Clark, I. M., Fu, Q., Abadie, M., Dixon, E. R., Blaud, A. and Hirsch, P. R. 2020. Edaphic factors and plants influence denitrification in soils from a long-term arable experiment. *Scientific Reports.* 10 (Article), p. 16053. https://doi.org/10.1038/s41598-020-72679-z

Supplementary Information

Edaphic factors and plants influence denitrification in soils from a long-term arable experiment

Ian M. Clark[a]; Qingling Fu[a,b]; Maïder Abadie[a]; Elizabeth R. Dixon[c]; Aimeric Blaud[a,d]; Penny R. Hirsch[a]*

[a] Sustainable Agriculture Sciences Department, Rothamsted Research, Harpenden, Hertfordshire AL5 2JQ, UK

[b] College of Resources and Environment, Huazhong Agricultural University, Wuhan 430070, Hubei Province, PR China

[c] Computational and Analytical Sciences Department, Rothamsted Research, North Wyke, Devon, EX20 2SB, UK

[d] Current address: School of Applied Sciences, Edinburgh Napier University, Sighthill Campus, Edinburgh, EH11 4BN, UK

*corresponding author

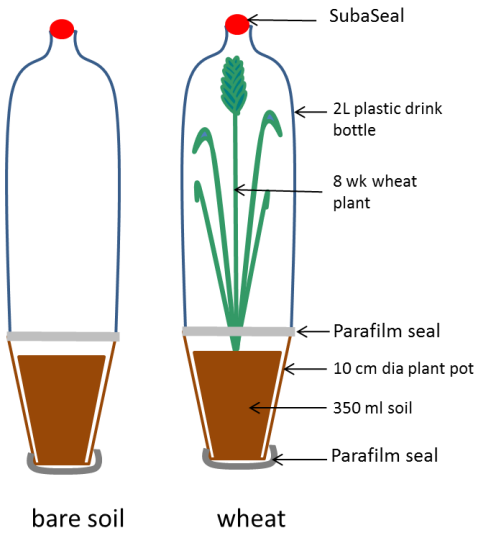
penny.hirsch@rothamsted.ac.uk

tel: 0044 (0)1582 938669

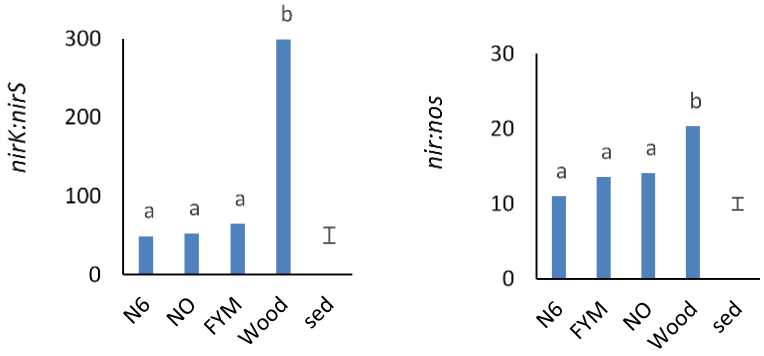
fax: 0044 (0)1582 760981

Supplementary Information

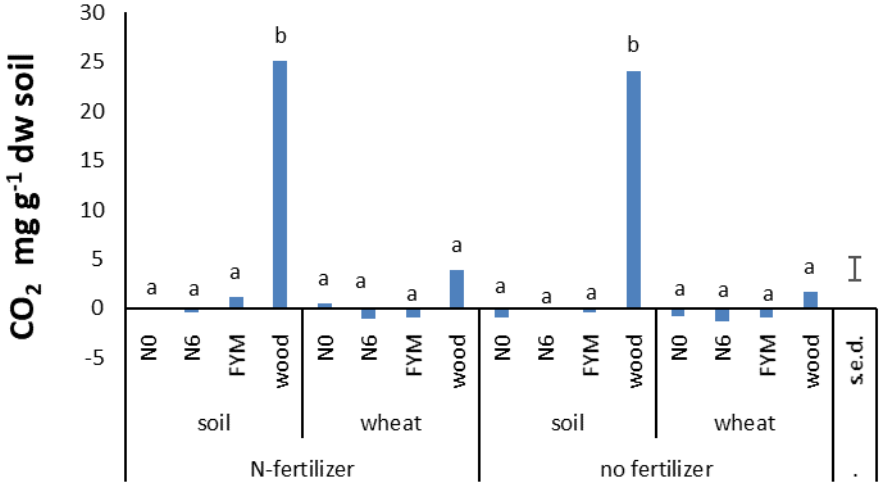
Supplementary Fig. S1. Plant pots set up for gas collection: gas samples collected though SubaSeals.



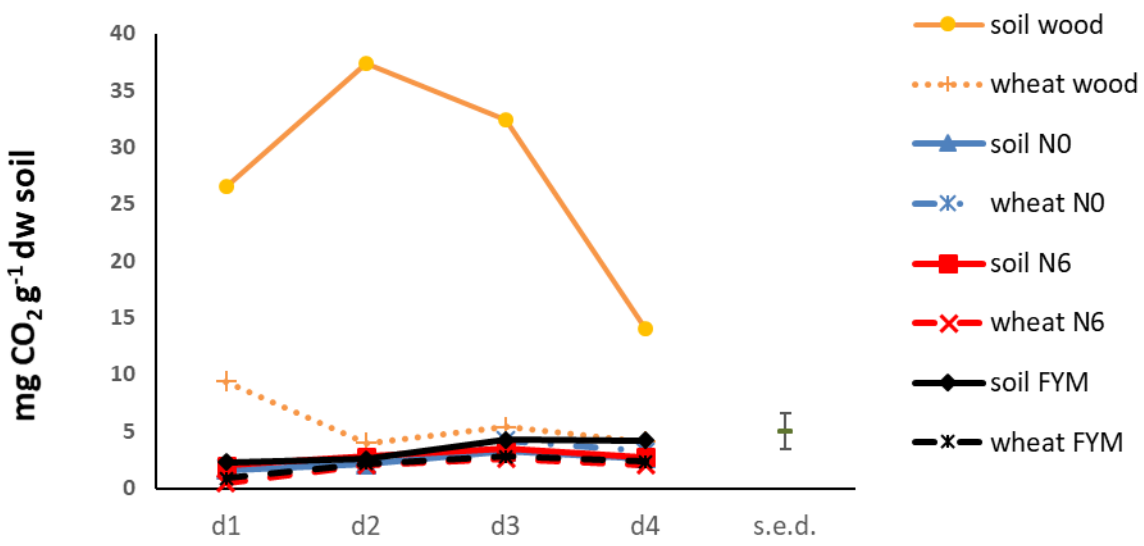
Supplementary Fig. S2. Ratio of nitrite reductase genes *nirK:nirS* (left) and nitrate reductase: nitrous oxide reductase genes (*nirS* + *nirK*) : (*nosZ*I + *nosZ*II)



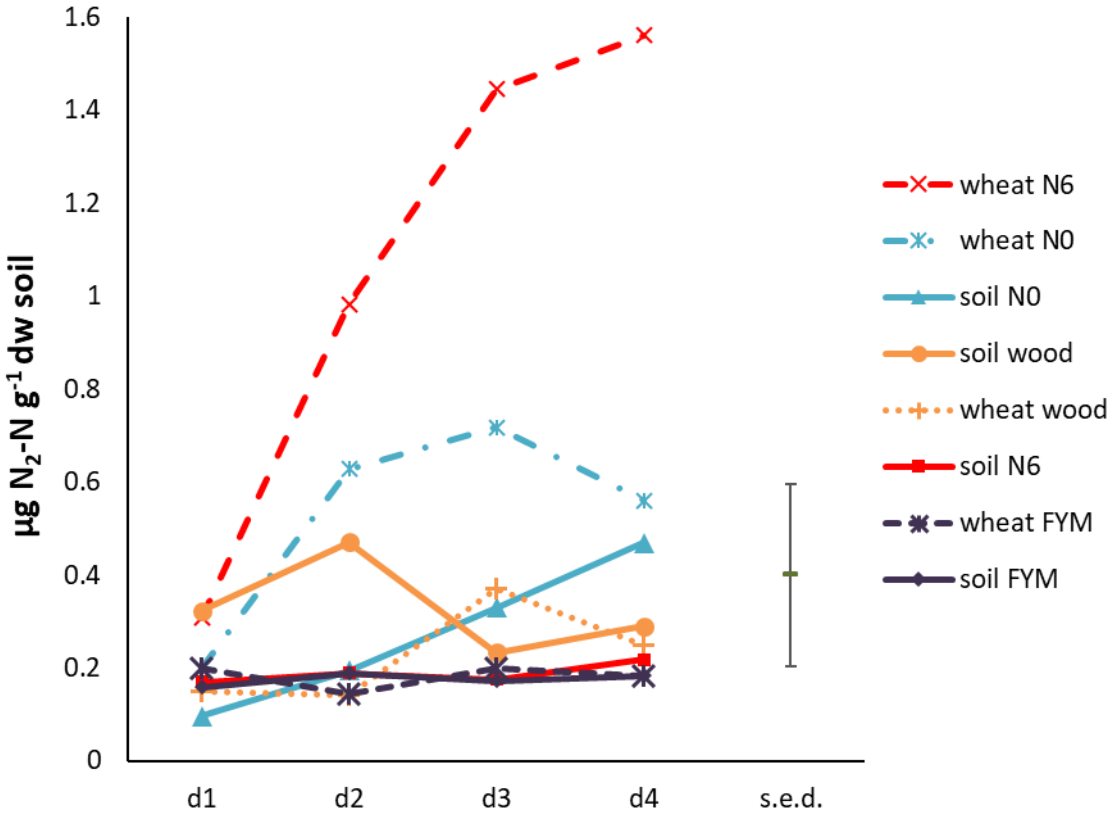
Supplementary Fig. S3. CO_2 production (mean measured over 4 days) with ambient CO_2 subtracted (hence negative values for some treatments).



Supplementary Fig S4. CO_2 measurements made 24 h after the chambers were sealed (d1) and at 24 h intervals subsequently (d2, d3, d4).



Supplementary Fig S5. N_2-N measurements made 24 h after $K^{15}NO_3$ fertilizer application (d1) and at 24 h subsequently (d2, d3, d4).



Supplementary Table S1. ANOVA for denitrification gene ratios.

		Ratio gene copy number from qPCR	
	d.f.	***nirK:nirS***	***nir:nos***
soil	$F_{3, 32}$	70.94, P <.001	11.58, P <.001
fertilizer	$F_{1, 32}$	NS	NS
plant	$F_{1, 32}$	NS	NS
soil x fertilizer	$F_{3, 32}$	NS	NS
soil x plant	$F_{3, 32}$	NS	NS
fertilizer x plant	$F_{1,32}$	NS	NS
soil x plant x fertilizer	$F_{3, 32}$	NS	NS

Supplementary Table S2a. Spearman's rank correlations for all 48 pots showing significant *r* values where *P* (no correlation) is < 0.05

	N_2O-N	CO_2	wfps	NO_3-N	NH_4-N	*16S*	*nirK*	*nirS*	*nosZ*I	*nosZ*II	*nir:nos*
CO_2	0.28										
wfps	0.42	0.04									
NO_3-N	0.61	0.58	0.43								
NH_4-N	-0.14	0.24	-0.40	-0.07							
16S	0.04	0.38	-0.05	0.16	0.70						
nirK	0.06	0.42	-0.14	0.22	0.72	0.87					
nirS	0.13	-0.25	0.38	0.06	0.07	0.34	0.26				
*nosZ*I	0.17	0.36	0.14	0.28	0.66	0.91	0.89	0.49			
*nosZ*II	0.12	0.27	0.15	0.21	0.60	0.93	0.79	0.59	0.92		
nir:nos	-0.17	0.34	-0.50	0.02	0.53	0.38	0.65	-0.27	0.27	0.21	
nirK:nirS	-0.03	0.45	-0.36	0.13	0.61	0.59	0.81	-0.23	0.54	0.41	0.87

Supplementary Table S2b. Spearman's rank correlations for 24 pots where $K^{15}NO_3$ was applied showing significant *r* values where *P* (no correlation) is < 0.05

	N_2O-N	N_2-N	total N	N_2O%	^{15}Natom%	CO_2	wfps	NO_3-N	NH_4-N	*16S*	*nirK*	*nirS*	*nosZ*I	*nosZ*II	*nir:nos*
N_2-N	0.47														
total N	0.54	0.99													
N_2O%	0.87	0.13	0.19												
^{15}Natom%	0.87	0.44	0.50	0.66											
CO_2	-0.24	-0.17	-0.18	-0.08	-0.48										
wfps	0.46	0.07	0.06	0.56	0.25	-0.14									
NO_3-N	0.01	-0.28	-0.30	0.30	-0.29	0.40	0.54								
NH_4-N	-0.39	-0.20	-0.22	-0.45	-0.49	0.30	-0.46	-0.21							
16S	-0.21	-0.22	-0.22	-0.13	-0.48	0.47	0.02	0.26	0.60						
nirK	-0.22	-0.15	-0.16	-0.23	-0.48	0.45	-0.03	0.17	0.66	0.90					
nirS	0.25	-0.20	-0.19	0.27	0.25	-0.27	0.41	0.16	-0.04	0.30	0.27				
*nosZ*I	-0.10	-0.18	-0.19	-0.08	-0.36	0.35	0.20	0.22	0.57	0.88	0.89	0.51			
*nosZ*II	-0.09	-0.27	-0.27	-0.01	-0.30	0.30	0.20	0.33	0.48	0.91	0.81	0.60	0.90		
nir:nos	-0.41	-0.15	-0.15	-0.44	-0.51	0.39	-0.51	0.03	0.64	0.51	0.65	-0.24	0.32	0.35	
nirK:nirS	-0.39	-0.13	-0.13	-0.38	-0.58	0.50	-0.39	0.01	0.67	0.66	0.78	-0.26	0.54	0.44	0.87

Total N = total gaseous N measured, i.e. N_2O-N + N_2-N; N_2O% = N_2O/total N; ^{15}N atom% is %^{15}N measured in N_2O-N. Mean values for gasses over 4 d sampling period were used; values that are related by co-derivation e.g. total N and N_2-N or N_2O-N are expected to correlate and are not commented on in the text.

Supplementary Table S3. Primers used for qPCR to assess denitrification gene abundance and activity

gene	primer	sequence	reference
Bacterial 16SrRNA	341F	CCT AYG GGR BGC ASC AG	Glaring et al., 2015[1]
	806R	GGA CTA CNN GGG TAT CTA AT	
nirK	nirK876F	ATY GGC GGV CAY GGC GA	Henry et al., 2004[2]
	nirK1040R	GCC TCG ATC AGR TTR TGG TT	Hallin and Lindgren, 1999[3]
nirS	cd3a F	GTS AAC GTS AAG GAR ACS GG	Michotey et al., 2000[4]
	R3cd R	GAS TTC GGR TGS GTC TTG A	Hallin and Lindgren, 1999[3]
nosZ Clade I	nosZ2F	CGC RAC GGC AAS AAG GTS MSS GT	Henry et al., 2006[5]
	nosZ2R	CAK RTG CAK SGC RTG GCA GAA	
nosZ Clade II	nosZIIF_1162-1178	CTI GGI CCI YTK CAY AC	Jones et al., 2013[6]
	nosZIIR_1889 -1907	GCI GAR CAR AAI TCB GTR C	

Supplementary Table S4. PCR primer efficiency in qPCR reactions

	gene target	efficiency (%)	r^2	slope	y-int
DNA	16S rRNA Bacteria	84.7	0.999	-3.751	35.845
	nirK	89	0.999	-3.618	35.832
	nirS	86.1	0.999	-3.708	33.375
	*nosZ*I	90.5	0.997	-3.572	35.427
	*nosZ*II	86.4	0.999	-3.697	32.52
RNA	16S rRNA Bacteria	82.8	0.996	-3.815	36.822
	nirK	87	0.989	-3.677	35.871
	nirS	83.8	0.999	-3.784	34.201
	*nosZ*I	82.9	0.998	-3.813	35.231
	*nosZ*II	87.5	0.998	-3.664	33.597

References

1 Glaring, M. A. *et al.* Microbial Diversity in a Permanently Cold and Alkaline Environment in Greenland. *Plos One* **10**, doi:10.1371/journal.pone.0124863 (2015).

2 Henry, S. *et al.* Quantification of denitrifying bacteria in soils by nirK gene targeted real-time PCR. *J. Microbiol. Methods* **59**, 327-335, doi:10.1016/j.mimet.2004.07.002 (2004).

3 Hallin, S. & Lindgren, P. E. PCR detection of genes encoding nitrile reductase in denitrifying bacteria. *Appl. Environ. Microbiol.* **65**, 1652-1657 (1999).

4 Michotey, V., Mejean, V. & Bonin, P. Comparison of methods for quantification of cytochrome cd(1)-denitrifying bacteria in environmental marine samples. *Appl. Environ. Microbiol.* **66**, 1564-1571, doi:10.1128/aem.66.4.1564-1571.2000 (2000).

5 Henry, S., Bru, D., Stres, B., Hallet, S. & Philippot, L. Quantitative detection of the nosZ gene, encoding nitrous oxide reductase, and comparison of the abundances of 16S rRNA, narG, nirK, and nosZ genes in soils. *Appl. Environ. Microbiol.* **72**, 5181-5189, doi:10.1128/AEM.00231-06 (2006).

6 Jones, C. M., Graf, D. R. H., Bru, D., Philippot, L. & Hallin, S. The unaccounted yet abundant nitrous oxide-reducing microbial community: a potential nitrous oxide sink. *Isme Journal* **7**, 417-426, doi:10.1038/ismej.2012.125 (2013).