Supplemental Information

Detailed Methods

Conventional qPCR for 16S rRNA gene

Conventional quantitative polymerase chain reaction (qPCR) of the bacterial 16S rRNA gene was used to assess DNA extraction quality and test for differences in bacterial abundance among treatments, collection timepoint, and location (Ishii et al., 2014). We used a previously validated protocol for SYBR green qPCR (Fukushima et al, 2003). Prior to running qPCR, we diluted each sample 10-fold using nuclease free water. All procedures were done in a PCR workstation that was sterilized before use for 30 minutes with a UV lamp. All PCR tubes, strips, and plates were similarly UV-irradiated prior to use for 10 minutes in a UV crosslinker. Pipettes, tip boxes, and all other materials were decontaminated with DNAZap (Life Technologies #AM9890).

Each reaction had a volume of 10 µl, which included 1X Sso Advanced Universal SYBR Green Supermix (Bio-Rad Laboratories, CA, USA), 0.5 µM each of forward and reverse primers, and 1 µl of DNA template. At least one negative control consisting of nuclease free water in place of template DNA was included on each plate. Serial dilutions consisting of known quantities of a gBlock standard were used, ranging from 10^0 to 10^7 copies/µl.

Conventional qPCR was performed using StepOnePlus Real-Time PCR System (Applied Biosystems, MA, USA) under the following conditions: initial annealing at 98°C for 3 minutes, followed by 40 cycles of 98°C for 10 seconds and 60°C for 30 seconds. Finally, melt curve analysis was performed from 60°C to 95°C. We compiled standard curves for each run and used them to calculate 16S rRNA gene copy number in our unknown samples. Data processing and quality control for conventional 16S rRNA gene qPCR were performed in R (version 4.0.3).

NiCE chip

Primer pair mix consisting of a final concentration of 5 µM (10X) of each primer and 1X Tris-EDTA (pH 8.0) was prepared in a 96-well PCR plate and then transferred to a 384-well plate (17.82 ul per well). Aliquot (4.51 µl) of assay mastermix (1.25X SmartChip TB Green Gene Expression Master Mix [Takara Bio]) was subsequently added to each well in the 384-well plate containing the primer mix. This 384-well 'assay source plate' was vortexed vigorously for 5 seconds and centrifuged for 5 minutes at 3,320 rpm or until all bubbles were removed. The assay source plate was kept at 4°C in the dark until loading onto the SmartChip Multisample NanoDispenser (MSND).

A 384-well 'sample source plate' was similarly prepared using the extracted DNA from our soil samples. We first prepared a sample mastermix which consisted of 1.25X SmartChip TB Green Gene Expression Master Mix and 2.86 µl of DNA sample (or gBlock standards; see below) in 96-well PCR plates. The sample mixture (13.1 µl) were subsequently transferred to the 384-well sample source plate, which was vortexed and centrifuged to remove bubbles as described above. To generate standard curves, gBlock standards of known concentration were serially diluted in a mixture (5×10^0 to 5×10^7 copies/µl) (Jang et al. 2022) and included in the sample source plate.

Samples and primer mix were dispensed from the 384-well source plates onto the SmartChip using the integrated MSND. The interior of the MSND is sealed and kept at a relative humidity

of 30-70% to ensure reproducibility of assay volume and thermal uniformity. After the NanoDispenser completes the transfer of sample and mastermix, the SmartChip was centrifuged at 4°C and kept in the dark until the final transfer into the SmartChip Real-Time PCR cycler. The final reaction volume in each well was 100 nl, consisting of 50 nl each of the sample and assay mixtures.

SmartChip data processing

Threshold cycle (C_t) values were determined for each assay using the SmartChip qPCR software. As described previously, standard curves for each assay were generated by linear regression of the C_t values versus the known quantities of gblock template (1,2). All standard curves had an R^2 value greater than or equal to 0.90 as part of our quality control pipeline.

Assays for which a standard curve failed to amplify were excluded from analysis. In addition, assays for which >50% of samples failed to amplify or assays that demonstrated strong batch effects between SmartChip runs were also excluded. We defined batch effects as cases in which >60% samples failed to amplify during one SmartChip run but <60% failed to amplify on a different run. Limit of quantification (LOQ) was defined as the lowest concentration of standards that were reliably detected, which ranged from 1.5-4.5 log copies/µl depending on the assay. For assays in which >60% of samples successfully amplified without apparent batch effects, samples with Ct values below the LOQ were given an imputed value of 0.5*LOQ (3).

Raw data processing removed 14 gene targets based on the previously described criteria for missingness or batch effects. Of the remaining assays, 2.3% of our samples failed to amplify despite having at least two biological replicates (same assay, treatment, location, timepoint) with successful amplification. We attributed missing values in these cases to machine error and assigned them a mean Ct value from within their biological replicate.

We normalized our results to a final unit of log copies g^{-1} soil using the following equation that accounts for the dilution factor, DNA extraction volume, and soil gravimetric water content (GWC):

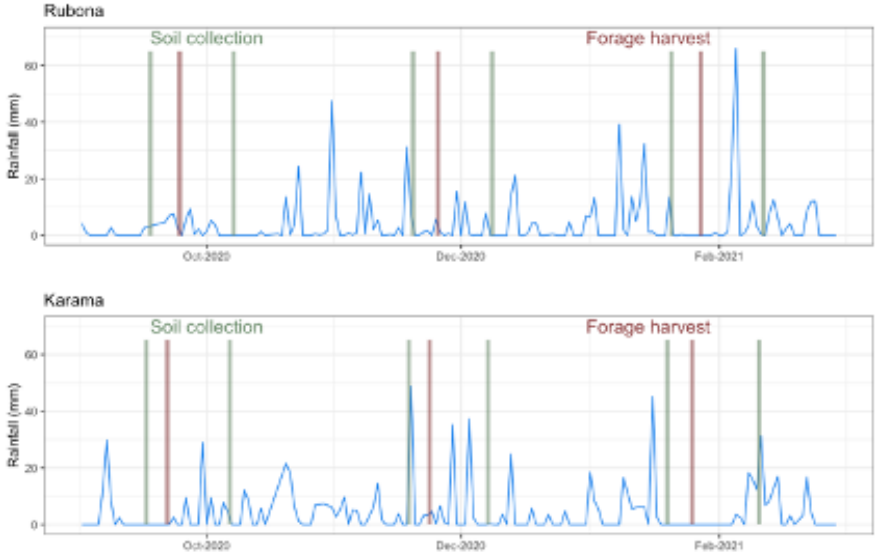
$$\log_{10}((\text{copies}/\mu\text{l}* 10 * 50\ \mu\text{l})/[0.25-(0.25*\text{GWC})]\ \text{g soil})$$

Supplemental Table 1. Soil properties

	Soil Type	pH	EC (uS/cm)	CEC (meq/100g)	P (ppm)	K (ppm)	Organic C (%)	Total N (%)	Sand (%)	Silt (%)	Clay (%)
Rubona	Haplic Acrisol	5.35	127	13.4	76.1	123	2.17	0.21	59.1	7.86	33.0
Karama	Haplic Ferralsol	6.17	108	9.61	81.7	360	2.25	0.19	61.0	6.0	33.0

Supplemental Figure 1. Map of study locations

Supplemental Figure 2. Rainfall during the sampling period



Supplemental Table 2. List of primers used in NiCE assays

Assay number	Assay ID	Target	Primer name	Sequence (5' > 3')	Used in analysis (Y/N)	References
2	16S_Arch	Total archaea	Archaea-F KO	CCCTAYGGGGYGCASCAGGC	Y	4
			Archaea-R KO	GCYCYCCCGCCAATTCMTTTA		
3	Gamo_F1R1	AOB (Gamma-proteobacteria)	Gamo172_F1	GGBGACTGGGAYTTCTGG	Y	5

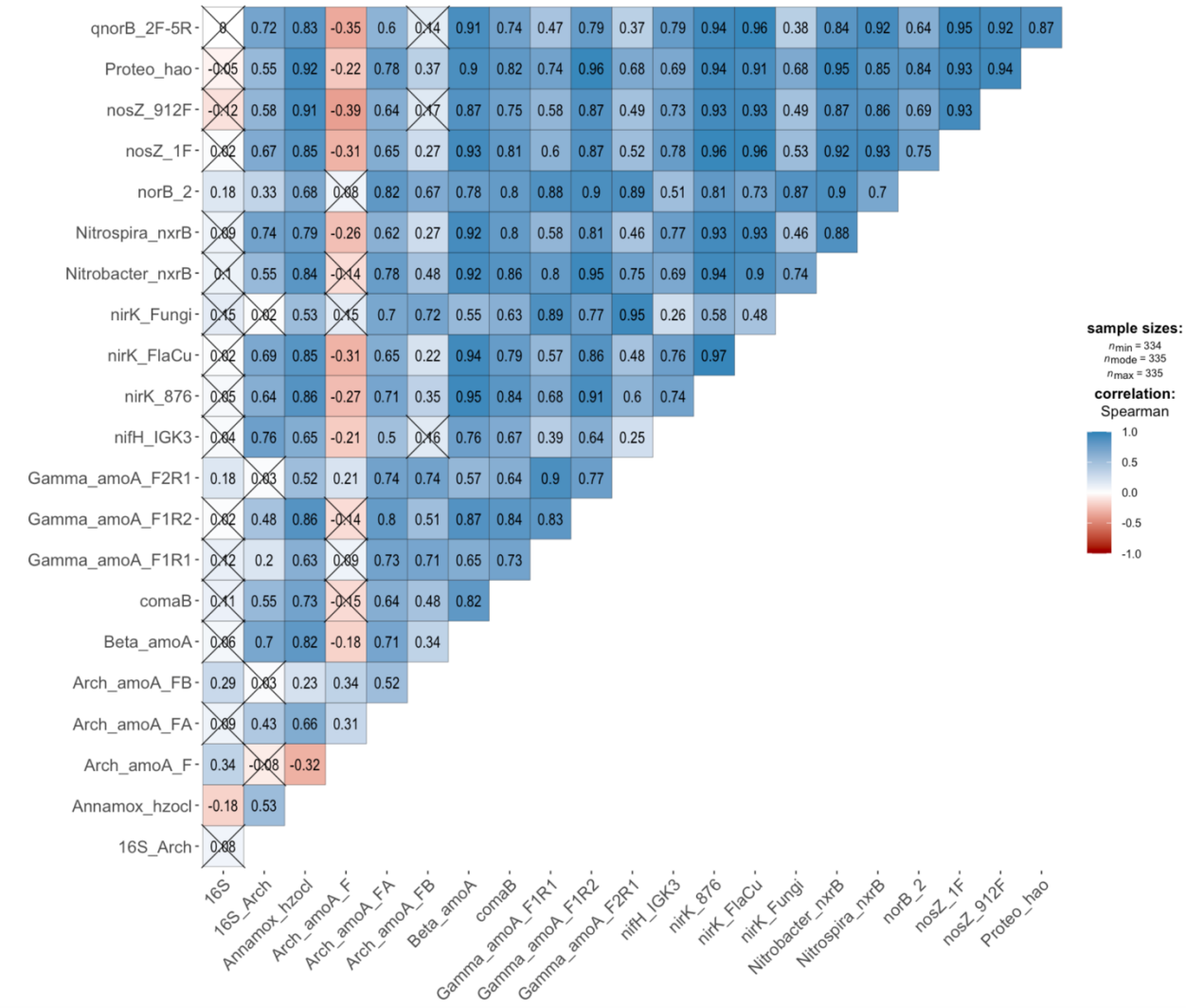
			Gamo172_F1_R1	AAARCCCGAGAAGAAMGC		
4	Gamo_F1R2	AOB (Gamma-proteobacteria)	Gamo172_F1	GGBGACTGGGAYTTCTGG	Y	5
			Gamo172_F1_R2	AAAACCCGCAAAAAAGGC		
5	Gamo_F2_R1	AOB (Gamma-proteobacteria)	Gamo172_F2	TGGGATTTCTGGATGGAC	Y	5
			Gamo172_F2_R1	TGATACGAACGCAGAGAA		
6	Bac_amoA	AOB (Beta-proteobacteria)	amoA_F1	GGGGHTTYTACTGGTGGT	Y	6
			amoA_2R	CCCCTCKGSAAAGCCTTCTTC		
7	Arch_amoA_F	AOA	Arch-amoAF	STAATGGTCTGGCTTAGACG	Y	7
			Arch-amoAR	GCGGCCATCCATCTGTATGT		
8	Arch_amoA_FA	AOA	Arch-amoAFA	ACACCAGTTTGGYTACCWTCDGC	Y	8
			Arch-amoAR	GCGGCCATCCATCTGTATGT		
9	Arch_amoA_FB	amoA / archaea_AOA	Arch-amoAFB	CATCCRATGTGGATTCCATCDTG	Y	8
			Arch-amoAR	GCGGCCATCCATCTGTATGT		
10		amoA / archaea_AOA	Arch-amoA-for	CTGAYTGGGCYTGGACATC	N	9
			Arch-amoA-rev	TTCTTCTTTGTTGCCCAGTA		
11	hzocl_1F1	hzo / anammox_bacteria	hzocl1F1	TGYAAGACYTGYCAYTGG	Y	10
			hzocl1R2	ACTCCAGATRTGCTGACC		
12	Proteo_hao	hao_hdh / proteobacteria_AOB	haoF4	AYCTKCGCTCRATGGG	Y	11
			haoR2	GGTTGGTYTTCTGKCCGG		
13	Annamox_hzocl		hzsA_1597F	WTYGGKTATCARTATGTAG	N	5,12

		hzs / anammox_bacteria	hzsA1857R	AAABGGYGAATCATARTGGC		
14	Nitrobacter_nxrB	nxrB / proteobacteria_Nitrobacter	NxrB1F	ACGTGGAGACCAAGCCGGG	Y	13
			NxrB1R	CCGTGCTGTTGAYCTCGTTGA		
15	Nitrospira_nxrB	nxrB / Nitrospirae_Nitrospira	NxrB169F	TACATGTGGTGGAACA	Y	14
			NxrB638R	CGGTTCTGGTCRATCA		
17		narG / bacteria	narG1960F	AYGTSGGSCARGARAA	Y	15
			narG2650R	TYTCRTACCABGTBGC		
18		nrfA / bacteria	nrfA2aw	CARTGYCAYGTBGARTA	N	16
			nrfAR1	TWNGGCATRTGRCARTC		
19		napA / bacteria	V66	TAYTTYYTNHSNAARATHATGTAYGG	N	17
			V67	DATNGGRTGCATYTCNGCCATRTT		
22		nirS / bacteria	nirSC1F	ATCGTCAACGTCAARGARACVGG	N	18
			nirSC1R	TTCGGGTGCGTCTTSAGAASAG		
23		nirS / bacteria	nirSC2F	TGGAGAACGCCGGNCARGTNTGG	N	18
			nirSC2R	GATGATGTCCACGGCNACRTANGG		
25	nirK_FlaCu	nirK / bacteria	FlaCu	ATCATGGTSCTGCCGCG	Y	19
			R3Cu	GCCTCGATCAGRTTGTGGTT		
26	nirK_876	nirK / bacteria	nirK876	ATYGGCGGVAYGGCGA	Y	20
			nirK1040	GCCTCGATCAGRTTRTGGTT		

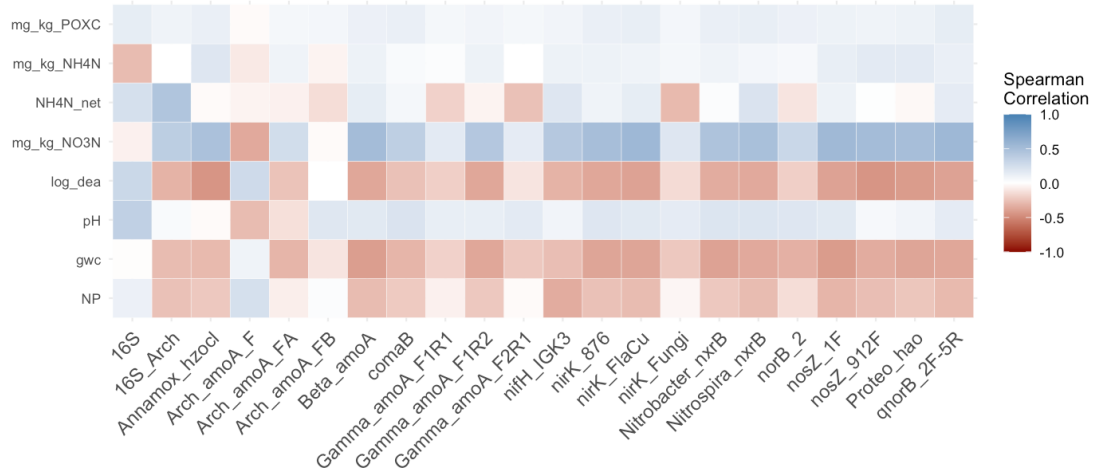
28		nirK / bacteria	nirKC2F	TGCACATCGCCAACggnatgtwygg	N	18
			nirKC2R	GGCGCGGAAGATGshrtgrtcnac		
31	norB_2	norB / denitrifier	norB2	GACAARHWVTAYTGGTGGT	Y	21
			norB6	TGCAKSARRCCCCABACBCC		
32		norB / denitrifier	cnorB-2F	GACAAGNNNTACTGGTGGT	N	22
			cnorB-6R	GAANCCCCANACNCCNGC		
33	qnorB_2F-5R	norB / bacteria	qnorB2F	GGNCAYCARGGNTAYGA	Y	22
			qnorB5R	ACCCANAGRTGNACNACCCACCA		
34		norB / bacteria	qnorB2F	GGNCAYCARGGNTAYGA	N	22
			qnorB7R	GGNGGRTTDATCADGAANCC		
35	nosZ_1F	nosZ / denitrifier_clade_I	nosZ1F	WCSYTGTTCMTCGACAGCCAG	Y	23
			nosZ1R	ATGTCGATCARCTGVKCRTTYTC		
36		nosZ / denitrifier_clade_I	nosZ-F-1181	CGCTGTTCITCGACAGYCAG	N	24
			nosZ-R-1880	ATGTGCAKIGCRTGGCAGAA		
37		nosZ / denitrifier_clade_II	nosZ-II-F	CTIGGICCIYTKCAYAC	N	25
			nosZ-II-R	GCIGARCARAAITCBGTRC		
38	nosZ_912F	nosZ / denitrifier_clade_II	nosZ912F	CGTCCCCGGCCTCGTGTA	Y	26
			nosZ853R	GAGCAGAAGTTCGTGCAGTAGTAGGG		
39		nifH / bacteria	nifHF	AAAGGYGGWATCGGYAARTCCACCAC	N	27
			nifHR	TTGTTSGCSGCRTACATSGCCATCAT		

40	nifH_IGK3	nifH / bacteria	IGK3	GCIWTHTAYGGIAARGGIGGIATH GGIAA	Y	28
			DVV	ATIGCRAAICCICCRCAIACIACRTC		
41		amoA / comammox	coma-244F	TAYAAYTGGGTSAAYTA	N	29
			coma-659R	ARATCATSGTGCTRTG		
42	comaB	amoA / comammox	comaB-244F	TAYAAYTGGGTSAAYTA	Y	29
			comaB-659R	ARATCCARACDGTGTG		
43	nirK_Fungi	nirK / fungi	nirKfF	TACGGGCTCATGTAYGTNSARCC	Y	30
			nirKfR	AGGAATCCCACASCNCCYTTNTC		

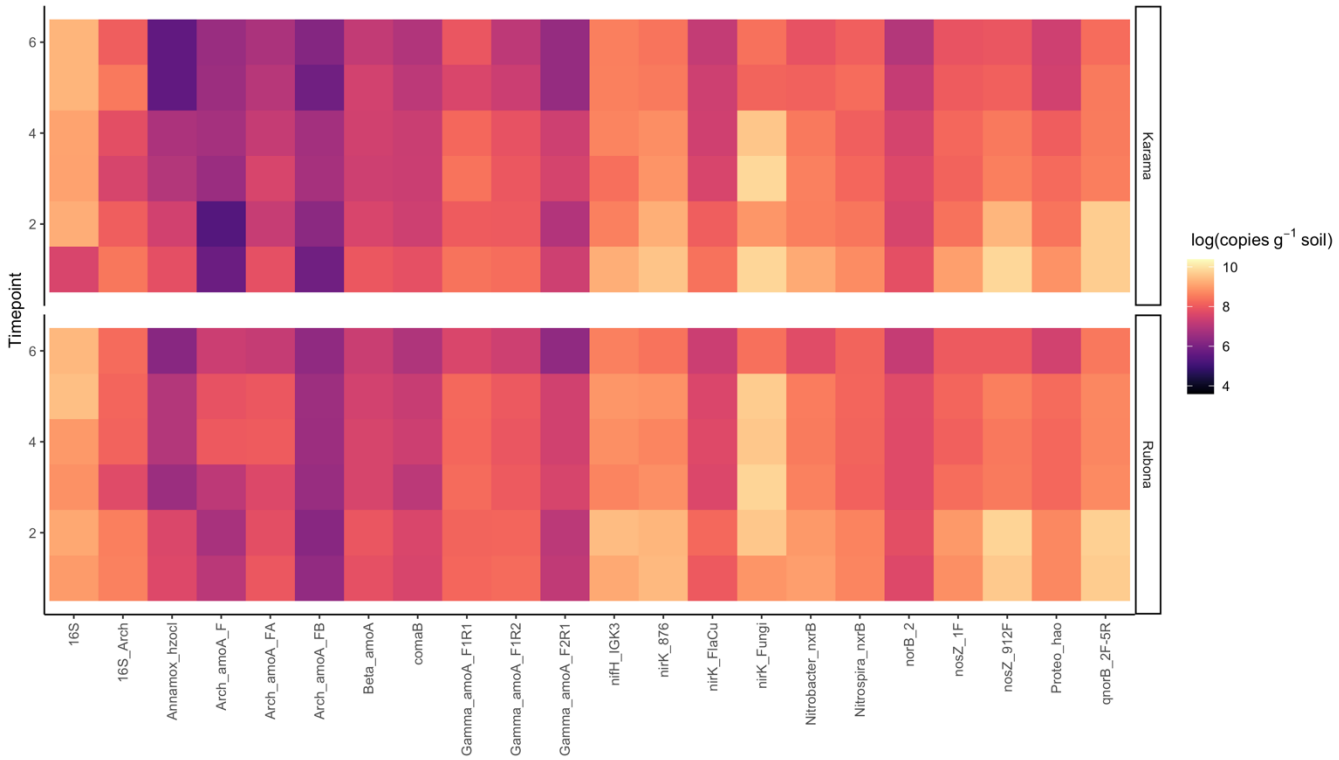
Supplemental Figure 3. Correlogram of gene abundances. An 'X' denotes a non-significant correlation at the level of p=0.05. Made with ggstatsplot (31).



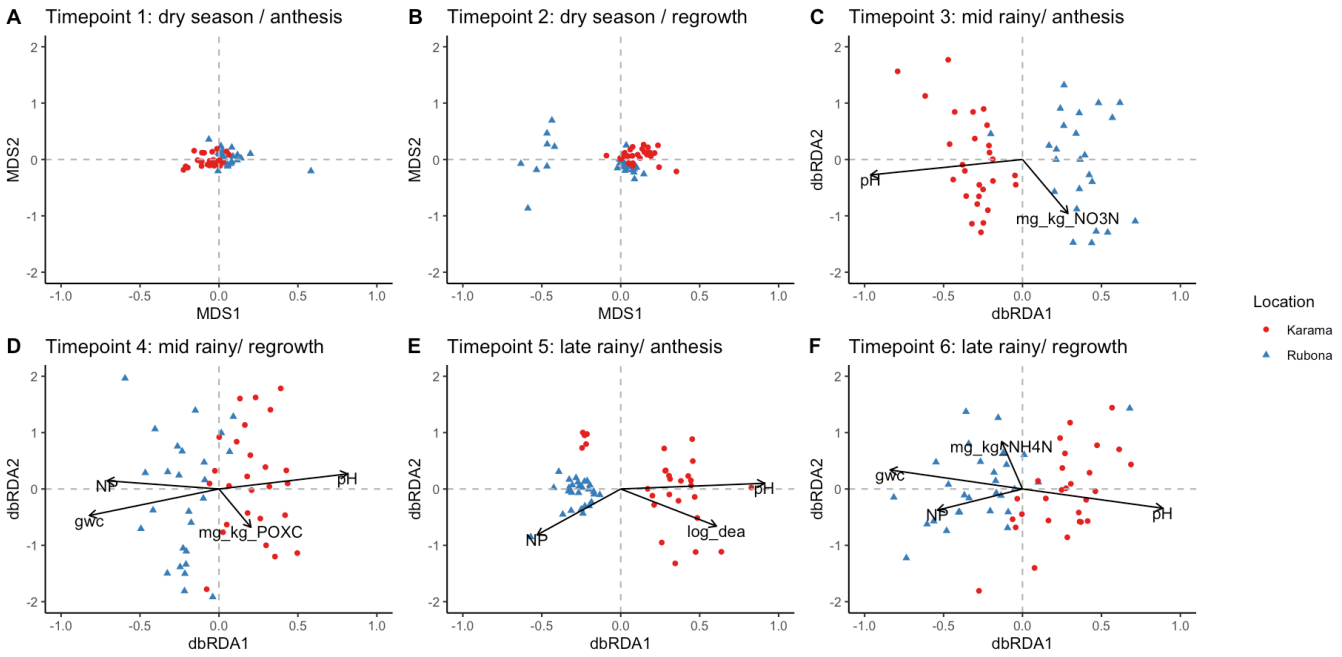
Supplemental Figure 4. Correlations between gene targets and soil features



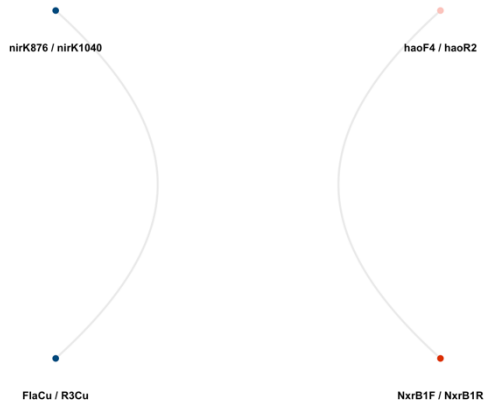
Supplemental Figure 5. Heatmap of gene target abundances



Supplemental Figure 6. Distance-based redundancy analysis within sampling timepoint (Spearman's dissimilarity)



Supplemental Figure 7. A global intersection network (both locations, all timepoints) indicates that nitrification and denitrification pathways do not have a conserved link across all timepoints and locations. The network consists of two disconnected diads.



Supplemental Table 3. Properties of each location x timepoint network

		Karama			Rubona		
Timepoint	**Season**	**Density**	**C_{obs}**	**L_{obs}**	**Density**	**C_{obs}**	**L_{obs}**
1	Late dry	0.89	0.93	1.11	0.91	0.92	1.09
2	Late dry	0.67	0.79	1.33	0.35	0.73	1.63
3	Early rainy	0.90	0.69	1.10	0.39	0.93	1.96
4	Early rainy	0.79	0.93	1.22	0.41	0.65	1.88
5	Late rainy	0.26	0.46	1.86	0.82	0.87	1.18
6	Late rainy	0.38	0.54	1.77	0.50	0.62	1.56

Supplemental Table 4. Keystone nitrification and denitrification gene targets by timepoint

Timepoint	Primer	Pathway	Target Gene	Node Degree	Betweenness Centrality	Closeness Centrality
1	qnorB2F / qnorB5R	Denitrification	Bacteria, nitric oxide reductase (norB)	17	3.72	0.06
1	nosZ1F / nosZ1R	Denitrification	Denitrifiers clade I, nitrous oxide reductase (nosZ)	17	3.72	0.06
1	norB2 / norB6	Denitrification	Denitrifiers, norB	17	3.72	0.06

1	Gamo172_F2 / Gamo172_F2_R1	Nitrification	Gamma proteobacteria, ammonia monooxygenase (amoA)	16	2.42	0.06
2	Gamo172_F2 / Gamo172_F2_R1	Nitrification	Gamma proteobacteria, amoA	7	16.68	0
2	norB2 / norB6	Denitrification	Denitrifiers, norB	3	12.00	0.01
3	Gamo172_F2 / Gamo172_F2_R1	Nitrification	Gamma proteobacteria, amoA	6	18.83	0.04
3	nosZ1F / nosZ1R	Denitrification	Denitrifiers clade I, nosZ	8	1.5	0.05
4	haoF4 / haoR2	Nitrification	Proteobacteria, hydroxylamine oxidoreductase (hao)	9	12.30	0.03
4	NosZ912F / NosZ853R	Denitrification	Denitrifiers clade II, nosZ	7	5.52	0.03
5	nirK876 / nirK1040	Denitrification	Bacteria, nitrite reductase (nirK)	10	30.67	0.02
5	haoF4 / haoR2	Nitrification	Proteobacteria, hao	4	11.00	0.02
6	FlaCu / R3Cu	Denitrification	Bacteria, nirK	4	4.33	0.08
6	NxrB1F / NxrB1R	Nitrification	Proteobacteria, *Nitrobacter*, nitrite oxidoreductase (nxr)	3	1.50	0.07

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