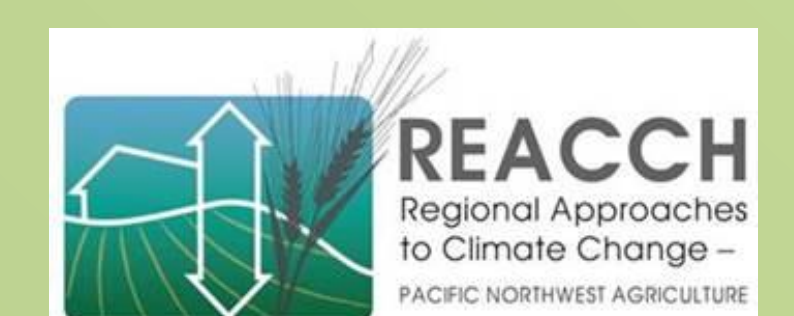


# Influence of Spatio-temporal Variation and Denitrifier Abundance on Denitrification Rate



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## Introduction

Nitrous oxide (N<sub>2</sub>O) is a potent greenhouse gas that is mediated by the soil microbial processes of denitrification and nitrification. Predicting soil denitrification rates is important for accurately estimating and managing field N<sub>2</sub>O emissions. However, denitrification rates exhibit large spatial and temporal variation, making prediction difficult.

The purpose of this study was to determine whether the inclusion of spatio-temporal variation and measurements of soil biology can improve prediction of denitrification activity for a wheat-based cropping system in the Palouse region. Stepwise multivariate regression models of denitrification rates were run using soil environmental characteristics and nitrite reductase gene (*nirK* and *nirS*) abundance as explanatory factors, with spatial and/or temporal variation included in samples.

## Methods

### Study Site

The study site was located within an area of rolling topography at the Washington State University R.J. Cook Agronomy Farm (CAF) in the dryland Palouse region of Washington State, USA. The field of study is cropped under a wheat, wheat, spring legume rotation that is characteristic of regional dryland agriculture.

### Soil Sampling

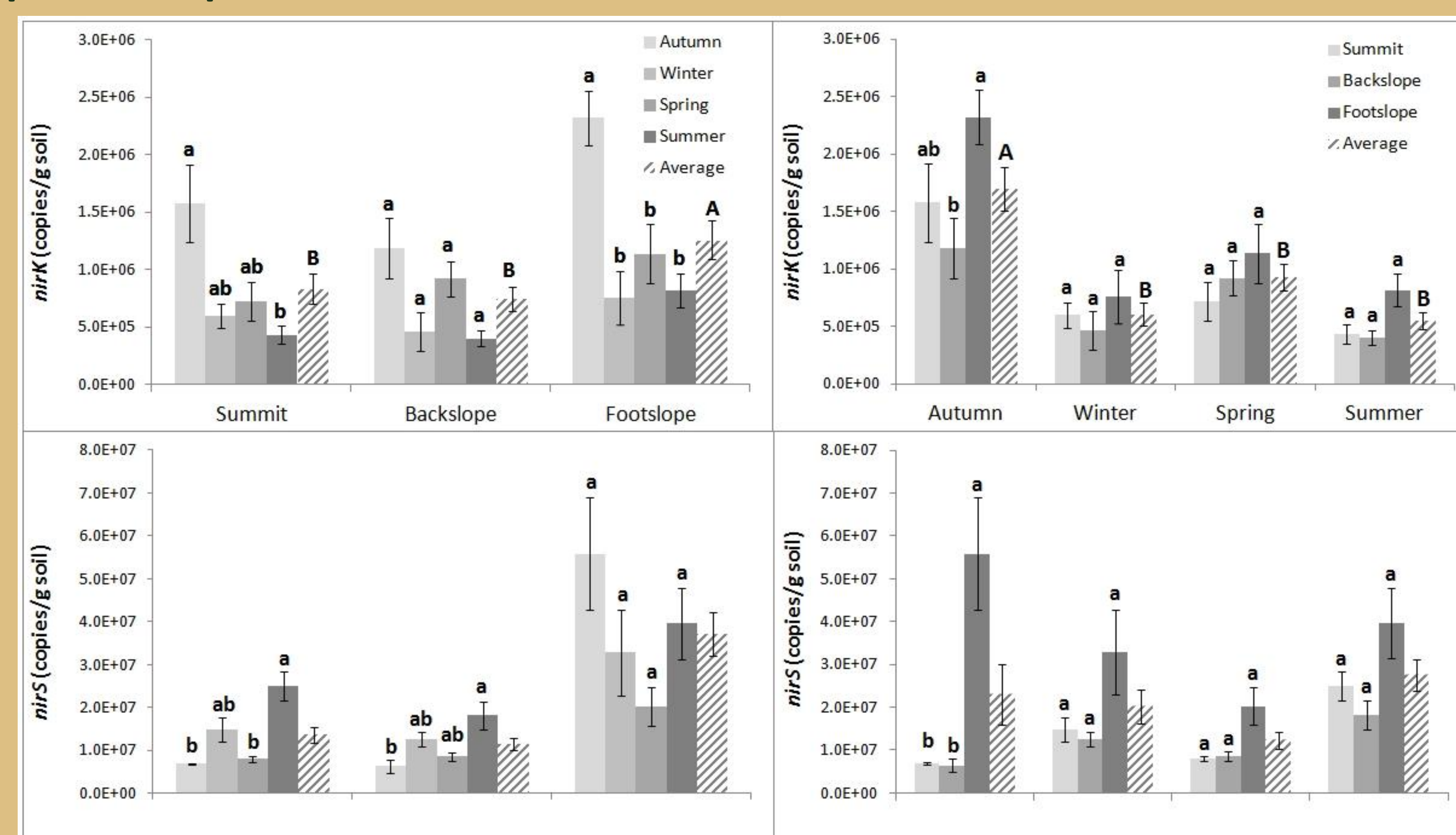
Soils were collected once per season (autumn, winter, spring, summer) at three topographical positions (summit, backslope, footslope) within a field to capture spatial and temporal variation. Each topographic position was sampled at six different locations during a sampling event, with the same locations used each time. Soil was collected from a 0 to 5 cm depth.

### Analyses

Soil chemical analyses included soil water content, nitrate (NO<sub>3</sub>-N), ammonium (NH<sub>4</sub>-N), soluble total nitrogen (N), soluble non-purgeable organic carbon (NPOC), pH, electrical conductivity (EC), and total C and N. Abundance of the nitrite reductase genes (*nirK* and *nirS*) was determined by quantitative polymerase chain reaction (qPCR). Potential denitrification rates of soil were assessed by short-term incubations using the acetylene inhibition method. Data were analyzed by a repeated measures analysis of variance (ANOVA) with PROC MIXED (SAS Institute, Cary, N.C., version 9.3) using Tukey's pairwise comparisons ( $P < 0.05$ ). PROC REG was used to develop stepwise multivariate regression models, with a significance level of  $P < 0.05$  for explanatory factors to enter and to stay in the model.

## Results

### Spatio-temporal variation of denitrifier abundance



Significant seasonal and topographical variation was observed for *nirK* abundance. Overall, the *nirK* abundance was significantly higher in the autumn than in other seasons, and was three times greater than the abundance in summer. The footslope had a significantly greater *nirK* abundance than the summit and backslope.

Significant seasonal and topographical variation was also observed for *nirS* abundance. The *nirS* abundance was typically greatest in the summer and lowest in the autumn, with the exception of the footslope. The *nirS* abundance was typically greatest at the footslope, with the difference significant in the autumn.

## Predicting denitrification rates

Explanatory variable	Variable removed	Parameter estimate	R <sup>2</sup>	p-value	n
<b>Spatio-temporal variation</b>					
Soil water content		-0.054	48%	<.0001	64
<i>nirS</i> abundance		0.50	20%	<.0001	
pH		-58050	6%	0.0003	
Soluble total N		0.09238	2%	0.0405	
Total			76%		
<b>Spatial variation</b>					
<b>Autumn</b>					
<i>nirK</i> abundance		4.6E-07	55%	0.0015	15
NO <sub>3</sub> -N		0.30	18%	0.0152	
Soil water content		0.093	8%	0.0463	
	<i>nirK</i> abundance	1.9E-07	-4%	0.1580	
EC		-0.45	9%	0.0179	
Total			87%		
<b>Winter</b>					
<i>nirS</i> abundance		0.69625	48%	0.0058	14
<b>Spring</b>					
<i>nirS</i> abundance		0.38529	30%	0.0198	18
<b>Summer</b>					
Soluble NPOC		-83	38%	0.0088	17
<b>Temporal variation</b>					
<b>Summit</b>					
Soil water content		-0.092	67%	<.0001	20
Total N		12	16%	0.0009	
<i>nirK</i> abundance		3.9E-07	4%	0.0288	
Total			88%		
<b>Backslope</b>					
Soil water content		-0.059	66%	<.0001	22
pH		-64439	10%	0.0088	
Total C		0.57	7%	0.0114	
Total			84%		
<b>Footslope</b>					
<i>nirS</i> abundance		0.65	39%	0.0018	22
Soil water content		-0.042	34%	<.0001	
EC		0.67	12%	0.0012	
Total			85%		

### Spatial and temporal variation

In predicting potential denitrification rates, the spatio-temporal model (which included both spatial and temporal variation) performed well in predicting measured values with an R<sup>2</sup> of 0.76.

Models including only temporal variation performed well, with R<sup>2</sup> ranging from 0.84 to 0.88. Models including only spatial variation typically have low predictive power, with R<sup>2</sup> below 0.5, although the autumn model was an exception with an R<sup>2</sup> of 0.87.

### *nirK* and *nirS* abundance

The *nirS* abundance contributed significantly to the spatio-temporal model, explaining 20% of variance of potential denitrification rates. It also contributed significantly to models with only spatial variation (winter and spring) and models with only temporal variation (footslope).

The *nirK* abundance was significant for only one model (summit). The *nirK* abundance was significant in the autumn model before being removed after the addition of NO<sub>3</sub>-N and soil water content as explanatory variables.

## Summary

- Including or assessing seasonal variation is necessary for accurately predicting denitrification rates in the region studied. Models including seasonal variation performed well, while models only including topographical variation typically had poor predictive power.
- Including both seasonal and topographical variation produces models that compare well to predicted values for the topographically diverse region studied.
- Measurement of *nirS* abundance improves prediction of denitrification rates when spatial and temporal variation are included.

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