Supplementary Material

Catch crop residues stimulate N_2O emissions during spring, without affecting the genetic potential for nitrite and N_2O reduction

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Alignment of terminal restriction fragments (T-RFs) using R package *fragalign*

T-RFs derived from soil samples in the present study were aligned using an in-house R package *fragalign*. The principals involved in the alignment algorism are described in the manuscript. Here, we list the parameters used in the analysis in the present study. The R source code of the package can be downloaded as a separate file.

Parameters:

```
color = "B"        # Fragment color.
frag.min = 50      # Minimum fragment size.
frag.max = 700     # Maximum fragment size.
noise.SD = 3       # Threshold for detecting noise peaks; default is 3, reduce to include more peaks.
norm = "h"         # Noise filtering option; h = filter noise based on peak height; a = filter based on peak area.
gaussR = 1.5       # Gaussian peak contrasts; blurs peaks - for PeakScanner, should be 1-3; GeneMapper should be 30-35.
merge.a = 0.04     # Merging parameter a - most useful for merging peaks with shoulders, increase to increase merging -
                   # set to 0 to turn off merging; default is 0.04 from Vähamää et al., 2007.
merge.b = 0.6      # Merging parameter b - default from Vähamää.
merge.c = 3.5      # Merging parameter c - default from Vähamää.
e = 0.05           # Alignment parameter e - default from Vähamää is 0.013; increase to allow greater range of fragments
                   # to be matched.
f = 0.8            # Alignment parameter f - default from Vähamää is 1.
g = 3.5            # Alignment parameter g - default from Vähamää is 3.5; absolute allowable size to be matched in bp.
min.n = 2          # Minimum number of fragments to keep sample - otherwise tossed.

# Call the fragment alignment function
out <- fragalign(fileName, color, frag.min, frag.max, noise.SD, norm, gaussR, merge.a, merge.b, merge.c, e, f, g, min.n)
```

Table S1. Primers and thermal cycling conditions for quantitative amplification of denitrifier genes.

Gene	Primers	Thermal cycling conditions for qPCR	Reference
nirK	F1aCu: ATCAT GGTSC TGCCG CG R3Cu: GCCTC GATCA GRTTR TGGTT	Initial denaturation at 95 °C for 10 min; 5 cycles of 95°C for 15 sec, 63°C for 30 sec (decreased by 1°C per cycle), and 72°C for 30 sec; 40 cycles of 95°C for 15 sec, 58°C for 30 sec, 72°C for 30 sec, 83°C for 15 sec, followed by plate reading.	Hallin and Lindgren (1999)
nirS	Cd3aF: AACGY SAAGG ARACS GG R3cd: GASTT CGGRT GSGTC TTSAY GAA	Initial denaturation at 95 °C for 10 min; 5 cycles of 95°C for 15 sec, 65°C for 30 sec (decreased by 1°C per cycle), 72°C for 30 sec; 40 cycles of 95°C for 15 sec, 60°C for 30 sec, 72°C for 30 sec, 83°C for 15 sec, followed by plate reading.	Throbäck et al. (2004)
nosZ Clade I	nosZ_1F: CGYTG TTCMT CGACA GCCAG nosZ_1R: CGSAC CTTST TGCCS TYGCG	Initial denaturation at 95 °C for 10 min; 5 cycles of 95°C for 15 sec, 65°C for 30 sec (decreased by 1°C per cycle), 72°C for 30 sec; 40 cycles of 95°C for 15 sec, 60°C for 30 sec, 72°C for 30 sec, 83°C for 15 sec, followed by plate reading.	Henry et al. (2006)
nosZ Clade II	nosZ-II_F: CTXGG XCCXY TKCAY AC nosZ-II_R: GCXGA RCARA AXTCB GTRC	Initial denaturation at 95 °C for 10 min; 40 cycles of 95°C for 30 sec, 54°C for 60 sec, 72°C for 60 sec, 82°C for 15 sec, followed by plate reading.	Jones et al. (2013)

References

Hallin, S., and Lindgren, P.-E. (1999). PCR Detection of Genes Encoding Nitrite Reductase in Denitrifying Bacteria. *Applied and Environmental Microbiology* 65(4), 1652-1657.

Henry, S., Bru, D., Stres, B., Hallet, S., and Philippot, L. (2006). 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. *Applied and Environmental Microbiology* 72(8), 5181-5189. doi: 10.1128/aem.00231-06.

Jones, C.M., Graf, D.R.H., Bru, D., Philippot, L., and Hallin, S. (2013). The unaccounted yet abundant nitrous oxide-reducing microbial community: A potential nitrous oxide sink. *The ISME Journal* 7(2), 417-426. doi: 10.1038/ismej.2012.125.

Léon, J. (1992). Crop Growth Rates and Durations of Spring Barley Cultivars as Affected by Varied N Supply and Seeding Rates. *Journal of Agronomy and Crop Science* 169(1‐2), 1-8. doi: doi:10.1111/j.1439-037X.1992.tb01179.x.

Throbäck, I.N., Enwall, K., Jarvis, Å., and Hallin, S. (2004). Reassessing PCR primers targeting *nirS*, *nirK* and *nosZ* genes for community surveys of denitrifying bacteria with DGGE. *FEMS Microbiology Ecology* 49(3), 401-417. doi: 10.1016/j.femsec.2004.04.011.

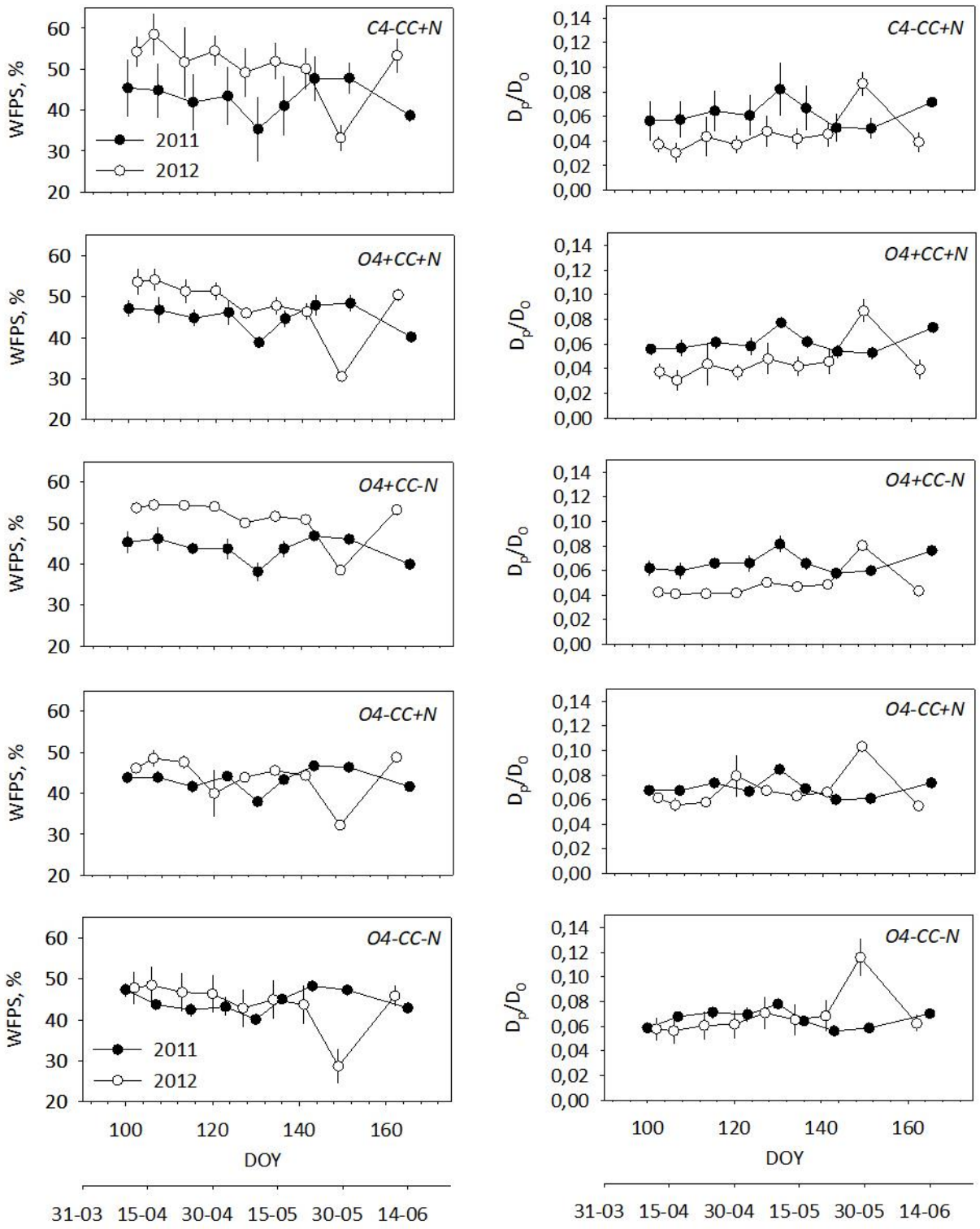

Figure S1. Soil water-filled pore space (WFPS) and relative soil gas diffusivity (D_p/D_0), as defined by Moldrup et al. (2005).

Moldrup, P., Olesen, T., Yoshikawa, S., Komatsu, T., and Rolston, D.E. (2005). Predictive-descriptive models for gas and solute diffusion coefficients in variably saturated porous media coupled to pore-size distribution: II. Gas diffusivity in undisturbed soil. *Soil Science* 170(11), 854-866. doi: 10.1097/01.ss.0000196768.44165.1f.

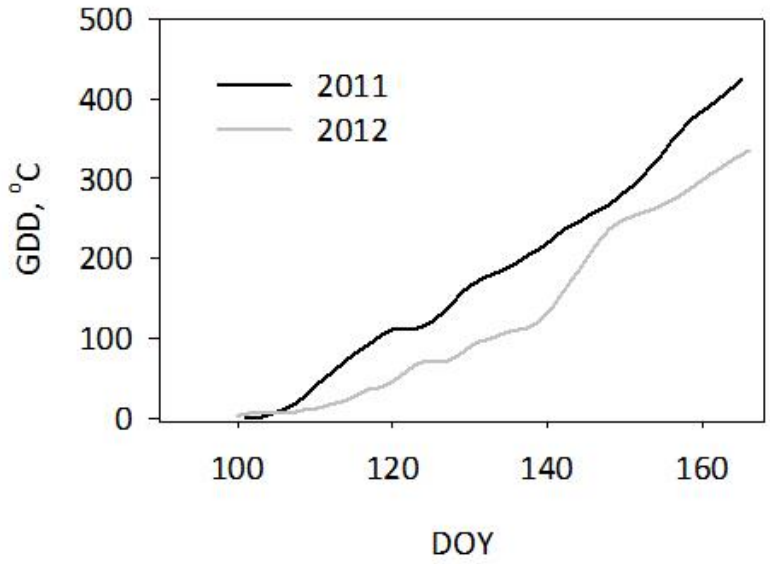

Figure S2. Growing degree days, GDD, calculated as: $GDD = \sum \max[T_d, T_{base}] - T_{base}$, where T_d is daily average temperature and T_{base} is a base temperature of 5°C (Léon, 1992).

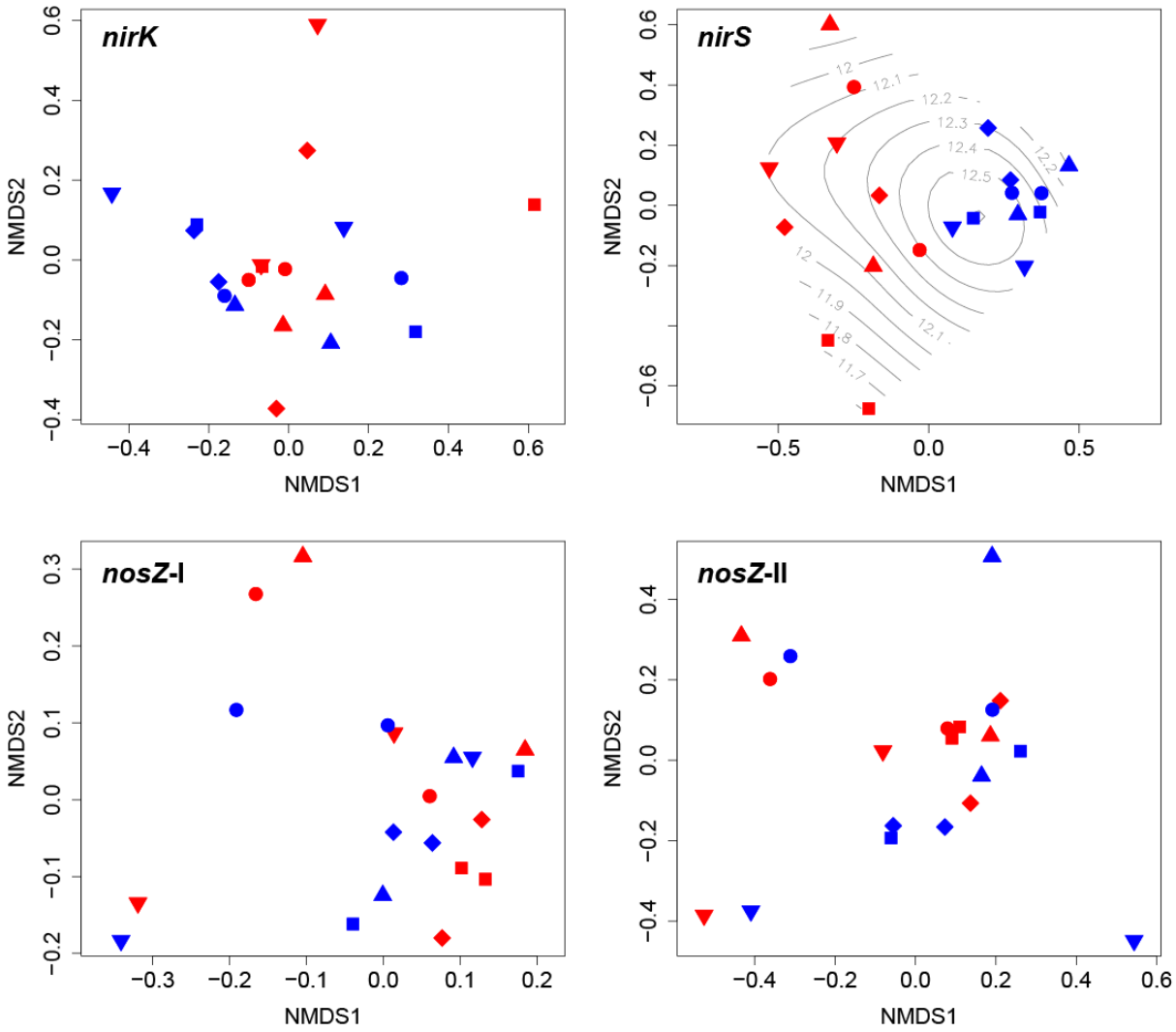

Figure S3: Non-metric multi-dimensional scaling (NMDS) ordination of T-RFLP profiles for *nirK* , *nirS*, *nosZ*-I, and *nosZ*-II genes. Each point represents the T-RFLP profile of one replicate plot. Average soil NO_3^- concentrations (mg N kg^{-1} soil) are fitted to the ordination of *nirS* genes ($p < 0.05$) and presented as gradients. ●: *C4-CC+N*; ■: *O4-CC-N*; ◆: *O4-CC+N*; ▲: *O4+CC-N*; ▼: *O4+CC+N*. Red points represent samples from 2011, and blue points those from 2012.