### 1 Electronic Supplementary Information

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### 3 Insights Gained into Activated Sludge Nitrification through Structural and

#### 4 Functional Profiling of Microbial Community Response to Starvation Stress

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#### 8 Nitrospira reference sequences and OTUs of interest

9 N. moscoviensis 16S rRNA gene (GenBank: X82558.1 base pairs 521-773): 10 TACGAAGGTGGCAAGCGTTGTTCGGATTCACTGGGCGTACAGGGAGCGTAGG CGGTTGGGTAAGCCCTCCGTGAAATCTCCGGGCCTAACCCGGAAAGTGCAGA 11 12 GGGGACTGCTCGGCTTGAGGATGGGAGAGGAGCGCGGAATTCCCGGTGTAGC 13 GGTGAAATGCGTAGAGATCGGGAGGAAGGCCGGTGGCGAAGGCGGCGCTCT <u>14</u> 16 N. defluvii 16S rRNA gene (GenBank: DQ059545.1 base pairs 522-774): 17 TACGAAGGTGGCAAGCGTTGTTCGGATTCACTGGGCGTACAGGGTGTGTAGG 18 CGGTTTGGTAAGCCTTCTGTTAAAGCTTCGGGCCCAACCCGGAAAGCGCAGA 19 GGGTACTGCCAGGCTAGAGGGTGGGAGAGGAGCGCGGAATTCCCGGTGTAG 20 CGGTGAAATGCGTAGAGATCGGGAGGAAGGCCGGTGGCGAAGGCGGCGCTC  $\frac{21}{22}$ TGGAACATACCTGACGCTGAGACACGAAAGCGTGGGGAGCAAACAGG 23 N. marina 16S rRNA gene (GenBank: X82559.1 base pairs 522-774): 24 TACGAAGGTGGCAAGCGTTGTTCGGATTTACTGGGCGTAAAGAGCACGTAGG 25 CGGTTGGGAAAGCCTTTTGGGAAATCTCCCGGCTTAACCGGGAAAGGTCGAG 26 AGGAACTATTCAGCTAGAGGACGGGGAGAGGAGCGCGGGAATTCCCGGTGTAG 27 CGGTGAAATGCGTAGATATCGGGAAGAAGGCCGGTGGCGAAGGCGGCGCTC <u>2</u>8 30 N. backiana 16S rRNA gene (GenBank: EU084879.1 base pairs 524-776): 31 TACGAAGGTGGCAAGCGTTGTTCGGATTTACTGGGCGTAAAGGGAGCGTAGG 32 CGGTTCGGTAAGACCGATGGGAAATCCCGGAGCTTAACTTCGGAAGGTCATC 33 GGTGACTGCCGGGCTAGAGGACGGGGAGAGGGGGGGGGAATTCCCGGTGTAG 34 CGGTGAAATGCGTAGAGATCGGGAGGAAGGCCGGTGGCGAAGGCGGCGCTC 35 TGGAACGTTCCTGACGCTGAGGCTCGAAAGCGTGGGGGAGCAAACAGG 37 N. calida 16S rRNA gene (GenBank: HM485589.1 base pairs 522-774): 38 TACGAAGGTGGCAAGCGTTGTTCGGATTTACTGGGCGTACAGGGTGCGTAGG 39 CGGTTCGGTAAGCCCTTCGGGAAAGCTCCGGGCTTAACCCGGAAAGGTCGGA

41 CGGTGAAATGCGTAGAGATCGGGAGGAAGGCCGGTGGCGAAGGCGGCGCTC 43 TGGAACGTTTCTGATGCTGAGGCACGAAAGCGTGGGGAGCAAACAGG 44 N. nitrosa 16S rRNA gene (European Nucleotide Archive: CZQA01000015.1 base pairs 45 522-774): 46 TACGAAGGTGGCAAGCGTTGTTCGGATTTACTGGGCGTACAGGGAGCGTAGG 47 CGGTTGGGTAAGCCCTCCGTGAAATCTCCGGGCCTAACCCGGAAAGTGCGGA 48 GGGGACTGCTCGGCTAGAGGATGGGAGAGGAGCGCGGAATTCCCGGTGTAG 49 CGGTGAAATGCGTAGAGATCGGGAGGAAGGCCGGTGGCGAAGGCGGCGCTC <del>3</del>0 TGGAACATTTCTGACGCTGAGGCTCGAAAGCGTGGGGAGCAAACAGG 52 *N. inopinata* 16S rRNA gene (European Nucleotide Archive: LN885086.1 base pairs 53 522-774): 54 TACGAAGGTGGCAAGCGTTGTTCGGATTTACTGGGCGTACAGGGAGCGTAGG 55 CGGTTGGGTAAGCCCTCCGTGAAATCTCCGGGCCTAACCCGGAAAGTGCGGA 56 GGGGACTGCTTGGCTAGAGGATGGGAGAGGAGCGCGGAATTCCCGGTGTAG 57 CGGTGAAATGCGTAGAGATCGGGAGGAAGGCCGGTGGCGAAGGCGGCGCTC ξĝ TGGAACATTTCTGACGCTGAGGCTCGAAAGCGTGGGGAGCAAACAGG 60 Leptospirillum ferroadiazotrophum 16S rRNA gene (GenBank: KT834983 base pairs 61 507-759): 62 GACAGGGGTGGCAAGCGTTGTTCGGAGTGACTGGGCGTAAAGGGTCTGTAGG 63 TGGTTTTTCAAGTCTTTGGTAAAAAGCCGTGGCTTAACCATGGTGAGGCCAAG GAGACTGGGAGACTCGAGGCTGGGAGAGGGGAAGCGGAATTTCTGGTGTAGC 64 65 GGTGAAATGCGTAGAGATCAGAAAGAAGGCCGGTGGCGAAGGCGGCTTCCT 69 GGAACAGACCTGACACTGAGAGACGAAAGCGTGGGGAGCAAACAGG 68 *Nitrospira* OTU A: 69 TACGAAGGTGGCAAGCGTTGTTCGGATTCACTGGGCGTACAGGGTGTGTAGG 70 CGGTTTGGTAAGCCTTCTGTTAAAGCTTCGGGCCCAACCCGGAAAGCGCAGA 71 GGGTACTGCCAGGCTAGAGGGTGGGAGAGGAGCGCGGAATTCCCGGTGTAG 72 CGGTGAAATGCGTAGAGATCGGGAGGAAGGCCGGTGGCGAAGGCGGCGCTC 73 TGGAACATACCTGACGCTGAGACACGAAAGCGTGGGGGAGCAAACAGG 75 Nitrospira OTU B: 76 TACGAAGGTGGCAAGCGTTGTTCGGATTTACTGGGCGTACAGGGAGCGTAGG 77 CGGTTGGGTAAGCCCTCCGTGAAATCTCCGGGCCTAACCCGGAAAGTGCGGA 78 GGGGACTGCTCGGCTAGAGGATGGGAGAGGAGCGCGGAATTCCCGGTGTAG 79 CGGTGAAATGCGTAGAGATCGGGAGGAAGGCCGGTGGCGAAGGCGGCGCTC 80 TGGAACATTTCTGACGCTGAGGCTCGAAAGCGTGGGGAGCAAACAGG

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# 82 Supplementary Tables

Trial	Experimental Temp (°C)	Date of Collection	Collection Temp (°C)	Frequency of NPR tests	Samples selected for 16S rRNA amplicon sequencing	
14 °C Trial 1	14	4/7/2014	15.17	Days 2, 3, 5, 8, 11, 14, 18	Days 2, 3, 5, 8, 11, 14, 18	
20 °C Trial 1	20	12/2/2013	18.38	Days 2, 3, 5, 7, 10, 14, 18	Days 2, 3, 5, 7, 10, 14, 18	
30 °C Trial 1	30	2/19/2014	15.17	Days 2, 3, 5, 7, 9, 12, 16	Days 2, 3, 5, 7, 9, 12, 16	
14 °C Trial 2	14	3/20/2015	14.36	Days 2, 3, 5, 7, 10, 14, 18 Recovery for 6hr, 12hr, 24hr, 36hr, 48hr	INF, Days 0, 2, 7, 18, Recovery for 12hr, 48hr	
20 °C Trial 2	20	5/11/2015	20.49	Days 2, 3, 5, 7, 10, 14, 18 Recovery for 6hr, 12hr, 24hr, 36hr, 48hr	INF, Days 0, 2, 7, 18, Recovery for 12hr, 48hr	
27 °C Trial 1	27	6/23/2015	23.72	Days 2, 3, 5, 7, 10, 14, 18 Recovery for 6hr, 12hr, 24hr, 36hr, 48hr	INF, Days 0, 2, 7, 18, Recovery for 12hr, 48hr	
Note: IN after Sta	Note: INF- sample taken at influent of DC Water nitrification basin, "Fed for" samples are those taken after Starvation Reactor was dosed with ammonia					

# 83 Table S1 Seed sludge collection dates, temperatures and sampling details

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87 Figure S1 Gene concentrations quantified in DNA extracted from MLSS samples

88 throughout all trials using qPCR of a) total 16S rRNA genes using universal primers

**b)** *amoA* gene encoding the ammonia monooxygenase enzyme used by bacteria for

- 90 oxidizing ammonia (AOBs) c) *Nitrospira* 16S rRNA targeting only *Nitrospira* species
- 91 and d) *Nitrobacter* 16S rRNA targeting only *Nitrobacter* species.
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95 Figure S2 Correlation analysis between metabolic activity (NO<sub>x</sub>PR and NPR) and

96 concentration of associated genes via qPCR. Statistical values are indicated in the

97 legend with significant p-values <0.05 and Spearman correlation coefficient (p) with

98 a value of 1 indicating perfect positive correlation and -1 indicating perfect negative correlation.

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102 Figure S3 Comparison of microbial community composition of all conditions and

103 trials applied across this study. MDS ordination of unifrac distance matrix of OTU

- 104 table rarefied to lowest sample sequencing depth (38,000 sequences). Intensity of red
- 105 shading increases with time in Starvation Reactor, purple points indicate samples
- 106 taken from the influent of the DC Water nitrification basin sampled after mixing of
- 107 return activated sludge, blue points represent samples collected during the
- 108 "recovery" phase, after feeding of ammonium to the Starvation Reactor.

	Log Relativ 14 °C Ex	re Abundance		
	DNA		cDNA	
Taxonomy (OMathulanhile and	D2 D3 D5 D8 D11 D14 D18	Taxonomy (a)Nannomytic	D2 D3 D5 D8 D11 D14 D18	Alphaproteobacteria
(f)Methylophilaceae		(g)Nannocystis	-1.62 -1.52 -1.40 -1.62 -2.40 -2.93 -3.47	Deltaproteobacteria
(o)Sphingobacteriales	-1.30 -1.23 -1.13 -1.08 -1.23 -1.48 -1.7	0 (f)Methylocystaceae	-1.06 -1.05 -1.12 -1.10 -1.00 -0.99 -0.96	Gammaproteobacteria
(g.s)Methylotenera, mobilis	-1.16 -1.22 -1.37 -1.56 -1.57 -1.66 -1.7	0 (o)Myxococcales	-1.49 -1.63 -1.71 -1.88 -2.07 -2.34 -2.19	Unclassified Proteorbacteria
(c)Betaproteobacteria	-1.39 -1.45 -1.68 -2.06 -2.17 -2.18 -2.3	6 (f)Methylophilaceae	<b>-1.52 -1.59 -1.71 -1.88 -1.96 -2.02 -2.08</b>	Acidobacteria
(f)Cytophagaceae	-1.83 -1.70 -1.71 -1.43 -1.40 -1.46 -1.3	3 (c)Betaproteobacteria	-1.74 -1.85 -2.17 -2.36 -2.38 -2.74 -2.87	Actinobacteria
(g,s)Methylotenera, mobilis	<b>-1.50 -1.54 -1.68 -1.90 -2.01 -2.01 -2.0</b>	4 (o)Myxococcales	-1.71 -1.85 -1.87 -2.02 -2.36 -2.37 -2.65	Bacteroidetes
(g)Nitrospira	-1.54 -1.45 -1.45 -1.49 -1.38 -1.35 -1.2	9 (o)Myxococcales	-1.72 -1.87 -1.60 -1.63 -1.81 -1.95 -2.07	Cyanobacteria
(o)Sphingobacteriales	-2.29 -2.30 -2.04 -1.80 -1.76 -1.64 -1.6	(o)Myxococcales		Deinococcus-Thermus
(g)Nabella	-2.20 -2.10 -2.11 -1.91 -1.02 -1.70 -1.0 14 °C Fy	periment 2	-1.45 -1.55 -1.55 -1.04 -1.05 -1.02 -1.56	Nitrospirae
	DNA	permient 2	cDNA	Planctomucates
Taxonomy	INF D0 D2 D7 D18 F12 F48	Taxonomyonomy	INF D0 D2 D7 D18 F12 F48	Unidentified
(f)Methylophilaceae	-0.72 -0.81 -0.71 -0.87 -1.12 -1.09 -1.1	1 (o)Myxococcales	-1.32 -1.33 -1.20 -1.20 -2.07 -2.12 -2.44	(k) kingdom
(f)Saprospiraceae	-2.44 -2.33 -2.34 -1.89 -0.98 -0.98 -1.0	1 (o)Myxococcales	-1.43 -1.41 -1.10 -1.43 -2.02 -2.34 -2.14	(p) phylum
(c)Betaproteobacteria	-1.02 -1.03 -0.93 -1.31 -1.43 -1.37 -1.4	1 (c)Betaproteobacteria	-1.26 -1.27 -1.22 -1.61 -1.74 -2.42 -2.19	(c) class
(c)Deltaproteobacteria	-2.43 -2.42 -2.15 -1.54 -1.87 -1.89 -1.8	2 (c)Deltaproteobacteria	-1.99 -1.91 -1.67 -1.28 -1.65 -1.26 -1.41	(o) order
(g)Rhodoferax	<b>-1.38 -1.43 -1.43 -1.59 -1.68 -1.59 -1.6</b>	6 (g)Thermus	ND ND ND -3.07 -3.54 -2.64 -1.32	(f) family
(o)Myxococcales	-2.02 -1.88 -1.72 -1.84 -2.96 -2.89 -3.1	2 (o)Phycisphaerales	-2.32 -2.42 -2.55 -1.96 -1.89 -1.28 -1.83	(g) genus
(f)Saprospiraceae	-2.08 -2.01 -2.06 -1.76 -1.60 -1.70 -1.6	8 (g)Nitrospira	-1.85 -1.89 -1.46 -1.63 -1.33 -1.41 -2.43	(g,s) genus, species
(f)Fiavobacteriaceae		(g)Pseudomonas	-3.10 -3.28 -3.09 -2.69 -2.49 -2.00 -1.40	
(f)Chitinophagaceae	-2.08 -1.93 -1.86 -1.63 -1.65 -1.62 -1.6	4 (c)Nannocystis	-4.40 -4.50 ##### -5.45 -5.00 -2.46 -1.43	
		n oniment 1	100 - 101 - 1016 - 1017 - 5.00 - 2.70	ha i
	20 °C Ex	periment 1	cDNA	D# Days
Taxonomy	D2 D3 D5 D7 D10 D14 D18	Taxonomy	D2 D3 D5 D7 D10 D14 D18	F# Hours after feeding
(g)Pseudomonas	-1.87 -1.39 -1.49 -0.92 -0.94 -1.17 -1.1	9 (f)Methylocystaceae	-0.48 -0.53 -0.56 -0.49 -0.63 -1.02 -1.57	
(f)Methylophilaceae	<b>-1.06 -0.99 -1.21 -1.39 -1.52 -1.64 -1.7</b>	4 (o)MLE1-12	-2.24 -2.13 -1.78 -1.64 -1.47 -1.60 -2.17	Least Relative Abundance
(g)Bdellovibrio	-2.45 -3.15 -2.22 -2.18 -1.36 -1.11 -1.7	3 (o)Myxococcales	<b>-1.61 -1.64 -1.71 -1.81 -1.98 -2.15 -2.58</b>	
(f)Methylocystaceae	-1.21 -1.25 -1.36 -1.59 -1.58 -2.20 -3.1	5 (o)HOC36	-1.51 -1.55 -1.68 -1.77 -1.79 -1.89 -2.07	
(g)Nitrospira	-1.24 -1.27 -1.19 -1.36 -1.47 -1.25 -1.0	6 (o)Myxococcales	-1.66 -2.03 -2.46 -2.82 -2.86 -3.63 -4.28	
(f)Saprospiraceae	-1.00 -1.54 -1.94 -2.19 -2.48 -2.56 ND	(g)Rhizobiales	-1.29 -1.20 -1.33 -1.28 -1.21 -1.18 -1.68 -1.75 -1.83 -1.76 -1.99 -2.15 -2.44	
(f)Saprospiraceae	-1.78 -1.77 -1.69 -1.50 -1.66 -2.45 -1.9	3 (g)Nitrospira	-1.73 -1.73 -1.77 -1.66 -1.56 -1.55	Most Relative Abundance
(o)Sphingobacteriales	-213 -230 -183 -173 ND ND -45	8 (g)Bdellovibrio	-3.54 -3.98 -3.13 -2.74 -2.09 -1.91 -2.52	most relative reperiod
(c)Betaproteobacteria	-1.67 -1.78 -2.12 -2.27 -2.49 -2.17 -3.3	8 (k)Bacteria	-2.51 -2.35 -2.18 -2.07 -2.30 -1.92 -1.94	
	20 °C Ex	periment 2		
	DNA		cDNA	
Taxonomy	INF D0 D2 D7 D18 F12 F48	Taxonomy	INF D0 D2 D7 D18 F12 F48	
(f)Methylophilaceae		1 (g)Staphylococcus	ND -4.58 ND -3.15 ND -0.77 -3.05	
(c)Betaproteobacteria		(c)Phizobialos	-0.96 -0.92 -1.03 -0.91 -0.80 -2.30 -4.58	
(g)Nitrospira	-1.49 -1.46 -1.42 -1.38 -1.16 -1.94 -1.8	(o)HOC36	-1.16 -1.12 -1.09 -1.53 -2.09 -3.28 ND	
(g,s)Bacillus, cereus	ND ND ND -1.73 -1.78 -1.46 -1.8	6 (g)Meiothermus	ND -3.98 -4.58 -2.52 -2.51 -1.32 -1.13	
(o)Sphingobacteriales	-2.21 -1.82 -1.73 -1.55 -1.95 ND -2.3	2 (g)Thermus	-3.80 -4.10 -4.10 -2.12 -2.19 -1.61 -1.18	
(o)HOC36	-1.75 -1.66 -1.83 -2.44 -2.61 -3.22 -3.2	4 (g)Corynebacterium	ND ND ND ND ND -1.20 ND	
(g,s)Methylotenera, mobilis	<b>-1.70 -1.68 -1.66 -1.87 -2.19 -2.44 -2.3</b>	3 (g)Pseudomonas	-2.76 -3.68 -3.63 -1.59 -1.58 -1.24 -1.32	
(o)Sphingobacteriales	-2.61 -2.52 -2.50 -1.92 -1.64 -1.91 -2.3	2 (g)Anaerococcus	ND ND ND ND ND -1.30 ND	
(p)Proteobacteria	-2.94 -3.12 -3.02 -2.34 -1.82 -1.95 -1.7	(c)Betaproteobacteria	ND ND ND -2.12 -3.24 -1.65 -1.39	
	27 °C E	xperiment		
	DNA	-	cDNA	
Taxonomy	INF D0 D2 D7 D18 F12 F48	Taxonomy	INF D0 D2 D7 D18 F12 F48	
(o)DH61	-4.28 ND ND ND -0.91 -1.16 -1.2	(f)Methylocystaceae	-0.82 -0.69 -0.72 -0.87 -1.84 -1.98 -2.28	
(g)rseudomonas	ND -4.28 ND -3.18 ND -2.64 -1.0	(g)HOC26	-4.55 ND ND -3.88 -3.63 -2.55 -1.00	
(f)Methylocystaceae	-1.10 -1.20 -1.20 -1.30 -1.71 -1.88 -1.7	8 (a)DH61	ND ND ND -4.58 -1.06 -1.64 -1.38	
(2)Meiothermus	ND ND ND -1.24 ND ND -2.9	1 (o)Rhizobiales	-1.18 -1.17 -1.16 -1.27 -1.97 -1.88 -2.17	
(g)Thermus	ND ND ND -1.31 ND ND ND	(g)Hyphomicrobium	-2.60 -2.50 -2.45 -2.44 -1.85 -1.36 -1.12	
(f)Cytophagaceae	-2.04 -2.14 -1.93 -1.91 -1.37 -1.31 -1.7	0 (o)Myxococcales	-1.36 -1.45 -1.41 -1.52 -2.37 -2.47 -2.82	
(c)Betaproteobacteria	ND ND ND -1.43 -3.80 ND ND	(0)MLE1-12	-2.29 -2.39 -2.31 -1.34 -2.21 -2.32 -2.86	
(f)Saprospiraceae	-1.96 -1.87 -1.77 -2.04 -1.40 -1.40 -1.4	8 (f)Rhodocyclaceae	-3.98 -3.35 -3.80 -3.80 -4.58 -2.52 -1.52	
(g)Nitrospira	-1.30 -1.25 -1.37 -1.79 -1.38 -1.50 -1.5 20 °C F	3 (g,s)Hyphomicrobium, zavarzi mont	nii -2.22 -2.01 -1.98 -2.05 -1.97 -1.73 -1.43	
	DNA	xperiment	cDNA	
Taxonomy	D2 D3 D5 D7 D9 D12 D16	Taxonomy	D2 D3 D5 D7 D9 D12 D16	
(f)Methylophilaceae	-0.76 -0.98 -1.31 -1.47 -1.79 -1.67 -2.0	4 (f)Methylocystaceae	-0.80 -0.77 -0.94 -1.12 -1.30 -1.77 -2.40	
(f)Sphingobacteriaceae	ND ND ND ND -0.91 -1.11 -1.3	0 (g)Nitrospira	-1.44 -1.45 -1.29 -1.40 -1.25 -1.14 -1.00	
(g)Pseudomonas	-1.79 -1.60 -1.02 -1.49 -0.81 -1.13 -1.1	6 (g)Pseudomonas	-2.96 -2.44 -2.09 -1.26 -1.45 -1.40 -1.39	
(g,s)Methylotenera, mobilis	<b>-1.35 -1.45 -1.84 -2.58 -2.47 -2.90 -2.9</b>	5 (o)MLE1-12	-1.63 -1.31 -1.28 -1.62 -1.86 -2.02 -2.76	
(g)Nitrospira	-1.38 -1.30 -1.20 -1.14 -1.13 -1.11 -1.0	(o)Myxococcales	-1.28 -1.80 -2.17 -2.76 -2.96 -2.98 -2.82	
(r)Methylocystaceae		(o)HOC26		
(g)Ceothrix	-1.00 -1.01 -1.00 -2.13 -2.12 -2.48 -2.3	(a)Myxococcales	-1.41 -1.03 -1.77 -1.79 -1.88 -2.10 -2.27	
(b)Sphingobacteriales	-2.73 ND ND -1.86 -2.00 -2.24 -2.34 -2.0	(g)Nannocystis	-1.50 -1.70 -1.61 -1.49 -1.75 -2.22 -2.75	
(f)Sphingobacteriaceae	ND ND -215 -158 -214 -213 ND	(g s)Bacillus cereus	ND ND -3.63 -1.80 -1.74 -1.96 -1.89	
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- 110 Figure S4 Ten most variable OTUs in each trial and nucleic acid type (DNA or
- 111 cDNA). Values are log relative abundance (normalized to sequencing depth
- 112 (38,000)). Heat mapping for each OTU indicates behavior of OTU in that trial
- 113 (intensity of color corresponds to relative abundance).
- 114