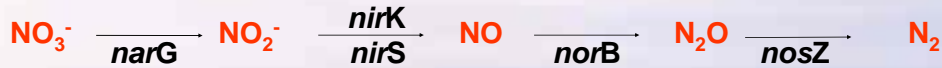


**Introduction:** Different agricultural management systems have direct impacts on soil structure and available nutrients. Do they also affect total microbial and denitrifying bacterial communities? Are differences in soil denitrifying potential due to changes in the denitrifying bacterial population or due to variations in agrophysical properties of the soil?

For a better understanding of the mechanisms controlling denitrification, denitrifying bacterial populations established on different long-term management systems were compared using PCR-DGGE analysis of genes involved in denitrification, *nirS*, *nirK* and *nosZ*. The long term experimental sites included Broadbalk, the classical field experiment at Rothamsted started in 1843, part of which, Wilderness, has reverted to woodland; the Russian Novgorod forest (>100 years); and Russian agricultural field plots established 10 years ago near St. Petersburg.

## FUNCTIONAL GENES INVOLVED IN THE ENZYMATIC STEPS OF DENITRIFICATION



**Methods:** Total microbial community DNA was extracted from soil samples (Table 1) when N<sub>2</sub>O fluxes were close to maximum measured in soil cores with (+N) or without the addition of nitrate. PCR products from *nirS* (*nirScd3aF*, *nirSR3cdGC*) *nirK* (*nirKFlaCu*, *nirKR3CuGC*) and *nosZ* (*nosZF*, *nosZ1622RGC*) were run on DGGE gels and profiles compared to determine the effects of long-term treatments on denitrifying bacterial populations.

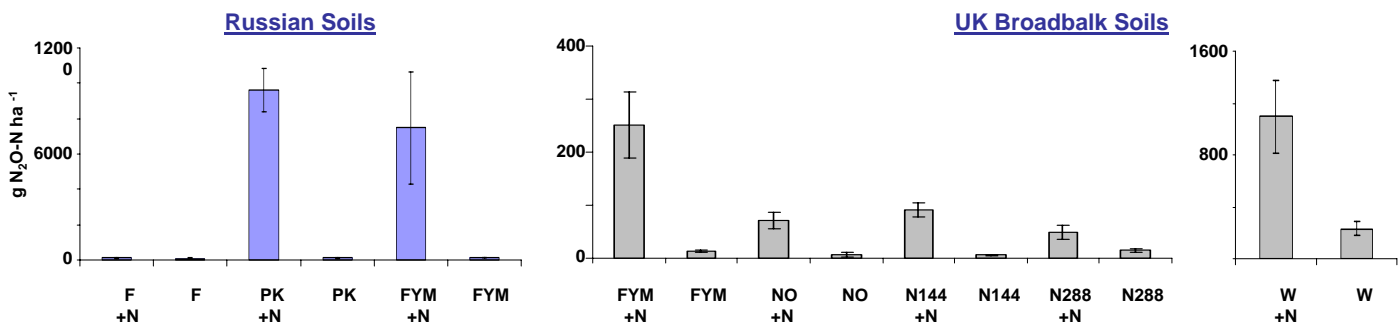
Table 1 Soil Samples

UK: Broadbalk: Wilderness from 1882	W
Continuous wheat fertilised from 1843	
- farm yard manure	FYM
- no nitrogen, + P, + K	N0
- 144 kg N/ha, + P, + K	N144
- 288 kg N/ha, + P, + K	N288
RUSSIA: Novgorod deciduous forest >100 years	F
Arable plots fertilised for >10 years with	
- farm yard manure	FYM
- no nitrogen, + P, + K	PK

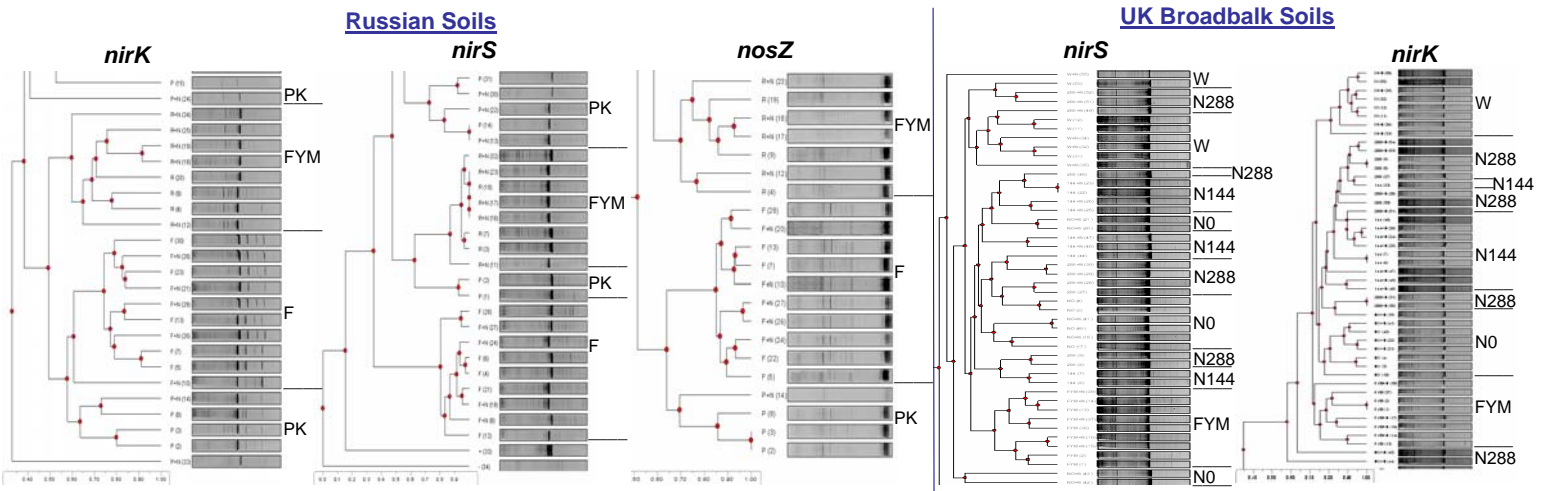


## Results

### Differences in cumulative N<sub>2</sub>O fluxes from soils 7 days after fertiliser application



### DGGE Analysis showing hierarchical similarities of denitrifying populations associated with different agricultural management systems



Denitrifying bacterial community analysis using *nirK*, *nirS* and *nosZ* DGGE profiling showed some differentiation according to the agricultural management system. For Russian soils, *nosZ* DGGE profiles grouped according to management system and for *nirK* and *nirS* the forest (F) and arable organic manure (FYM) treatments clustered separately with the arable without N fertiliser (PK) profiles falling into two or more groups. For Broadbalk soils the cluster analysis was more complicated but the profiles for both *nirS* and *nirK* tended to group according to fertiliser treatments and management regime: arable organic manure (FYM); arable mineral fertilisers including no N (N0, N144, N288, ); uncultivated woodland (W). No obvious associations were observed that related to high or low N<sub>2</sub>O emissions. Predominant denitrifying populations were found for all three functional genes tested and were common to all treatments suggesting that they were not affected by soil management practices. However, soil structural differences were observed between woodland, mineral fertiliser, and organic manure-treated plots and may be the main factor affecting denitrification rates.