Supplementary Materials

Bioremediation of Nitrate in Agricultural Drainage Ditches: Impacts of Low-Grade Weirs on Microbiomes and Nitrogen Cycle Gene Abundance

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This file contains seven figures, three tables, and references.

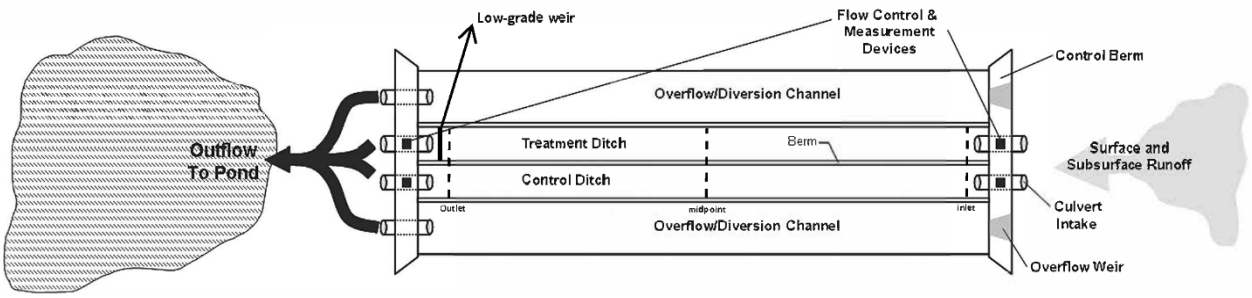

Figure S1. Schematic diagram of the paired experimental drainage channels (not to scale). The locations of sampling points and the low-grade weirs are also shown. Adapted from Strock et al. 2005 [2].

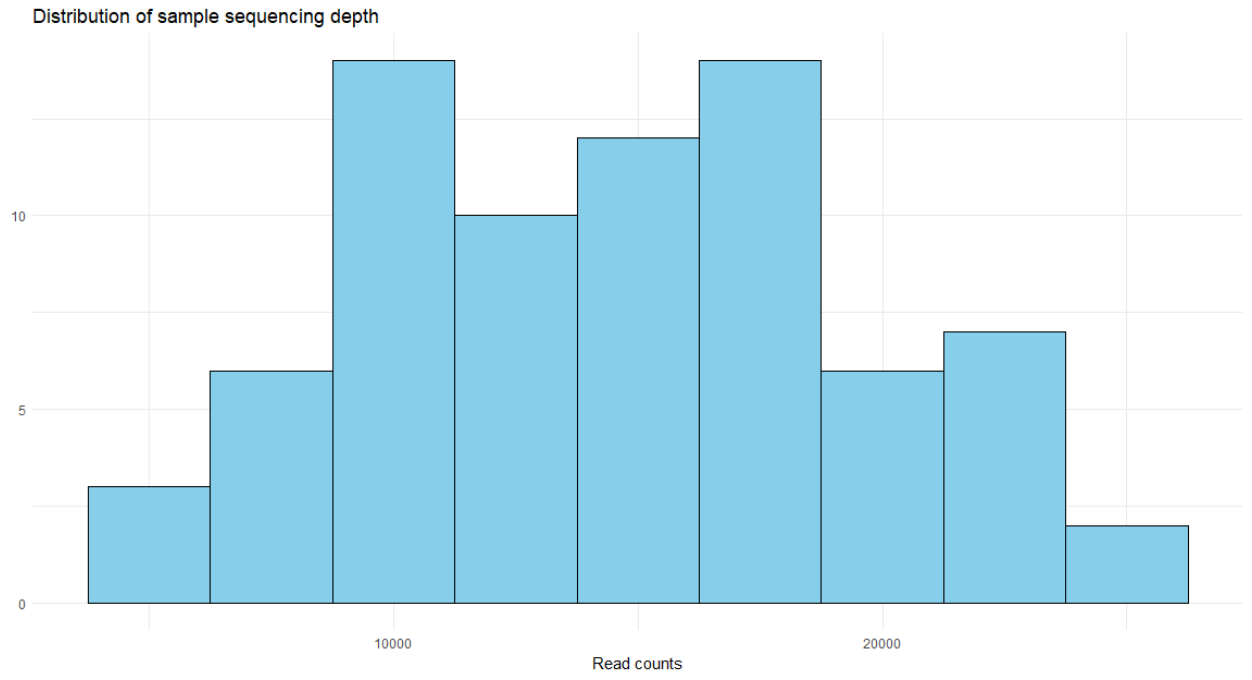

Figure S2. Distribution of sample sequencing depth (n = 74; mean = 14,596 reads; median = 14,896 reads)

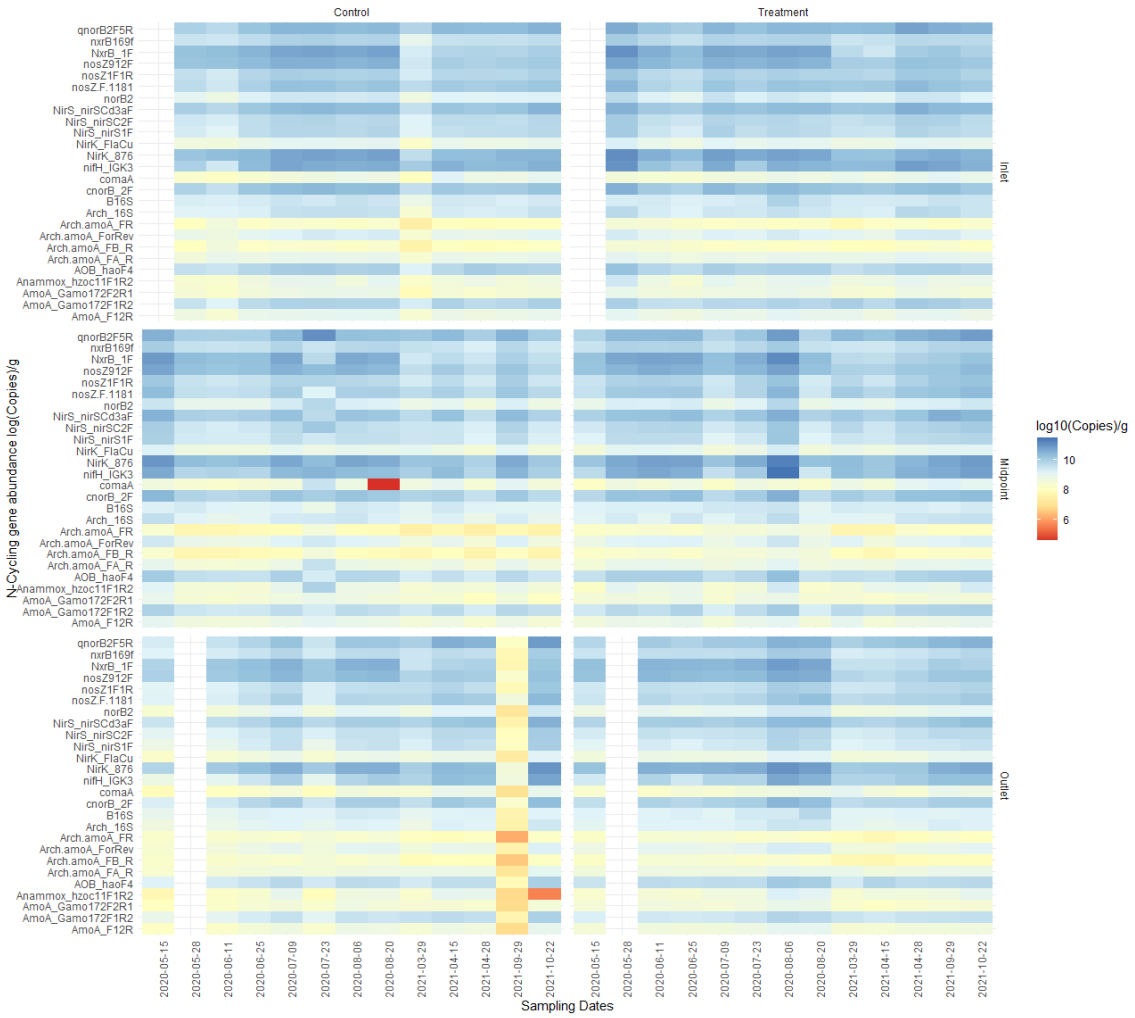

Figure S3. Heatmap showing the abundances of the N cycle-associated genes in the sediment collected at each channel location over two years. Gene concentration was measured by the Nitrogen Cycle Evaluation (NiCE) chip. See Table S1 for sample ID.

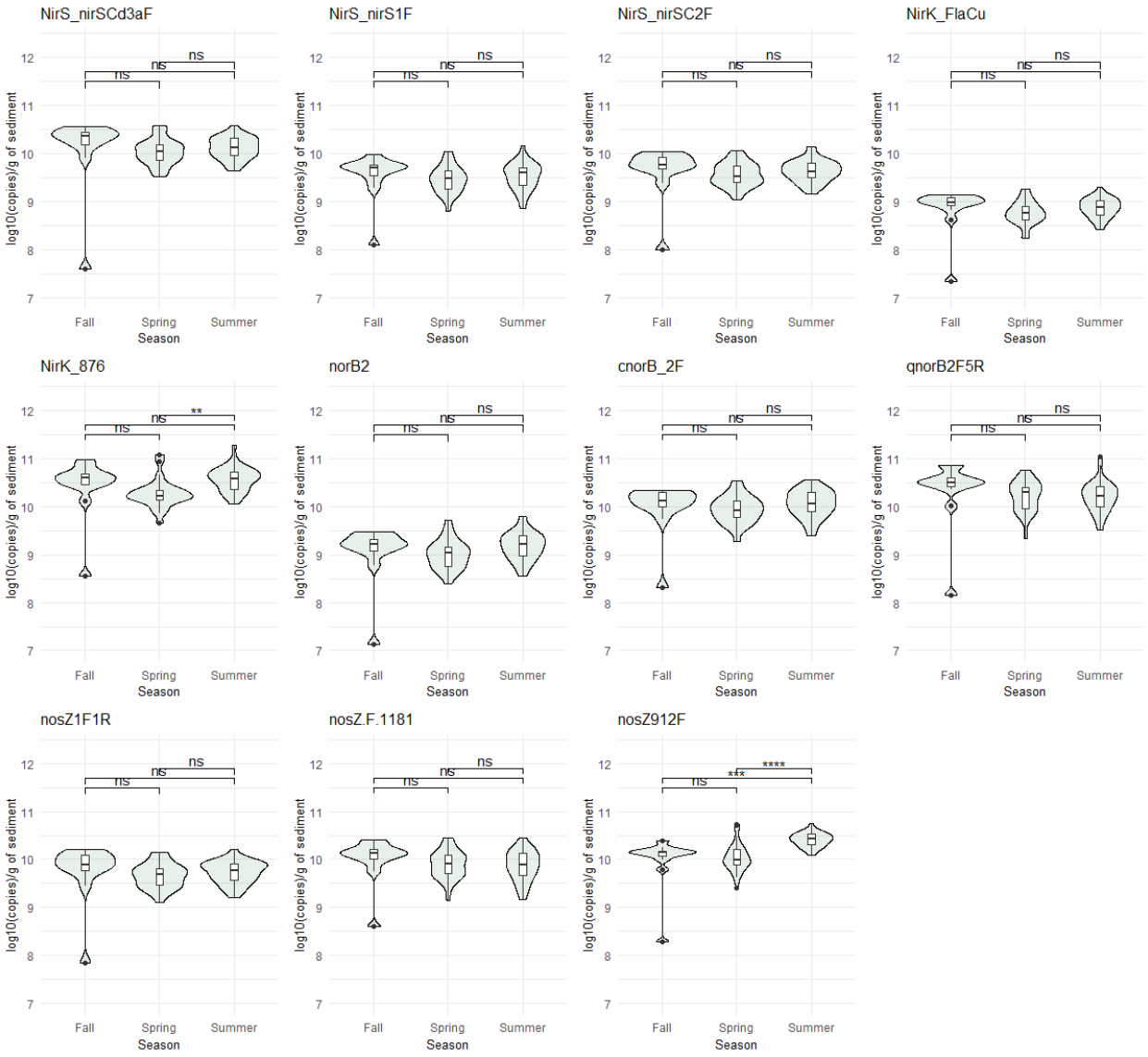

Figure S4. Violin plots showing the average abundance of denitrification genes in the samples collected in spring (i.e., March-May), summer (i.e., June-August), and fall (i.e., September-October). Significance brackets were determined using ANOVA followed by Tukey's HSD.

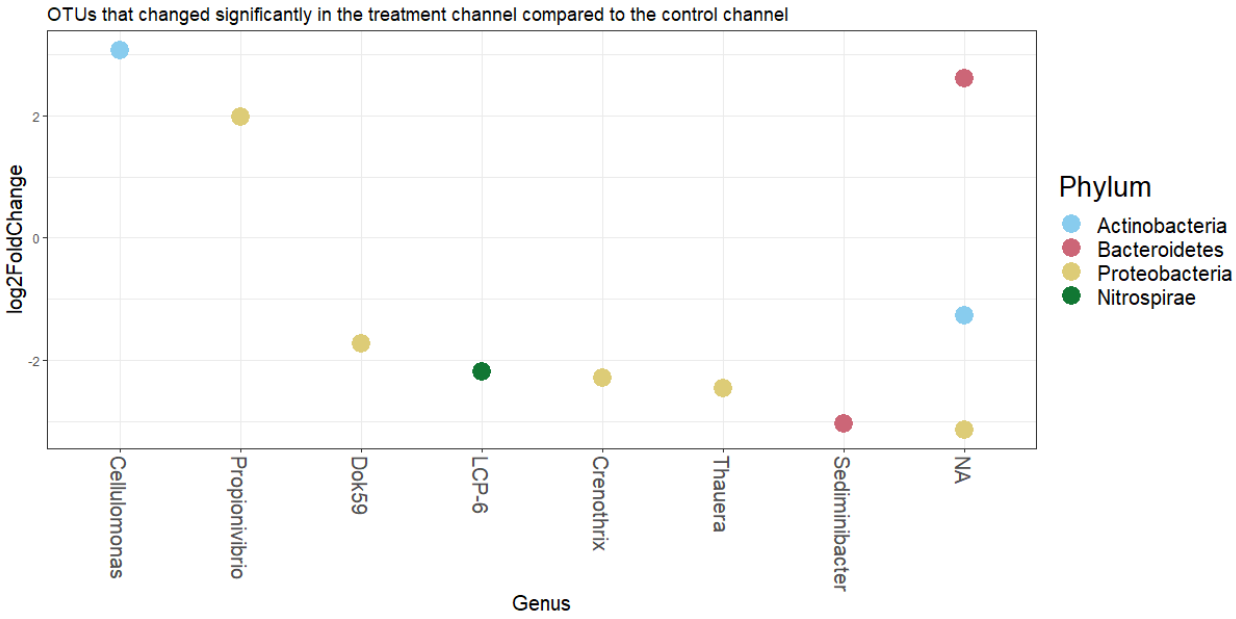

Figure S5. DeSeq2 results showing the OTUs that changed significantly in the treatment ditch compared to the control ditch (α = 0.01)

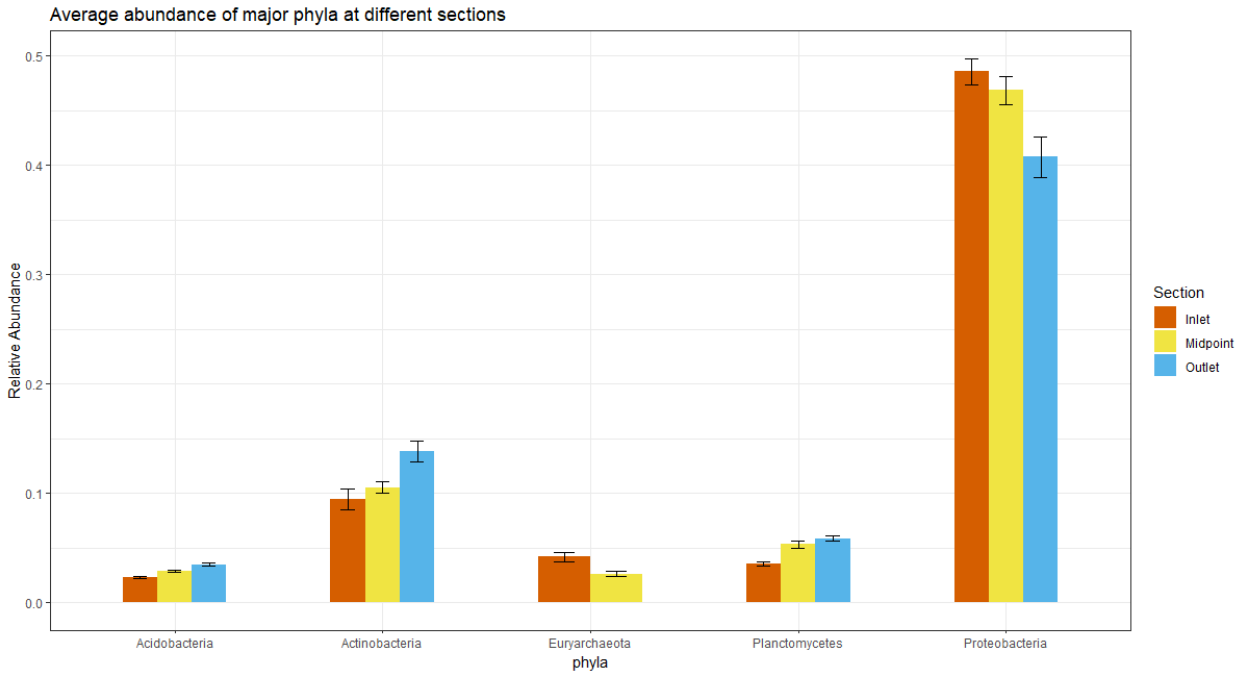

Figure S6. Relative abundance of the phylum that was significantly different between samples collected from the different channel locations ($p < 0.01$ by Kruskal-Wallis test)

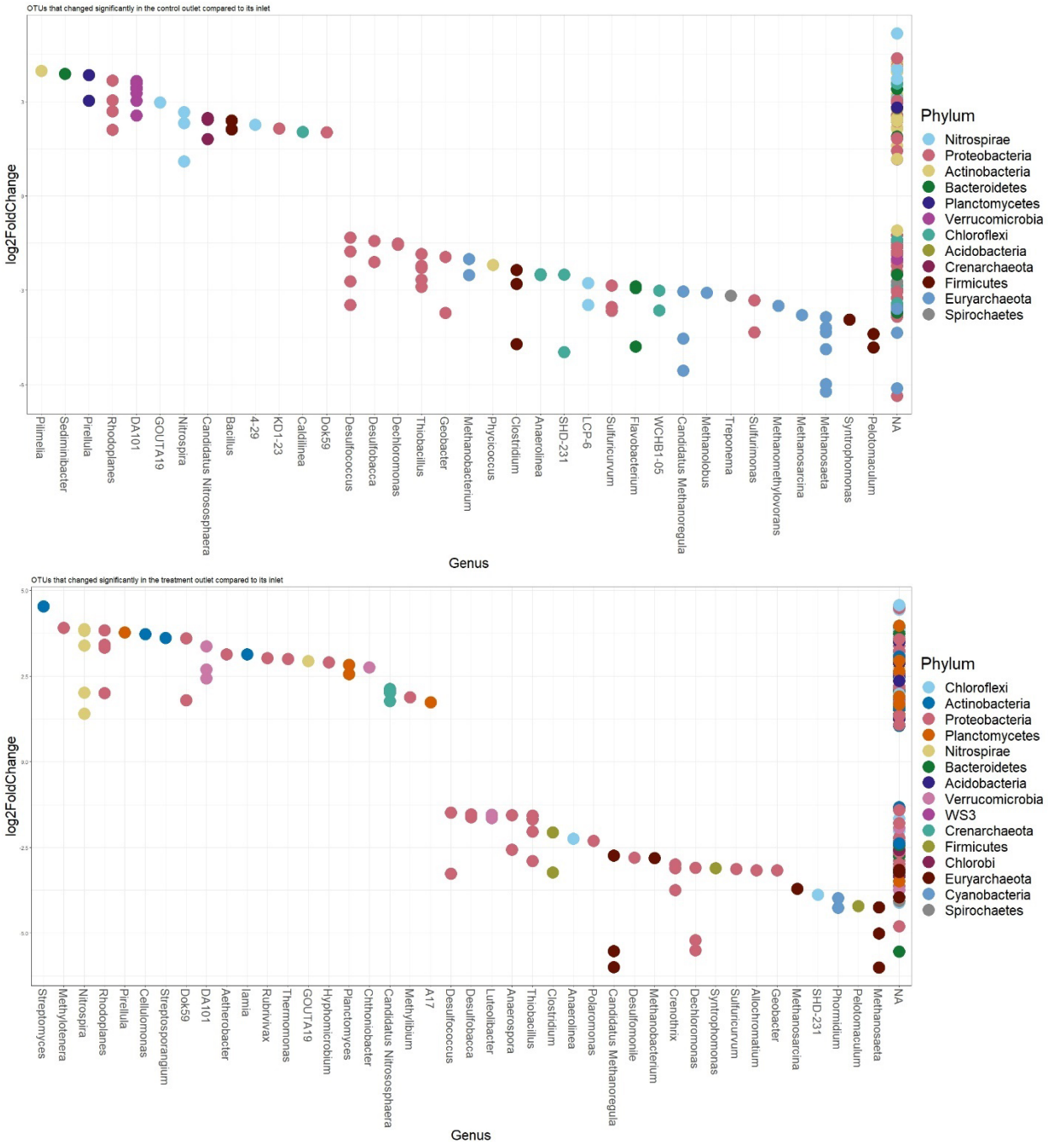

Figure S7. DeSeq2 results showing the OTUs that changed significantly at the outlet compared to the inlet of each channel (α = 0.01)

Table S1. Sample description. Asterisk (*) indicate the samples included in the final analysis (n=74)

Sample ID	ExpGroup	Location	Section	Date
0515C1	Control	C1	Inlet	2020-05-15
0515C2*	Control	C2	Midpoint	2020-05-15
0515C3*	Control	C3	Outlet	2020-05-15
0515T1	Treatment	T1	Inlet	2020-05-15
0515T2*	Treatment	T2	Midpoint	2020-05-15
0515T3*	Treatment	T3	Outlet	2020-05-15
0528C1*	Control	C1	Inlet	2020-05-28
0528C2*	Control	C2	Midpoint	2020-05-28
0528C3	Control	C3	Outlet	2020-05-28
0528T1*	Treatment	T1	Inlet	2020-05-28
0528T2*	Treatment	T2	Midpoint	2020-05-28
0528T3	Treatment	T3	Outlet	2020-05-28
0611C1*	Control	C1	Inlet	2020-06-11
0611C2*	Control	C2	Midpoint	2020-06-11
0611C3*	Control	C3	Outlet	2020-06-11
0611T1*	Treatment	T1	Inlet	2020-06-11
0611T2*	Treatment	T2	Midpoint	2020-06-11
0611T3*	Treatment	T3	Outlet	2020-06-11
0625C1*	Control	C1	Inlet	2020-06-25
0625C2*	Control	C2	Midpoint	2020-06-25
0625C3*	Control	C3	Outlet	2020-06-25
0625T1*	Treatment	T1	Inlet	2020-06-25
0625T2*	Treatment	T2	Midpoint	2020-06-25
0625T3*	Treatment	T3	Outlet	2020-06-25
0709C1*	Control	C1	Inlet	2020-07-09
0709C2*	Control	C2	Midpoint	2020-07-09
0709C3*	Control	C3	Outlet	2020-07-09
0709T1*	Treatment	T1	Inlet	2020-07-09
0709T2*	Treatment	T2	Midpoint	2020-07-09
0709T3*	Treatment	T3	Outlet	2020-07-09
0723C1*	Control	C1	Inlet	2020-07-23
0723C2*	Control	C2	Midpoint	2020-07-23
0723C3*	Control	C3	Outlet	2020-07-23
0723T1*	Treatment	T1	Inlet	2020-07-23
0723T2*	Treatment	T2	Midpoint	2020-07-23
0723T3*	Treatment	T3	Outlet	2020-07-23
0806C1*	Control	C1	Inlet	2020-08-06
0806C2*	Control	C2	Midpoint	2020-08-06
0806C3*	Control	C3	Outlet	2020-08-06

0806T1*	Treatment	T1	Inlet	2020-08-06
0806T2*	Treatment	T2	Midpoint	2020-08-06
0806T3*	Treatment	T3	Outlet	2020-08-06
0820C1*	Control	C1	Inlet	2020-08-20
0820C2*	Control	C2	Midpoint	2020-08-20
0820C3*	Control	C3	Outlet	2020-08-20
0820T1*	Treatment	T1	Inlet	2020-08-20
0820T2*	Treatment	T2	Midpoint	2020-08-20
0820T3*	Treatment	T3	Outlet	2020-08-20
0903C1	Control	C1	Inlet	2020-09-03
0903C2	Control	C2	Midpoint	2020-09-03
0903C3	Control	C3	Outlet	2020-09-03
0903T1	Treatment	T1	Inlet	2020-09-03
0903T2	Treatment	T2	Midpoint	2020-09-03
0903T3	Treatment	T3	Outlet	2020-09-03
0917C1	Control	C1	Inlet	2020-09-17
0917C2	Control	C2	Midpoint	2020-09-17
0917C3	Control	C3	Outlet	2020-09-17
0917T1	Treatment	T1	Inlet	2020-09-17
0917T2	Treatment	T2	Midpoint	2020-09-17
0917T3	Treatment	T3	Outlet	2020-09-17
1001C1	Control	C1	Inlet	2020-10-01
1001C2	Control	C2	Midpoint	2020-10-01
1001C3	Control	C3	Outlet	2020-10-01
1001T1	Treatment	T1	Inlet	2020-10-01
1001T2	Treatment	T2	Midpoint	2020-10-01
1001T3	Treatment	T3	Outlet	2020-10-01
1016C1	Control	C1	Inlet	2020-10-16
1016C2	Control	C2	Midpoint	2020-10-16
1016C3	Control	C3	Outlet	2020-10-16
1016T1	Treatment	T1	Inlet	2020-10-16
1016T2	Treatment	T2	Midpoint	2020-10-16
1016T3	Treatment	T3	Outlet	2020-10-16
1119C1	Control	C1	Inlet	2020-11-19
1119C2	Control	C2	Midpoint	2020-11-19
1119C3	Control	C3	Outlet	2020-11-19
1119T1	Treatment	T1	Inlet	2020-11-19
1119T2	Treatment	T2	Midpoint	2020-11-19
1119T3	Treatment	T3	Outlet	2020-11-19
0329C1*	Control	C1	Inlet	2021-03-29
0329C2*	Control	C2	Midpoint	2021-03-29
0329C3*	Control	C3	Outlet	2021-03-29
0329T1*	Treatment	T1	Inlet	2021-03-29

0329T2*	Treatment	T2	Midpoint	2021-03-29
0329T3*	Treatment	T3	Outlet	2021-03-29
0415C1*	Control	C1	Inlet	2021-04-15
0415C2*	Control	C2	Midpoint	2021-04-15
0415C3*	Control	C3	Outlet	2021-04-15
0415T1*	Treatment	T1	Inlet	2021-04-15
0415T2*	Treatment	T2	Midpoint	2021-04-15
0415T3*	Treatment	T3	Outlet	2021-04-15
0428C1*	Control	C1	Inlet	2021-04-28
0428C2*	Control	C2	Midpoint	2021-04-28
0428C3*	Control	C3	Outlet	2021-04-28
0428T1*	Treatment	T1	Inlet	2021-04-28
0428T2*	Treatment	T2	Midpoint	2021-04-28
0428T3*	Treatment	T3	Outlet	2021-04-28
0520C1	Control	C1	Inlet	2021-05-20
0520C2	Control	C2	Midpoint	2021-05-20
0520C3	Control	C3	Outlet	2021-05-20
0520T1	Treatment	T1	Inlet	2021-05-20
0520T2	Treatment	T2	Midpoint	2021-05-20
0520T3	Treatment	T3	Outlet	2021-05-20
0604C1	Control	C1	Inlet	2021-06-04
0604C2	Control	C2	Midpoint	2021-06-04
0604C3	Control	C3	Outlet	2021-06-04
0604T1	Treatment	T1	Inlet	2021-06-04
0604T2	Treatment	T2	Midpoint	2021-06-04
0604T3	Treatment	T3	Outlet	2021-06-04
0723C1	Control	C1	Inlet	2021-07-23
0723C2	Control	C2	Midpoint	2021-07-23
0723C3	Control	C3	Outlet	2021-07-23
0723T1	Treatment	T1	Inlet	2021-07-23
0723T2	Treatment	T2	Midpoint	2021-07-23
0723T3	Treatment	T3	Outlet	2021-07-23
0830C1	Control	C1	Inlet	2021-08-30
0830C2	Control	C2	Midpoint	2021-08-30
0830C3	Control	C3	Outlet	2021-08-30
0830T1	Treatment	T1	Inlet	2021-08-30
0830T2	Treatment	T2	Midpoint	2021-08-30
0830T3	Treatment	T3	Outlet	2021-08-30
0929C1*	Control	C1	Inlet	2021-09-29
0929C2*	Control	C2	Midpoint	2021-09-29
0929C3*	Control	C3	Outlet	2021-09-29
0929T1*	Treatment	T1	Inlet	2021-09-29
0929T2*	Treatment	T2	Midpoint	2021-09-29

0929T3*	Treatment	T3	Outlet	2021-09-29
1022C1*	Control	C1	Inlet	2021-10-22
1022C2*	Control	C2	Midpoint	2021-10-22
1022C3*	Control	C3	Outlet	2021-10-22
1022T1*	Treatment	T1	Inlet	2021-10-22
1022T2*	Treatment	T2	Midpoint	2021-10-22
1022T3*	Treatment	T3	Outlet	2021-10-22
1116C1	Control	C1	Inlet	2021-11-16
1116C2	Control	C2	Midpoint	2021-11-16
1116C3	Control	C3	Outlet	2021-11-16
1116T1	Treatment	T1	Inlet	2021-11-16
1116T2	Treatment	T2	Midpoint	2021-11-16
1116T3	Treatment	T3	Outlet	2021-11-16

Table S2. Primers used for the high-throughput N cycle quantification (Nitrogen Cycle Evaluation [NiCE] chip).

Target	Assay ID	Primer name	Sequence (5' --> 3')	Reference
Total Archaea	Arch_16S	Archaea-F KO	CCCTAYGGGGYGCASCAGGC	(1)
		Archaea-R KO	GCYCYCCCGCCAATTCMTTTA	
AOB (Gamma-proteobacteria)	Gamo_F1R2	Gamo172 F1	GGBGACTGGGAYTTCTGG	(2)
		Gamo172 F1_R2	AAAACCCGCAAAAAAGGC	
	Gamo_F2R1	Gamo172 F2	TGGGATTTCTGGATGGAC	
		Gamo172 F2_R1	TGATACGAACGCAGAGAA	
AOB (Beta-proteobacteria)	Bac_amoA	amoA_F1	GGGGHTTYTACTGGTGGT	(2, 3)
		amoA_2R	CCCCTCKGSAAAGCCTTCTTC	
AOB hao	hao	haoF4	AYCTKCGCTCRATGGG	(4)
		haoR2	GGTTGGTYTTCTGKCCGG	
AOA	Arch_amoAF	Arch-amoAF	STAATGGTCTGGCTTAGACG	(5)
		Arch-amoAR	GCGGCCATCCATCTGTATGT	
	Arch_amoAFA	Arch-amoAFA	ACACCAGTTTGGYTACCWTCDGC	(6)
		Arch-amoAR	GCGGCCATCCATCTGTATGT	
	Arch_amoAFB	Arch-amoAFB	CATCCRATGTGGATTCCATCDTG	
		Arch-amoAR	GCGGCCATCCATCTGTATGT	
	Arch_amoA-for	Arch-amoA-for	CTGAYTGGGCYTGGACATC	(7)
		Arch-amoA-rev	TTCTTCTTTGTTGCCCAGTA	
NOB (Nitrobacter)	nxrBF	NxrB 1F	ACGTGGAGACCAAGCCGGG	(8)
		NxrB 1R	CCGTGCTGTTGAYCTCGTTGA	
NOB (Nitrospira)	nxrB169f	nxrB169f	TACATGTGGTGGAACA	(9)
		nxrB638r	CGGTTCTGGTCRATCA	
Comammox	comaA*	comaA-244F	TAYAAYTGGGTSAAYTA	(10)
		comaA-659R	ARATCATSGTGCTRTG	

	comaB	comaB-244F	TAYTTCTGGACRTTYTA	
		comaB-659R	ARATCCARACDGTGTG	
Anammox	hzocl	hzocl1F1	TGYAAGACYTGYCAYTGG	(11)
		hzocl1R2	ACTCCAGATRTGCTGACC	
	hzsA*	hzsA_1597F	WTYGGKTATCARTATGTAG	(2, 12)
		hzsA1857R	AAABGGYGAATCATARTGGC	
DNRA	nrfA*	nrfAF2aw	CARTGYCAYGTBGARTA	(13)
		nrfAR1	TWNGGCATRTGRCARTC	
Denitrification	nirSCd3aF	nirSCd3aF	AACGYSAAGGARACSGG	(14)
		nirSR3cd	GASTTCGGRTGSGTCTTSAYGAA	
	narG_1960f*	narG1960f	AYGTSGGSCARGARAA	(15)
		narG2650r	TYTCRTACCABGTBGC	
	napA_V66*	V66	TAYTTYYTNHSNAARATHATGTAYGG	(16)
		V67	DATNGGRTGCATYTCNGCCATRTT	
	napA_v17m*	V17m	TGGACVATGGGYTTYAAYC	(28)
		napA4r	ACYTCRCGHGCVGTRCCRCA	
	nirSC1F	nirSC1F	ATCGTCAACGTCAARGARACVGG	(17)
		nirSC1R	TTCGGGTGCGTCTTSAGAASAG	
	nirSC2F	nirSC2F	TGGAGAACGCCGGNCARGTNTGG	
		nirSC2R	GATGATGTCCACGGCNACRTANGG	
	nirK_FlaCu	FlaCu	ATCATGGTSCTGCCGCG	(18)
		R3Cu	GCCTCGATCAGRTTGTGGTT	
	nirK876	nirK876	ATYGGCGGVAYGGCGA	(19)
		nirK1040	GCCTCGATCAGRTTRTGGTT	
	nirKC2F*	nirKC2F	TGCACATCGCCAACggnatgtwygg	(17)
		nirKC2R	GGCGCGGAAGATGshrtgrtcnac	
	norB2	norB2	GACAARHWVTAYTGGTGGT	(20)
		norB6	TGCAKSARRCCCCABACBCC	

	cnorB-2F	cnorB-2F	GACAAGNNNTACTGGTGGT	(21)
		cnorB-6R	GAANCCCCANACNCCNGC	
	qnorB2F-5R	qnorB2F	GGNCAYCARGGNTAYGA	
		qnorB5R	ACCCANAGRTGNACNACCCACCA	
	qnorB2F-7R*	qnorB2F	GGNCAYCARGGNTAYGA	
		qnorB7R	GGNGGRTTDATCADGAANCC	
	nosZ1F	nosZ1F	WCSYTGTTCMTCGACAGCCAG	(22)
		nosZ1R	ATGTCGATCARCTGVKCRTTYTC	
	nosZ-F-1181	nosZ-F-1181	CGCTGTTCITCGACAGYCAG	(23)
		nosZ-R-1880	ATGTGCAKIGCRTGGCAGAA	
	nosZ-II-F*	nosZ-II-F	CTIGGICCIYTKCAYAC	(24)
		nosZ-II-R	GCIGARCARAAITCBGTRC	
	nosZ912F	NosZ912F	CGTCCCCGGCCTCGTGTA	(25)
		NosZ1853R	GAGCAGAAGTTCGTGCAGTAGTAGGG	
Fungal denitrification	nirKfF*	nirKfF	TACGGGCTCATGTAYGTNSARCC	(26)
		nirKfR	AGGAATCCCACASCNCCYTTNTC	
N fixation	nifHF*	nifHF	AAAGGYGGWATCGGYAARTCCACCAC	(27)
		nifHR	TTGTTSGCSGCRTACATSGCCATCAT	
	nifH_IGK3	IGK3	GCIWTHTAYGGIAARGGIGGIATHGGIAA	(29)
		DVV_correct	ATIGCRAAICCICCRCAIACIACRTC	

* These assays did not produce amplifications on more than half of the samples or did not have a standard curve with at least three points and an r^2 value >0.95; therefore, they were not used for the downstream analyses.

Table S3. List of the NiCE chip assays used for downstream analyses and the associated r^2 values of the standard curves.

Target	Assay ID	Standard Curve r^2 value	
		Chip 1	Chip 2
Total Archaea	Arch_16S	0.9981	0.9953
AOB (Gammaproteobacteria)	Gamo_F1R2	0.9930	0.9998
	Gamo_F2R1	0.9995	0.9996
AOB (Betaproteobacteria)	Bac_amoA	0.9971	0.9960
AOB hao	hao	0.9920	0.9989
AOA	Arch_amoAF	0.9999	0.9882
	Arch_amoAFA	0.9943	0.9938
	Arch_amoAFB	0.9998	0.9982
	Arch_amoA-for	0.9855	0.9941
NOB (Nitrobacter)	nxrBF	0.9986	0.9938
NOB (Nitrospira)	nxrB169f	0.9987	0.9927
Comammox	comaB	0.9931	0.9984
Anammox	hzocl	0.9973	0.9965
Denitrification	nirSCd3aF	0.9956	0.9800
	nirSC1F	0.9964	0.9994
	nirSC2F	0.9965	0.9965
	nirK_FlaCu	0.9947	0.9948
	nirK876	0.9984	0.9984
	norB2	0.9989	0.9813
	cnorB-2F	0.9842	0.9966
	qnorB2F-5R	0.9812	0.9934
	nosZ1F	0.9883	0.9995
	nosZ-F-1181	0.9677	0.9972
	nosZ912F	0.9934	0.9769
Nitrogen Fixation	IGK3	0.9651	0.9990

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