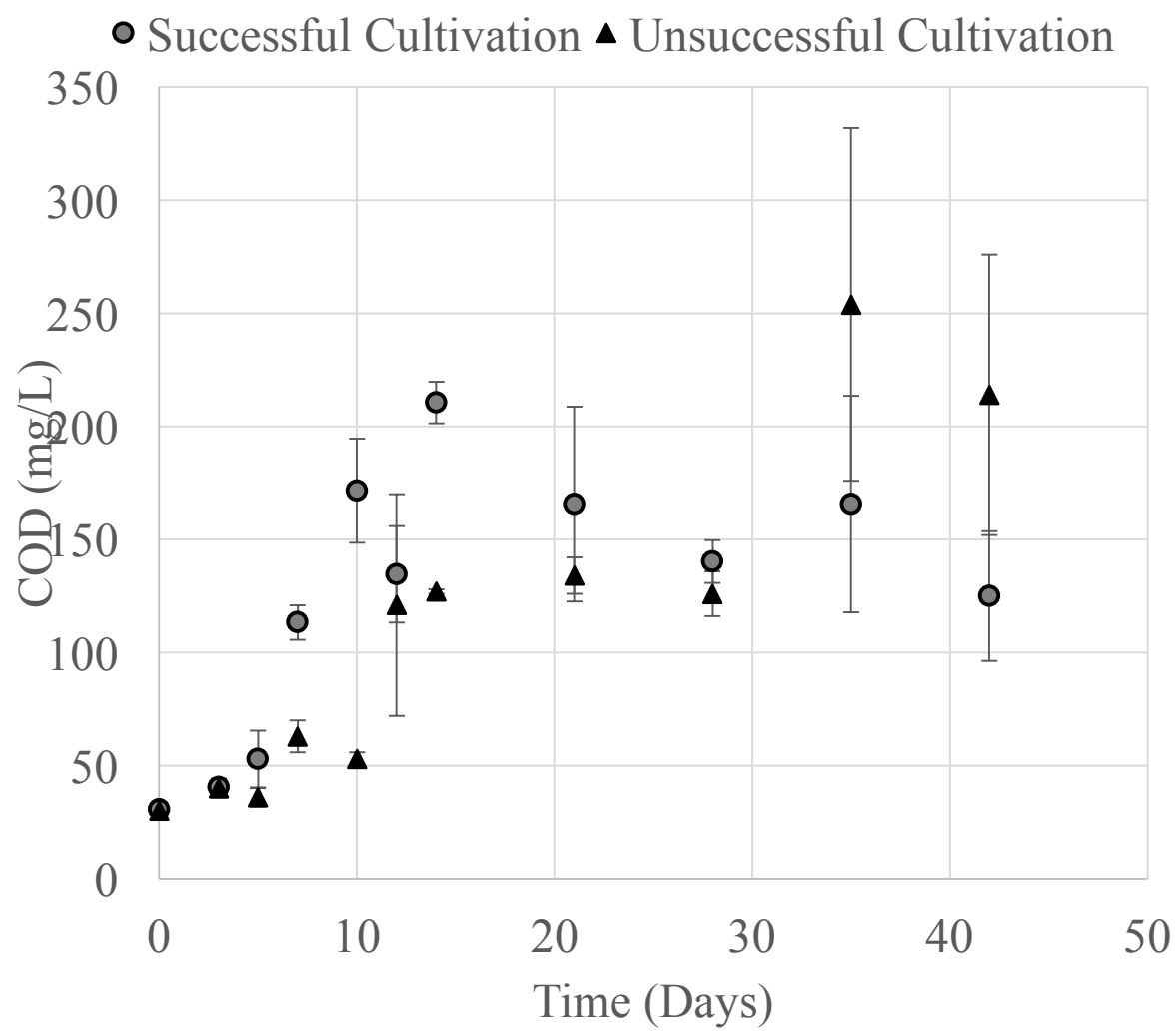


Figure S2. Soluble COD concentration for successful and unsuccessful cultivations

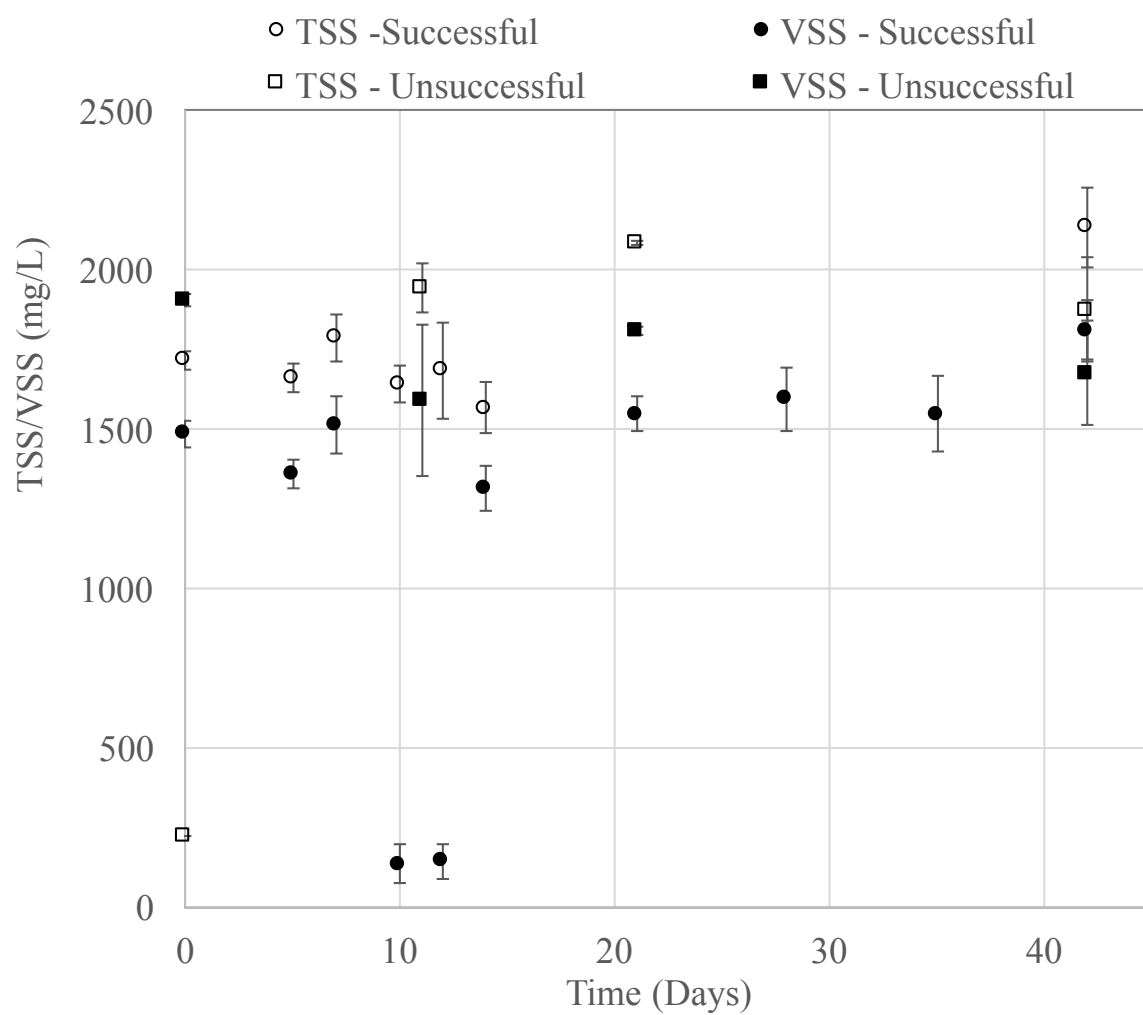
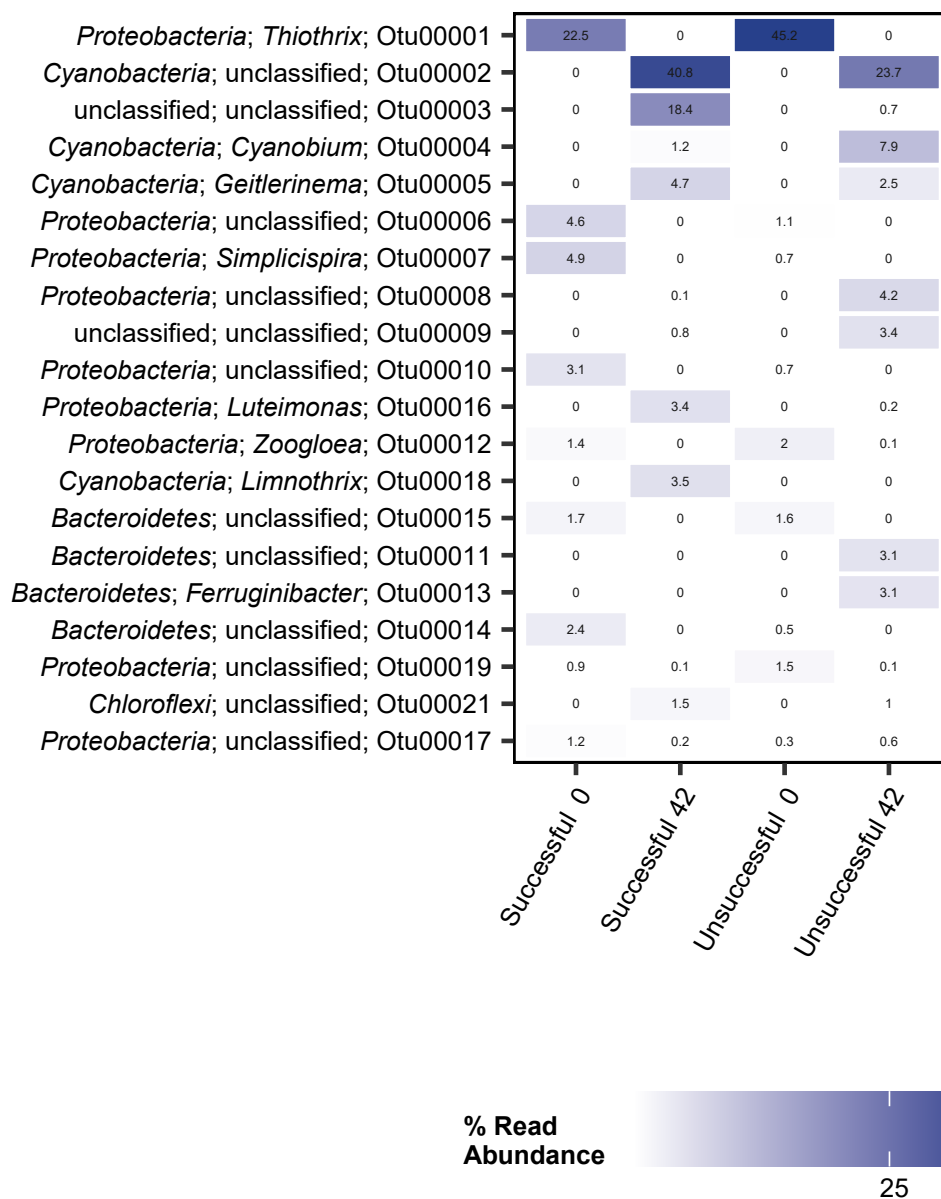


Figure S3. TSS and VSS concentration for successful and unsuccessful cultivations

Figure S4. Heat map for the top twenty OTUs for the initial and final communities at times 0 and 42 days.

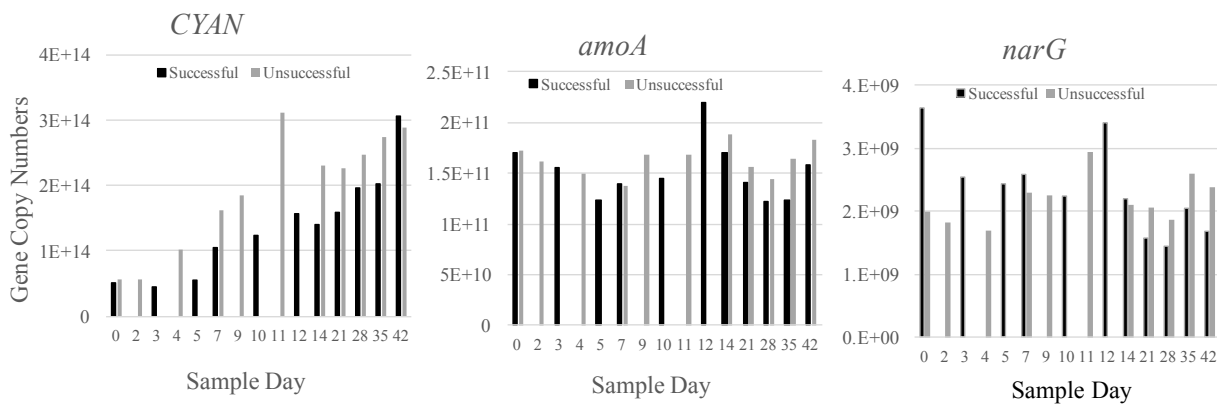


Figure S5. Gene Copy numbers of the target genes *CYAN*, *amoA*, and *narG* for the successful community and unsuccessful community for each time point. Absolute copy numbers were calculated using standard curves constructed qPCR reactions

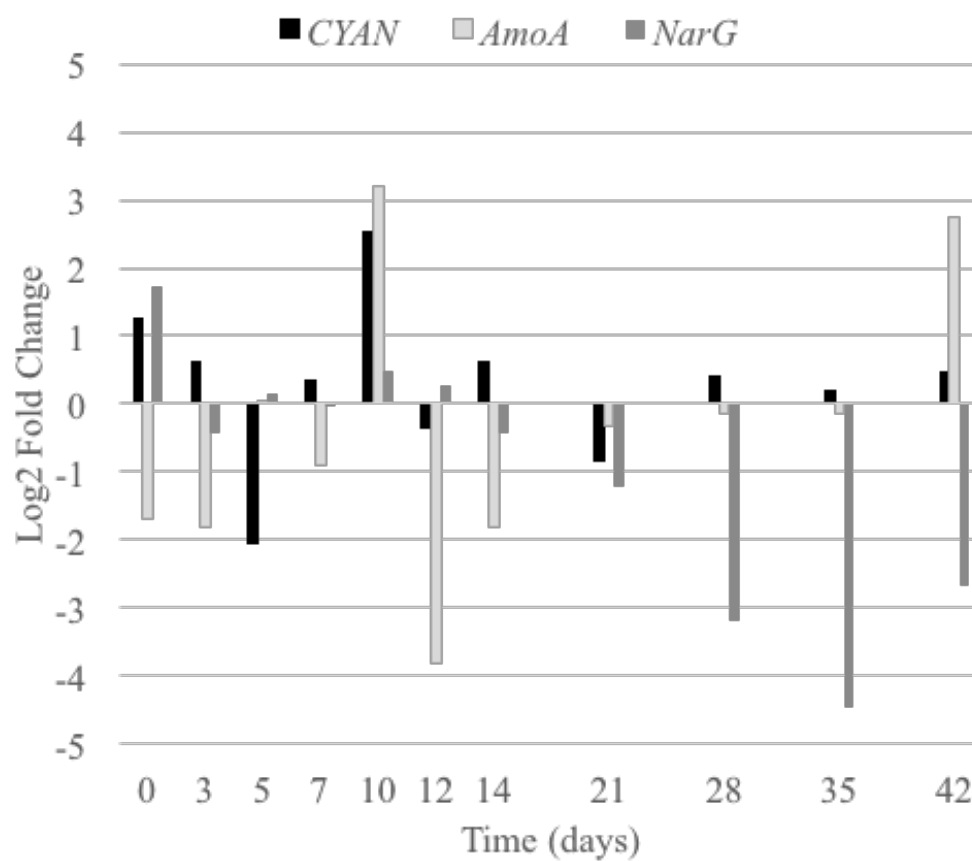


Figure S6. Log₂ fold change of the target genes *CYAN*, *amoA*, and *narG* using 16S rDNA as a reference for the successful community and compared to the unsuccessful community for each time point. R_2 values were > 0.98 for all calibrations and reaction efficiencies were greater than 90%