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Supporting Information

Title: Effect of model root exudate on denitrifier community dynamics and activity at different WFPS levels

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Results of day 0 sample analysis and comparison with water-only controls

DOC and pH

Soil pH at day 0 was approximately equal for all WFPS levels at 5.96 ± 0.02. At the end of the experiment, pH remained unchanged in the water-only controls and all other treatments except rhizospheric soil at 90% WFPS. DOC measured in the day 0 microcosms for the 50, 70 and 90% WFPS microcosms were 13.34 ± 2.19, 25.68 ± 1.99 and 17.97 ± 5.79 mg C kg^{-1} dry soil, respectively.

Nitrate and ammonia

NO_3-N levels in the control microcosms sampled at day 0 were high (105.4 ± 3.3 mg N kg^{-1} dry soil) due to the nitrate supplemented as KNO_3. At the end of the experiment, NO_3-N levels were maintained in the water-only controls for the 90 and 70% WFPS, but showed a significant decrease ($p<0.01$) at 50% WFPS (84.21 ± 3.0 mg N kg^{-1} dry soil). NH_4-N levels in the control microcosms sampled at day 0 were low among all WFPS treatments (0.20 ± 0.01 mg N kg^{-1} dry soil), and did not significantly change in the water-only controls at the end of the experiment (Fig S2A).

Total denitrification and N_2O emissions rates

Total denitrification rates assessed using the acetylene inhibition technique showed denitrification activity was low in the day 0 and water-only controls (0 C) at all WFPS levels

(<1.5 ng N_2O-N g dry soil^{-1} h^{-1}). When actual rates of N_2O emission rates were assessed, values were again very low (<1.6 ng N_2O-N g dry soil^{-1} h^{-1}) in the day 0 and water-only control samples.

Soil microbial community structure and size

No significant difference was observed between the day 0 samples and the water-only controls at the end of the experiment for any target in either community structure or gene copy number. This indicates that both the total bacterial and denitrifier community size and structure were stable after microcosm formation and pre-incubation.

Average 16S rRNA gene copy number of the water-only controls ranged from 1.61 x 10^9 to 2.05 x 10^9 copies g^{-1} dry weight soil across treatments (Fig 3A). nirK gene copy numbers in the water-only controls ranged from 2.20 to x 10^7 to 4.34 x 10^7 copies g^{-1} dry weight soil in the different treatments representing 1.3 to 2.1% of the 16S gene copy number (Table S5).

nirS gene copy numbers in the water-only controls ranged from 1.34 x 10^7 to 1.91 x 10^7 copies g^{-1} dry weight soil (Fig 3E) (1.2 to 2.3-fold lower than nirK) and accounting for 0.8 to 1% of the 16S copy number (Table S5).

Gene copy number of nosZ-I and nosZ-II in the water-only controls ranged from 5.48 x 10^6 to 9.78 x 10^6 copies g^{-1} dry weight soil for nosZ-I and 1.67 x 10^7 to 2.14 x 10^7 copies g^{-1} dry weight soil for nosZ-II. nosZ-I copy numbers accounted for 0.3 to 0.6% of the 16S gene copy count with nosZ-II being 2.0 to 3.6-fold higher accounting for 0.9 to 1.2% of the 16S (Table S5).

Fig. S1. Schematic representation of the soil microcosm setup used in the experiment. The large cylinder indicates the total volume occupied by soil in the microcosm. The ARE injection point (2 cm depth in the centre) is indicated by the circle at the end of the red line. The dotted cylinder indicates the subsample taken using a soil core to represent the model "rhizospheric" (R) soil compartment. The remaining surrounding soil was termed "bulk" (B) soil compartment and was sampled separately.

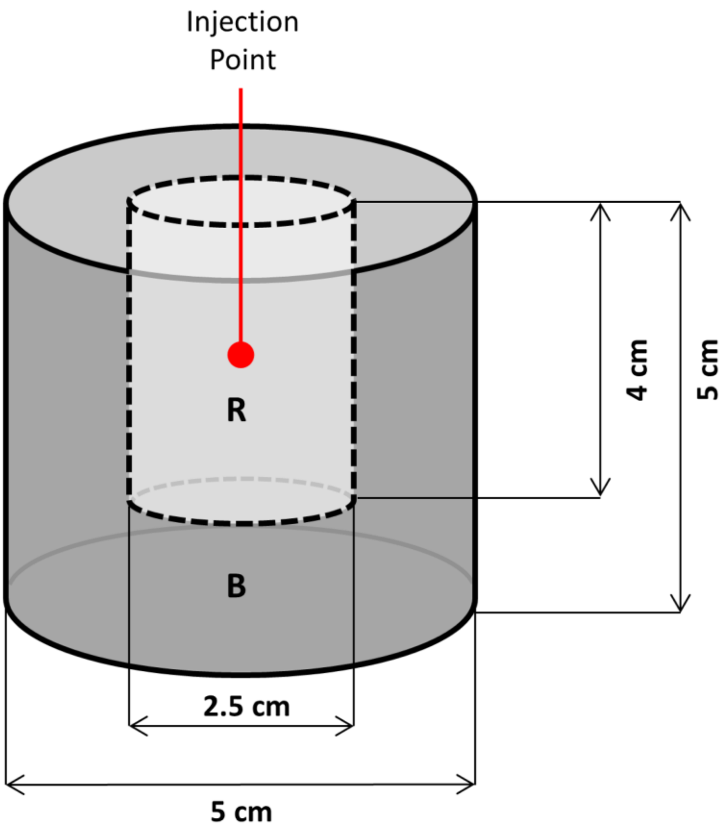

Fig. S2. Interaction plots showing NH_4-N (A), pH (B), and WFPS (C) data for the "bulk" and "rhizospheric" soil compartments after daily addition of ARE (0 to 3 mg C day^{-1}) for 7 consecutive days at 50, 70 and 90% WFPS. Filled symbols (■) represent "rhizospheric" soil and empty symbols "bulk" soil (□). The different WFPS levels are given by the symbol shape (50%: ■, 70%: ● and 90%:▲). Error bars indicate the 5% LSDs calculated using ANOVA.

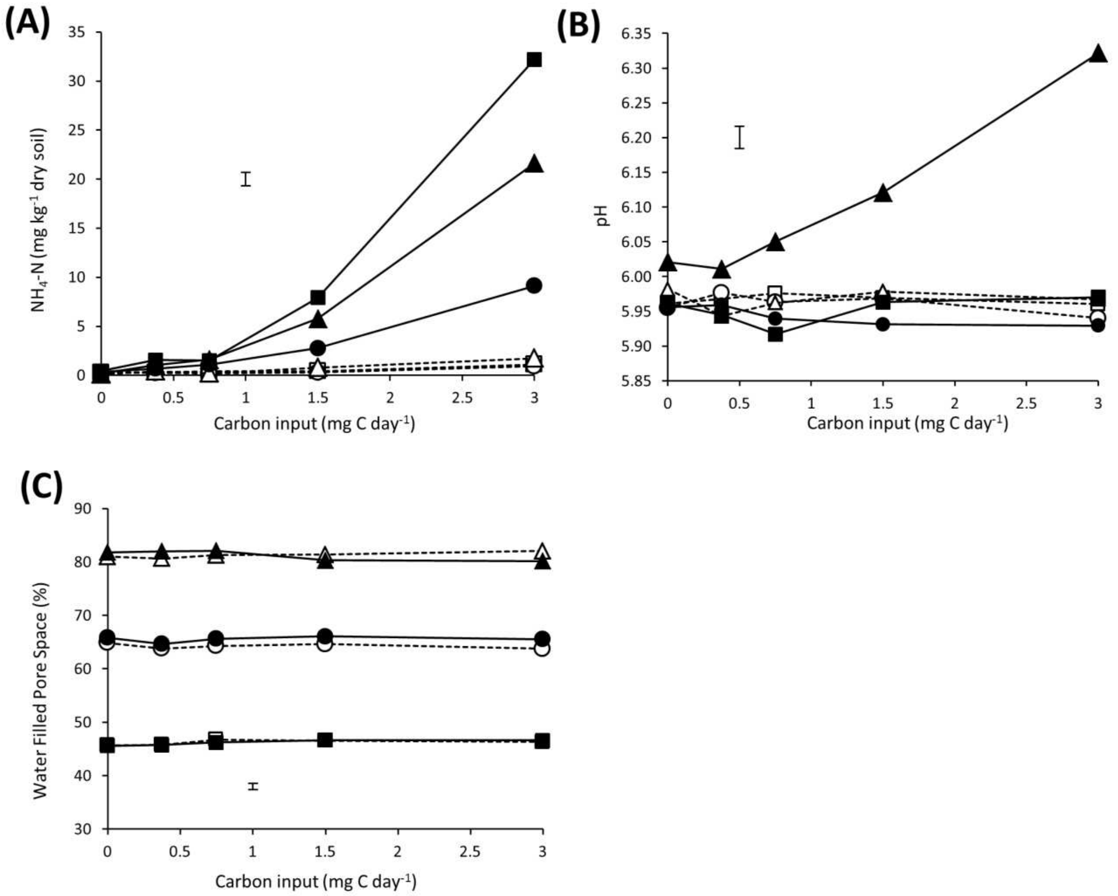

Table S1. Primer pairs and reaction conditions used for T-RFLP and relative real time PCR analysis.

Target	Primer	Sequence	Reaction conditions	Primer reference
T-RFLP				
16S	8F - FAM	AGAGTTTGATCCTGGCTCAG - FAM	94 ℃ for 4 min 30 sec; 30 cycles of 94 ℃ for 30 sec, 57 ℃ for 30 sec, 68 ℃ for 1min 30 sec; 68 ℃ for 10 min	(Lane, 1991)
	1392R (modified)	ACGGGCGRTGTGTACA		
nirK (1st round)	nirK F1	TTCGTCTAYCAYTGYGC	94 ℃ for 3 min; 40 cycles of 94 ℃ for 30 sec, 54 ℃ for 30 sec, 72 ℃ for 45 sec; 72 ℃ for 10 min	(Casciotti and Ward, 2001)
	nirK R1	GCCTCGATCAGRTTRTG		(Braker et al., 1998)
nirK (2nd round)	nirK F2 – FAM	ATGGTSCTKCCSCGYGA – FAM	94 ℃ for 3 min; 35 cycles of 94 ℃ for 30 sec, 54 ℃ for 30 sec, 72 ℃ for 35 sec; 72 ℃ for 10 min	(Braker et al., 1998)
	nirK R1 – ATTO 565	GCCTCGATCAGRTTRTG - ATTO 565		
nirS (1st round)	nirS F1	CCTAYTGGCCGCCRCART	94 ℃ for 5 min; 35 cycles of 94 ℃ for 30 sec, 60 ℃ for 30 sec, 72 ℃ for 1 min; 72 ℃ for 10 min	(Braker et al., 1998)
	nirS R1 (modified)	CGTTGAACTTRCCGGTSGG		
nirS (2nd round)	nirS F1 – FAM	CCTAYTGGCCGCCRCART-FAM	94 ℃ for 5 min; 35 cycles of 94 ℃ for 30 sec, 55 ℃ for 30 sec, 72 ℃ for 1 min; 72 ℃ for 10 min	(Braker et al., 1998)
	nirS R2 – ATTO 565	CGGRTGSGTCTTGAYGAASAG – ATTO 565		This study
nosZ -I	nosZ 1F – FAM	WCSYTGTTCMTCGACAGCCAG	95 ℃ for 2 min; 35 cycles of 95 ℃ for 30 sec, 62 ℃ for 30 sec, 68 ℃ for 1 min; 68 ℃ for 10 min	(Henry et al., 2006)
	nosZ 2R– ATTO 565	CAKRTGCAKSGCRTGGCAGAA		
nosZ-II	nosZ-II-F – FAM	CTIGGICCIYTKCAYAC	95 ℃ for 5 min; 38 cycles of 95 ℃ for 30 sec, 53 ℃ for 1 min, 68 ℃ for 1 min; 68 ℃ for 10 min	(Jones et al., 2013)
	nosZ-II-R – ATTO 565	GCIGARCARAAITCBGTRC		
Relative real time PCR				
Reference spike	Mut-F	CCTACGGGAGGCAGGTC	95 ℃, 15 min; 40 cycles: 95 ℃ for 10 s, 54 ℃ for 10 s, 72 ℃ for 10 s acquiring at 81 ℃ for 5 sec after each elongation	Daniell et al. (2012)
	Mut-R	ATTACCGCGGCTGCACC		
16S	Primer-1	CCTACGGGAGGCAGCAG	As above	Muyzer et al. (1993)
	Primer-2	ATTACCGCGGCTGCTGG		
nirK	nirK876	ATYGGCGGVCAYGGCGA	95 ℃ for 15 min; 6 cycles of 95 ℃ for 10 sec, 63 ℃ for 10 sec, 72 ℃ for 10 sec; 40 cycles of 60 ℃ for 10 sec, 72 ℃ for 20 sec acquiring at 86 ℃ for 5 sec after each elongation	Hallin et al. (2009)
	nirK1040	GCCTCGATCAGRTTRTGGTT		
nirS	Cd3aF	GTSAACGTSAAGGARACSGG	95 ℃ for 10 min; 40 cycles of 95 ℃ for 30 sec, 57 ℃ for 20 sec, 72 ℃ for 20 sec acquiring for 5 sec at 72 ℃ after each elongation	Michotey et al. (2000)
	R3cd	GASTTCGGRTGSGTCTTGA		Throback et al. (2004)
nosZ-I	nosZ2F	CGCRACGGCAASAAGGTSMSSGT	95 ℃ for 10 min then 50 cycles of 95 ℃ for 30 sec, 62 ℃ for 15 sec, 72 ℃ for 30 sec acquiring at 82 ℃ for 5 sec after each elongation.	Henry et al. (2006)
	nosZ2R	CAKRTGCAKSGCRTGGCAGAA		
nosZ-II	nosZ-II-F	CTIGGICCIYTKCAYAC	95 ℃ for 15 min then 50 cycles of 95 ℃ for 15 sec, 53 ℃ for 30 sec, 72 ℃ for 30 sec acquiring at 80 ℃ for 30 sec after each elongation.	Jones et al. (2013)
	nosZ-II-R	GCIGARCARAAITCBGTRC		

Table S2. P-values for the main effects of the different factors (Compartment, C Input, and WFPS) and their interactions for the total denitrification rates, N_2O emission rates and the different soil chemical parameters measured as determined by three-way ANOVA. Significance level of p-values is indicated as follows: *** = p <0.001, ** = p <0.01, * = p <0.05, n.s. = p >0.05 (not significant).

	DF	Total denitrification rates ($N_2 + N_2O$)[a]	N_2O emission rates[a]	Moisture content	pH	DOC	NO_3-N	NH_4-N [a]
Compartment	1	NA[b]	NA[b]	***	***	***	***	***
C Input	4	***	n.s.	n.s.	***	n.s.	n.s.	***
WFPS	2	***	***	***	***	***	***	***
Compartment × C Input	4	NA[b]	NA[b]	n.s.	***	n.s.	*	***
Compartment × WFPS	2	NA[b]	NA[b]	***	***	n.s.	***	n.s.
C input × WFPS	8	***	*	n.s.	***	n.s.	***	*
Compartment × C input × WFPS	8	NA[b]	NA[b]	***	***	n.s.	***	n.s.

[a] Total denitrification rates, N_2O emission rates and NH_4-N data were transformed in order to satisfy the requirement for normally distributed residuals

[b] Not applicable. The factor Compartment did not exist for the gas measurements dataset since microcosms were separated into “rhizosphere” and “bulk” only after this step.

Table S3. Soil physicochemical data (NH_4-N, NO_3-N, moisture, pH and DOC) for the "bulk" and "rhizospheric" compartments after daily addition of ARE (0 to 3 mg C day^{-1}) for 7 days at 50, 70 and 90% WFPS. Values are averages (n=10) with the S.E.M. in brackets.

WFPS C input (mg C day^{-1})	NH_4-N mg kg^{-1} dry soil	NO_3-N mg kg^{-1} dry soil	Moisture %	pH -	DOC mg kg^{-1} dry soil
Bulk Soil					
50% WFPS					
0	0.31 (0.07)	90.73 (3.31)	20.79 (0.15)	5.96 (0.02)	10.01 (1.90)
0.375	0.30 (0.06)	96.26 (5.05)	20.87 (0.16)	5.97 (0.01)	11.31 (2.66)
0.75	0.42 (0.11)	87.94 (2.52)	21.32 (0.22)	5.98 (0.01)	9.48 (2.56)
1.5	0.43 (0.08)	100.73 (4.12)	21.23 (0.17)	5.97 (0.01)	9.68 (1.87)
3	1.13 (0.15)	107.76 (6.63)	21.11 (0.30)	5.96 (0.02)	8.35 (2.17)
70% WFPS					
0	0.26 (0.11)	101.59 (2.72)	29.57 (0.09)	5.96 (0.02)	16.25 (2.90)
0.375	0.25 (0.07)	105.04 (2.45)	29.08 (0.32)	5.98 (0.01)	10.48 (2.07)
0.75	0.21 (0.06)	103.44 (3.28)	29.30 (0.25)	5.96 (0.01)	10.33 (0.66)
1.5	0.31 (0.07)	98.30 (4.79)	29.47 (0.17)	5.97 (0.01)	12.93 (2.01)
3	0.93 (0.34)	109.33 (3.77)	29.07 (0.24)	5.94 (0.01)	15.31 (2.17)
90% WFPS					
0	0.14 (0.09)	102.11 (3.12)	37.11 (0.34)	5.98 (0.01)	16.74 (2.48)
0.375	0.44 (0.15)	102.05 (2.23)	36.77 (0.30)	5.94 (0.01)	18.00 (1.37)
0.75	0.27 (0.11)	99.71 (3.10)	37.01 (0.54)	5.96 (0.02)	15.47 (1.05)
1.5	0.80 (0.26)	98.63 (3.86)	37.19 (0.28)	5.98 (0.01)	13.23 (2.37)
3	1.78 (0.49)	83.81 (3.64)	37.42 (0.45)	5.97 (0.01)	14.41 (2.01)
Rhizosphere Soil					
50% WFPS					
0	0.48 (0.15)	76.16 (4.33)	20.74 (0.36)	5.96 (0.00)	7.52 (1.47)
0.375	1.56 (0.80)	86.43 (4.00)	20.86 (0.19)	5.94 (0.02)	7.15 (1.74)
0.75	1.53 (0.19)	83.73 (6.52)	21.08 (0.21)	5.92 (0.02)	8.71 (2.37)
1.5	7.92 (0.92)	86.57 (4.39)	21.27 (0.25)	5.96 (0.02)	8.11 (1.36)
3	32.28 (1.61)	88.50 (3.07)	21.25 (0.33)	5.97 (0.02)	10.48 (1.95)
70% WFPS					
0	0.35 (0.10)	99.37 (2.29)	30.03 (0.16)	5.96 (0.01)	11.54 (1.86)
0.375	0.69 (0.13)	102.37 (3.92)	29.48 (0.39)	5.96 (0.01)	11.34 (2.27)
0.75	1.12 (0.19)	99.18 (2.35)	29.92 (0.40)	5.94 (0.02)	10.05 (2.14)
1.5	2.78 (0.53)	104.49 (2.76)	30.13 (0.34)	5.93 (0.01)	10.98 (2.53)
3	9.12 (1.21)	112.60 (4.31)	29.87 (0.24)	5.93(0.01)	12.17 (2.22)
90% WFPS					
0	0.25 (0.06)	98.51 (4.15)	37.31 (0.36)	6.02 (0.01)	6.95 (1.84)
0.375	1.05 (0.19)	107.06 (5.46)	37.39 (0.26)	6.01 (0.02)	11.51 (1.78)
0.75	1.63 (0.33)	95.75 (2.83)	37.43 (0.28)	6.05 (0.02)	6.65 (2.14)
1.5	5.77 (0.73)	88.95 (4.51)	37.39 (0.34)	6.12 (0.01)	13.04 (2.83)
3	21.59 (1.45)	59.30 (5.12)	36.56 (0.32)	6.32 (0.03)	18.53 (4.26)
5% LSD	1.39	11.07	0.54	0.032	6.05

Table S4. Gene copy number of the 16S rRNA, nirK, nirS, nosZ-I and nosZ-II genes in the "bulk" and "rhizospheric" soil compartments after daily addition of ARE (0 to 3 mg C day^{-1}) for 7 consecutive days at 50, 70 and 90% WFPS. Values are averages (n=10) with the S.E.M. in brackets.

	Gene abundance (copies soil g^{-1} dw)				
WFPS C input (mg C day^{-1})	**16S rRNA**	**nirK**	**nirS**	**nosZ-I**	**nosZ-II**
Bulk Soil					
50% WFPS					
0	1.68E+09 (1.10E+08)	2.20E+07 (5.52E+06)	1.77E+07 (2.19E+06)	5.48E+06 (5.75E+05)	1.88E+07 (1.50E+06)
0.375	1.74E+09 (9.43E+07)	3.00E+07 (7.57E+06)	1.77E+07 (1.68E+06)	5.80E+06 (5.76E+05)	1.79E+07 (2.10E+06)
0.75	1.95E+09 (1.18E+08)	2.84E+07 (8.27E+06)	1.78E+07 (1.51E+06)	5.89E+06 (4.92E+05)	2.03E+07 (1.83E+06)
1.5	1.78E+09 (1.09E+08)	3.88E+07 (5.72E+06)	1.44E+07 (1.73E+06)	6.45E+06 (7.75E+05)	1.82E+07 (2.03E+06)
3	1.67E+09 (1.58E+08)	1.91E+07 (5.32E+06)	1.51E+07 (1.37E+06)	6.48E+06 (8.47E+05)	1.48E+07 (2.23E+06)
70% WFPS					
0	2.02E+09 (1.52E+08)	3.07E+07 (5.71E+06)	1.91E+07 (2.46E+06)	6.99E+06 (7.74E+05)	1.83E+07 (2.01E+06)
0.375	2.31E+09 (3.45E+08)	3.86E+07 (1.12E+07)	2.44E+07 (3.93E+06)	8.93E+06 (1.35E+06)	2.50E+07 (3.46E+06)
0.75	1.98E+09 (1.78E+08)	5.08E+07 (7.02E+06)	1.76E+07 (1.31E+06)	7.44E+06 (9.30E+05)	2.51E+07 (3.78E+06)
1.5	2.17E+09 (2.15E+08)	3.89E+07 (6.65E+06)	1.71E+07 (1.88E+06)	6.74E+06 (6.77E+05)	2.16E+07 (3.21E+06)
3	2.18E+09 (2.46E+08)	6.13E+07 (6.73E+06)	1.97E+07 (2.47E+06)	1.19E+07 (2.53E+06)	2.07E+07 (1.82E+06)
90% WFPS					
0	1.61E+09 (2.27E+08)	2.66E+07 (5.02E+06)	1.57E+07 (1.66E+06)	4.79E+06 (6.23E+05)	1.77E+07 (2.17E+06)
0.375	1.70E+09 (1.45E+08)	3.20E+07 (6.05E+06)	1.88E+07 (2.01E+06)	7.04E+06 (1.08E+06)	2.22E+07 (2.97E+06)
0.75	1.64E+09 (7.08E+07)	2.44E+07 (6.63E+06)	1.74E+07 (1.66E+06)	5.55E+06 (4.87E+05)	1.60E+07 (2.41E+06)
1.5	1.54E+09 (1.55E+08)	2.75E+07 (6.01E+06)	1.56E+07 (1.58E+06)	4.91E+06 (5.94E+05)	2.23E+07 (2.60E+06)
3	1.67E+09 (1.85E+08)	3.93E+07 (5.42E+06)	2.12E+07 (4.11E+06)	6.41E+06 (1.43E+06)	1.83E+07 (1.36E+06)
Rhizosphere Soil					
50% WFPS					
0	2.05E+09 (1.38E+08)	4.34E+07 (6.87E+06)	1.88E+07 (1.70E+06)	6.57E+06 (3.38E+05)	2.14E+07 (2.46E+06)
0.375	1.98E+09 (9.47E+07)	5.14E+07 (5.80E+06)	1.80E+07 (1.25E+06)	8.85E+06 (8.13E+05)	2.09E+07 (2.48E+06)
0.75	2.32E+09 (2.31E+08)	4.01E+07 (8.93E+06)	2.32E+07 (3.44E+06)	1.56E+07 (1.98E+06)	2.02E+07 (2.36E+06)
1.5	2.81E+09 (1.68E+08)	5.88E+07 (1.07E+07)	2.91E+07 (3.82E+06)	3.42E+07 (5.47E+06)	1.76E+07 (1.82E+06)
3	3.87E+09 (2.44E+08)	5.15E+07 (9.53E+06)	4.40E+07 (4.60E+06)	6.20E+07 (6.00E+06)	2.23E+07 (2.57E+06)
70% WFPS					
0	1.71E+09 (1.24E+08)	2.96E+07 (4.18E+06)	1.34E+07 (2.34E+06)	9.78E+06 (1.73E+06)	1.98E+07 (3.34E+06)
0.375	2.12E+09 (1.29E+08)	3.61E+07 (7.89E+06)	2.02E+07 (1.93E+06)	1.17E+07 (1.37E+06)	2.22E+07 (3.75E+06)
0.75	2.42E+09 (2.30E+08)	5.40E+07 (1.08E+07)	2.31E+07 (1.94E+06)	2.04E+07 (2.68E+06)	2.34E+07 (2.98E+06)
1.5	2.69E+09 (2.63E+08)	5.83E+07 (1.00E+07)	2.81E+07 (2.83E+06)	3.49E+07 (4.61E+06)	2.05E+07 (3.06E+06)
3	3.04E+09 (3.07E+08)	5.54E+07 (1.42E+07)	3.23E+07 (2.90E+06)	4.76E+07 (4.54E+06)	2.21E+07 (3.05E+06)
90% WFPS					
0	1.68E+09 (1.61E+08)	2.81E+07 (5.41E+06)	1.60E+07 (1.23E+06)	5.63E+06 (6.94E+05)	1.67E+07 (1.61E+06)
0.375	1.75E+09 (1.47E+08)	5.02E+07 (7.47E+06)	3.40E+07 (5.34E+06)	1.13E+07 (1.46E+06)	1.82E+07 (2.52E+06)
0.75	1.90E+09 (1.46E+08)	3.41E+07 (7.36E+06)	5.23E+07 (1.22E+07)	1.24E+07 (2.35E+06)	1.53E+07 (1.95E+06)
1.5	2.42E+09 (2.98E+08)	5.10E+07 (1.11E+07)	8.83E+07 (1.39E+07)	2.41E+07 (3.63E+06)	1.90E+07 (3.15E+06)
3	2.72E+09 (3.21E+08)	4.14E+07 (6.12E+06)	9.75E+07 (1.49E+07)	2.48E+07 (4.82E+06)	1.97E+07 (2.48E+06)
5% LSD	5.55E+08	2.20E+07	1.33E+07	3.85E+06	7.23E+06

Table S5. Gene ratios of the different denitrification genes studied when compared to 16S rRNA in the "bulk" and "rhizospheric" soil compartments after daily addition of ARE (0 to 3 mg C day^{-1}) for 7 consecutive days at 50, 70 and 90% WFPS. Values are averages (n=10).

WFPS C input (mg C day^{-1})	Gene ratios					
	nirK/16S	nirS/16S	nosZ-I/16S	nosZ-II/16S	nirK/nirS	nosZ-I/nosZ-II
Bulk Soil						
50% WFPS						
0	0.013	0.010	0.003	0.011	1.25	0.29
0.375	0.017	0.010	0.003	0.010	1.70	0.32
0.75	0.015	0.009	0.003	0.010	1.59	0.29
1.5	0.022	0.008	0.004	0.010	2.69	0.35
3	0.011	0.009	0.004	0.009	1.26	0.44
70% WFPS						
0	0.015	0.009	0.003	0.009	1.61	0.38
0.375	0.017	0.011	0.004	0.011	1.58	0.36
0.75	0.026	0.009	0.004	0.013	2.88	0.30
1.5	0.018	0.008	0.003	0.010	2.28	0.31
3	0.028	0.009	0.005	0.010	3.12	0.58
90% WFPS						
0	0.017	0.010	0.003	0.011	1.69	0.27
0.375	0.019	0.011	0.004	0.013	1.70	0.32
0.75	0.015	0.011	0.003	0.010	1.40	0.35
1.5	0.018	0.010	0.003	0.015	1.76	0.22
3	0.024	0.013	0.004	0.011	1.85	0.35
Rhizosphere Soil						
50% WFPS						
0	0.021	0.009	0.003	0.010	2.31	0.31
0.375	0.026	0.009	0.004	0.011	2.86	0.42
0.75	0.017	0.010	0.007	0.009	1.73	0.77
1.5	0.021	0.010	0.012	0.006	2.02	1.94
3	0.013	0.011	0.016	0.006	1.17	2.79
70% WFPS						
0	0.017	0.008	0.006	0.012	2.22	0.49
0.375	0.017	0.010	0.006	0.010	1.79	0.53
0.75	0.022	0.010	0.008	0.010	2.34	0.87
1.5	0.022	0.010	0.013	0.008	2.08	1.70
3	0.018	0.011	0.016	0.007	1.71	2.15
90% WFPS						
0	0.017	0.010	0.003	0.010	1.76	0.34
0.375	0.029	0.019	0.006	0.010	1.48	0.62
0.75	0.018	0.028	0.007	0.008	0.65	0.81
1.5	0.021	0.036	0.010	0.008	0.58	1.28
3	0.015	0.036	0.009	0.007	0.42	1.26

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