Denitrifier abundance has a greater influence on denitrification rates at larger landscape scales but is a lesser driver than environmental variables

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Abstract

Nitrous oxide is a potent greenhouse gas and its production is mediated by the soil microbial processes of denitrification and nitrification. A thorough understanding of denitrification drivers is necessary to accurately predict and manage nitrous oxide emissions. However, studies disagree on the utility of quantifying the denitrifier community to predict denitrification rates. This study examines the influence of nitrite reductase gene (*nirK* and *nirS*) abundance on denitrification rates across a topographically sloping region. The study was carried out over different seasons (autumn, winter, spring, summer) and topographic positions (shoulder and backslope) within a field cropped to spring wheat. A footslope cropped to winter wheat was included in certain analyses to introduce additional variation at a broader scale. We measured denitrification enzyme activity (DEA) and basal denitrification (BD), *nirK* and *nirS* abundance, bacterial and archaeal ammonia monooxygenase gene (*amoA*) abundance, and soil environmental and chemical characteristics which included gravimetric water content, pH, electrical conductivity, and concentrations of nitrate (NO₃-N), ammonium (NH₄-N), total nitrogen, total soluble nitrogen, total carbon and soluble non-purgeable organic carbon (NPOC). Stepwise multivariate regression (SMR) models of DEA and BD were performed using the measured soil characteristics and denitrifier and nitrifier abundance as explanatory factors to determine whether the microbial community size influenced prediction of denitrification activity. Two SMR models were generated: one SMR model was that with the greatest R^2 from utilizing 1-3 significant variables ($P < 0.05$) and another model was developed solely from a few proposed primary drivers of denitrification, which we described as soluble NPOC, total soluble N, and denitrifier abundance. The SMR models were also generated at two different landscape scales (with and without

footslope). We found *nirS* abundance to be a significant explanatory variable ($P < 0.05$) of DEA rates when footslope samples were included in the analysis, explaining 9 % and 16 % of variation depending upon whether soil gravimetric water content (greatest R^2 model) or soluble NPOC (proposed drivers model) was accepted as the primary explanatory variable, respectively. This was however lower than the variance explained by environmental variables (53 % and 37 %, respectively). When only shoulder and backslope samples were included in the analysis, *nirS* abundance was only a significant explanatory variable of DEA rates when soluble NPOC (proposed drivers model) was the primary explanatory variable. The proposed drivers models that included soluble NPOC, soluble total N, and *nirS* population size consistently produced robust predictive models of DEA at the two different field scales ($R^2 = 0.53$ and 0.70 compared to our greatest- R^2 models of $R^2 = 0.61$ and 0.79 , with and without footslope inclusion, respectively). The *nirK* abundance was insignificant in the stepwise multivariate regression models of DEA, although it was significantly correlated to DEA ($r = 0.35$, $P = 0.003$) when footslope samples were included in the analysis. The *nirK+nirS* abundance was found to be a significant explanatory variable of BD rates (explaining 5 % of variance) with footslope inclusion, but total soluble nitrogen concentration explained much more variance (43 %). Although we found that denitrifier abundance can be a significant predictor of denitrification rates at the field scale, we conclude that measurements of soil moisture, carbon and nitrogen availability are more useful. Additionally, our study gives evidence that greater landscape variation results in a stronger correlation between denitrification rates and denitrifier abundance, indicating that measurements of the denitrifier community are likely important and useful predictors of denitrification rates at larger scales.

1. Introduction

Nitrous oxide (N_2O) is a potent long-lived greenhouse gas and the most significant ozone-depleting substance (Ravishankara et al., 2009). The majority of direct and indirect N_2O emissions come from agricultural lands and continue to increase linearly each year (Forster et al., 2007). Climate change mitigation strategies must consider N_2O emissions in addition to CO_2 as carbon (C) sequestering processes may still contribute to climate change because of co-production of N_2O (Six et al., 2004; Stöckle et al., 2012). The two main sources of soil N_2O emissions are microbial nitrification and denitrification (Bremner, 1997). Nitrification pathways produce N_2O as a byproduct whereas N_2O is generated through incomplete denitrification. The relative N_2O contribution of denitrification and nitrification depends upon soil environmental conditions. The majority of N_2O produced under oxygen-limited conditions is from denitrification pathways (Zhu et al., 2013) and overall N_2O emissions increase with increasing water-filled pore space (Linn and Doran, 1984; Smith et al., 1998). For soil at water contents below saturation, nitrification is the primary source of N_2O production (Mummey et al., 1994).

The usefulness of measuring the denitrifier community to predict denitrification rates is debatable as studies disagree as to whether measurements of the denitrifier community are better predictors of denitrification rates than measurable soil properties. Petersen et al. (2012) observed greatly variable denitrification rates across a gradient of vegetation and found denitrifier population size was a better predictor of denitrification rates than measured soil properties. A survey of studies indicated that the utility of population size as a predictor of denitrification enzyme activity (DEA) is dependent on the range of measured DEA; a range in DEA of at least

five-fold was generally needed in order to detect a significant correlation between DEA and denitrifier abundance (Petersen et al., 2012). Wallenstein et al. (2006) described the denitrifier community as an intermediate by which soil environmental variables control denitrification rates, such that the denitrifier community is an integration of environmental drivers over time. Thus, measurements of the denitrifier community may be moot in predicting denitrification if the key environmental drivers can be sufficiently measured and modeled. To the contrary, the denitrifier community may be a more readily quantifiable integrated factor in diverse landscapes where soil environmental characteristics are complex and greatly variable.

The denitrifier population can be identified by the gene sequences for seven different enzymes involved in the denitrification pathway (Philippot et al., 2007). Gene sequences utilized for studying denitrifiers include those that encode enzymes for nitrite reduction (*nirK* targets copper nitrite reductase and *nirS* targets *cd₁* nitrite reductase), nitric oxide reduction (*norB* targets nitric oxide reductase and *qnorB* targets quinol nitric oxide reductase), and nitrous oxide reduction (*nosZ* targets nitrous oxide reductase). The enzymes for nitrate reduction (*napA* targets periplasmic nitrate reductase and *narG* targets membrane bound nitrate reductase) are also part of the denitrification pathway, but are observed widely among both denitrifying and non-denitrifying proteobacteria (Potter et al., 2001). Because of the diversity of enzymes involved in the denitrification pathway, targeting a single enzyme does not capture the entire denitrifier community. Jones et al. (2008) found that out of 68 complete genomes that contained *nirS* and *nirK* sequences, only 43 of them also contained a *nosZ* sequence. Moreover, *nirK* and *nirS* both encode evolutionarily distinct but functionally equivalent nitrite reductase enzymes and only one may be present in a bacterium (Glockner et al., 1993). The sum of *nirK* and *nirS* populations is

often used as an approximation of the total denitrifier population (Philippot et al., 2011), which we have also done in this study.

In our study, we assessed the usefulness of measuring the denitrifier community size to predict denitrification rates along a hillslope. We hypothesized that *nirK* and *nirS* abundance would be significant predictors for field scale denitrification rates if both spatial and temporal variation were evaluated, as other studies that measured a wide range of denitrification rates find denitrifier abundance to be a primary predictor of denitrification rates (Čuhel et al., 2010; Petersen et al., 2012). We also measured nitrifier abundance (specifically ammonia-oxidizing bacteria and archaea) as a possible predictor of denitrification rates because nitrifier denitrification has been shown to be a major component of denitrification pathways (Zhu et al., 2013).

The temperate Palouse region of the inland Pacific Northwest provides an ideal study location by virtue of its large topographical variability. The Thatuna and Palouse soil series common to the region can have slopes as great as 55 and 60 %, respectively (USDA, 1978). We measured both DEA and basal denitrification (BD) rates. The DEA rates were measured under anaerobic conditions with an abundant supply of available C and nitrogen (N), reflective of conditions that cause spikes in N₂O flux in the field. Correspondingly, DEA rates have been found to correlate to N₂O emissions in several field studies (van Kessel et al., 1993; Attard et al., 2011). The BD rate was assessed without supplementing soil available C and N, and provides an understanding of denitrification rates when C and N substrate may be limiting under anaerobic conditions. However, our BD rates were measured from sieved soil, and therefore do not entirely represent field conditions. Our secondary goal was to develop a better understanding of the spatio-temporal dynamics of soil environmental characteristics, the denitrifier and nitrifier

communities, and denitrification rates in this variable landscape. The findings presented here will be used to develop better predictions of field denitrification rates and N₂O emissions in the topographically diverse region, both of which are essential to climate change prediction and mitigation strategies.

2. Materials and methods

2.1. Description of study site and soil sampling

Samples were collected from the Washington State University R.J. Cook Agronomy Farm in the dryland Palouse region of Washington State, USA (46° 46' 44" N, 117° 05' 19" W). The farm has been managed in no-tillage cropping systems since 1998. Based on 30-year normals (1981-2010) from the nearby PULLMAN 2 NW National Weather Service station (46° 45' 37" N, 117° 11' 10" W), average annual precipitation is 517 mm, mean annual high temperature is 14.7°C and the mean annual low temperature is 2.8°C. Soils are either Palouse silt loam, a fine-silty, mixed, superactive, mesic Pachic Ultic Haploxeroll; Thatuna silt loam, a fine-silty, mixed, superactive, mesic Oxyaquic Argixerolls; or Latah silt loam, a fine, mixed, superactive, mesic Xeric Argialbolls (USDA, 1978). The field of study is cropped under a wheat-wheat-spring legume rotation that is characteristic of regional dryland agriculture.

Soil samples were taken within a field from shoulder, backslope, and footslope topographical positions (Ruhe, 1969) at four separate sampling events. Sampling events occurred on November 4, 2012; February 26, 2013; May 6, 2013 and July 29, 2013 to represent the seasons of autumn, winter, spring and summer, respectively. Each topographic position was sampled at six different locations during a sampling event (Figure S1). The shoulder samples were located from 784-788 m elevation and ranged from 7-15 % slope, backslope samples were located from 778-781 m

elevation ranged from 6-19 % slope, and footslope samples were located from 774-779 m elevation and ranged from 2-7 % slope. The shoulder and backslope sampling points were 40-80 m apart, while the backslope and footslope sampling points were 30-50 m apart; distance between sampling points at a topographic position ranged from 20-200 m. For each sample, approximately 500 g of soil was collected from a 0 to 5 cm depth using a sharpshooter shovel. An unsieved field moist subsample was taken from each soil sample for molecular applications and stored at -20°C until DNA extraction. Remaining soil samples were sieved to 4 mm and stored at 4°C until analyzed.

The shoulder and backslope were planted to soft white spring wheat (*Triticum aestivum* L. var. "Louise") in early April 2013 as part of a winter wheat-spring wheat-spring legume rotation. The footslope was planted to Clearfield soft winter wheat (*T. aestivum* L. var. "AP 700") in October 2012, following winter wheat in a winter wheat-winter wheat-spring legume rotation. Both winter and spring wheat were fertilized at the time of planting with 135 kg N ha⁻¹ urea ammonium (NH₄⁺) nitrate (NO₃⁻). Fertilizer at planting was banded at 10.0 cm and 11.4 cm depth and seeding depth was at 2.5 and 3.8 cm depth for spring wheat and winter wheat, respectively. Small foliar applications of ammonium sulfate (less than 17 kg N ha⁻¹) were made twice in the fall and once in the spring for winter wheat, and twice in the spring for spring wheat. Harvest occurred after the July 2013 soil sampling.

2.2. Soil analyses

Gravimetric soil water content (g water g⁻¹ dry soil) was determined from soil sample weights before and after oven drying at 105°C for at least 48 h. Soil pH and electrical conductivity were determined using a Denver Instruments Model 250 pH ISE conductivity meter (Denver

Instruments Company, Denver, CO) in 1:1 soil:water extract. Soil $\text{NO}_3\text{-N}$ and $\text{NH}_4\text{-N}$ concentrations were quantified from 2 M KCl extracts (Keeney and Nelson, 1982) using a Technicon flow-injection auto-analyzer (Lachat Quickchem FIA 8000 series, Lachat Instruments, Milwaukee, WI). Soluble non-purgeable organic C (NPOC) and total soluble N were extracted in 3 volumes of 17 m Ω nanopure water and filtered to 0.2 μm following centrifugation, then measured using a total C and N analyzer (TOC-VCSH, Shimadzu, Kyoto, Japan). Soils were homogenized and air-dried before total C and N analysis. Particulate organic matter (POM) fraction and mineral-associated fraction were separated by shaking air-dried soil in 5 g l⁻¹ sodium hexametaphosphate solution for 24 h and sieving to 53 μm . POM (> 53 μm) and mineral fraction (< 53 μm) total C and N were analyzed on a subset of samples ($n = 25$). Total C and N was quantified by dry combustion using a Costech Elemental Combustion System 4010 (Valencia, CA).

2.3. Short-term denitrification incubations

Soil DEA and BD rates were measured using the acetylene inhibition method (Yoshinari et al., 1977). Soil moisture was not modified prior to sample analysis. Sieved field moist soil equivalent to 20 g dry soil was sealed in half pint wide mouth canning jars with a septum in the lid and pre-incubated overnight at 20°C. Incubations were performed in triplicate. The headspace of each jar was flushed with N_2 gas to create an anaerobic environment. For the DEA incubation, soil was wetted with a solution of 25 ml of 240 $\mu\text{g ml}^{-1}$ glucose-C and 40 $\mu\text{g ml}^{-1}$ $\text{NO}_3\text{-N}$ for a final concentrations of 300 and 50 $\mu\text{g g}^{-1}$ dry soil, respectively (Luo et al., 1996). The BD incubation soil did not receive any C or N solution and remained at field water content. The incubation was initiated by injection of acetylene into the headspace (10 % v/v). Gas samples were taken from the headspace at 2 and 4 h and analyzed on a gas chromatograph equipped with

an electron capture detector (GC-2014, Shimadzu, Kyoto, Japan). Gas samples were taken within 4 h of the start of the incubation (addition of acetylene) to ensure minimal synthesis of new enzymes (Luo et al., 1996). N₂O concentration was determined using calculations described in Drury et al. (2008). Denitrification rates were calculated as the change in N₂O between the 2 and 4 h measurements.

2.4. DNA extraction and quantification of denitrifier and nitrifier abundance

DNA was extracted from field moist soil equivalent of 0.2 g dry weight soil using PowerLyzer PowerSoil DNA Isolation Kit (MO BIO Laboratories, Carlsbad, CA). Extraction was performed according to manufacturer's instructions, with the exception of DNA elution being performed with the buffer heated to 80°C and then re-eluted with the eluant. Extracts were visualized by 1 % agarose gel electrophoresis with a 1 kb ladder (New England Biolabs, Ipswich, MA). DNA concentration was measured using the Qubit ds DNA HS Assay (Life Technologies, Grand Island, NY). DNA concentration of samples ranged from 2.6 to 28.2 µg ml⁻¹.

The ammonia-oxidizing bacterial (AOB) and archaeal (AOA) communities were quantified by bacterial and archaeal ammonia monooxygenase (*amoA*) genes and the denitrifier community was quantified by bacterial nitrite reductase (*nirS* and *nirK*) genes using quantitative PCR (qPCR). DNA extracts were first tested for the presence of PCR inhibitors using a method described previously (Reardon et al., 2013). Briefly, the quantification cycle (C_q) of a known concentration of pUC19 plasmid (Invitrogen, Carlsbad, CA) was determined by qPCR using the universal sequencing forward primer M13(-40) (5'—GTT TTC CCA GTC ACG AC—3') and reverse primer M13(-48) (5'—AGC GGA TAA CAA TTT CAC ACA GGA—3') at 60°C annealing temperature in the presence and absence of soil DNA diluted 1:20 with H₂O. The

mean C_q of duplicate reactions containing soil extract were no greater than 0.4 C_q of the extract-free control, indicating little to no significant inhibition. The primers and conditions for qPCR reactions are shown in Table S1. Soil extracts were measured in triplicate. The 10 μ l qPCR reactions contained 0.8 $\times$ Power SYBR Green PCR Master Mix (Life Technologies, Grand Island, NY), 0.1 μ g μ l⁻¹ bovine serum albumin (Roche Applied Science, Indianapolis, IN), forward and reverse primer, and 1 μ l DNA diluted 1:20 in H₂O. The standard curves were generated by 10-fold dilution of plasmids containing cloned amplification products over 5 to 6 orders of magnitude. Thermal cycling was performed using a StepOnePlus Real-Time PCR System (Life Technologies) with either 2- or 3-step amplification protocols as indicated in Table S1. The reaction was initiated at 95°C for 10 min followed by 40 cycles of a 2-step amplification at 95°C melting for 15 s and annealing for 1 min or 3-step amplification at 95°C for 15 s, annealing for 1 min and extension at 72°C for 30 s. Melt curve analysis was performed by cycling 15 s at 95°C and 1 min at 60°C with an increase of 0.3°C to a final temperature of 95°C. Fluorescence was measured prior to each melt step for both the 2- and 3-step protocols. The standard deviation of the C_q for replicate reactions was below 0.5. The standard curve efficiencies were 90-98 % with R^2 values ranging from 0.994-0.999 for all targets. Samples below the standard curve, which were omitted from analysis, included twelve archaeal and one bacterial *amoA* samples, but none of the *nirK* and *nirS* samples.

2.5. Statistical analysis

Data were analyzed by a repeated measures analysis of variance (ANOVA) using PROC MIXED (SAS Institute, Cary, N.C., version 9.3) with season and topographical position as main effects. ANOVA of topographic comparisons did not include the footslope because a difference in crop and management at the footslope prevented direct comparisons based on topography. ANOVA

of seasonal comparisons was performed with and without the footslope to highlight and acknowledge that footslope inclusion meant that a difference in crop and management was incorporated, providing greater landscape variation. Further, our seasonal comparisons mirror our Stepwise Multivariate Regression (SMR) models which were performed at two landscape scales: shoulder and backslope; and shoulder, backslope and footslope. Normality was tested using the Anderson-Darling test ($P < 0.05$). Data was transformed as necessary to address normality for ANOVA and stepwise multivariate regression analysis. As a repeated measures study, a compound symmetric covariance structure could not be assumed for the ANOVA. The suitability of a compound symmetric covariance structure was tested by Mauchly's Sphericity test ($P < 0.05$). If a compound symmetric covariance structure was found to be inappropriate, the covariance structure with the lowest Bayesian Information Criterion (BIC) and the most conservative estimate of significant differences was used. Tukey's pairwise comparisons were used to compare topographical or seasonal differences ($P < 0.05$). Pearson's correlations were determined using PROC CORR ($P < 0.05$). Denitrifier specific activity was calculated as DEA divided by the sum of *nirK* and *nirS* gene copies per gram soil (modified from Attard et al., 2011). Wherever BD rates were analyzed, unless otherwise noted, summer samples were excluded from analysis since it measured an unnatural soil condition of an anaerobic, dry soil.

A stepwise multivariate regression (SMR) analysis of DEA and BD rates was used to identify and rank predictors. PROC REG was used with a significance level of $P < 0.05$ for explanatory factors to enter and to stay in the model. The variable with the greatest F -statistic value was added at each step. The model with the greatest R^2 from 1-3 significant variables ($P < 0.05$) was generated from potential explanatory variables, which included: gravimetric water content, pH, electrical conductivity, $\text{NO}_3\text{-N}$, $\text{NH}_4\text{-N}$, total nitrogen, total soluble nitrogen, total carbon and

soluble non-purgeable organic carbon (NPOC), and denitrifier and nitrifier abundance. The greatest R^2 models had soil gravimetric water content as the leading driver of DEA rates. Since soil wetting of the DEA may have influenced denitrification activities, a second "proposed drivers" model was developed solely from denitrifier population, soluble NPOC, and total soluble N as potential explanatory variables, which we found to be the strongest predictors of DEA rates when gravimetric water content was not included in analysis. The SMR models were developed with the footslope excluded and included to compare different landscape scales. If model assumptions were violated (normality, homoscedasticity or lack of correlation between variables), explanatory factors were removed as necessary from the model.

3. Results

3.1. Spatio-temporal variation of soil chemical characteristics

Measurements of soluble soil C and N, pH and water content varied by season across the shoulder and backslope (Table 1). Soluble NPOC was generally greatest in summer. Total C was also greatest in summer with differences between summer and autumn significant although marginal. Total soluble N was greater in summer and spring compared to autumn and winter, although the differences were not always significant. In contrast, concentration of $\text{NO}_3\text{-N}$ and $\text{NH}_4\text{-N}$ were greatest in autumn and winter, with the concentration of $\text{NH}_4\text{-N}$ peaking in autumn and the concentration of $\text{NO}_3\text{-N}$ subsequently peaking in winter. Soil pH was generally greatest in autumn. Soil was significantly drier in summer than the rest of the year. Seasonal variations were not observed for total N or mineral and POM fraction total C and N. For the year of study, mineral fraction total C and N was on average $1.8 \pm 0.1 \%$ and $0.16 \pm 0.01 \%$, respectively, and POM fraction total C and N was on average $7.6 \pm 0.3 \%$ and $0.48 \pm 0.01 \%$, respectively ($n = 25$;

data not shown). Measurements across the shoulder, backslope and footslope were analyzed for greater landscape scale although interactions between season and topography or insufficient normalization after transformation precluded statistical comparisons (Table 2).

Differences in soil characteristics were observed among topographic positions within certain seasons (Table 3). Total soluble N was significantly greater at the footslope than at the shoulder and backslope in autumn. However, in spring total soluble N was significantly lower at the footslope than at the backslope. Total inorganic N followed a similar trend in spring, and was significantly lower at the footslope than at the shoulder and backslope. Across all seasons, total N was significantly greater at the footslope than at the backslope, although the magnitude of difference was marginal (0.20 ± 0.01 % and 0.18 ± 0.01 % for the footslope and backslope, respectively; data not shown). Soluble NPOC did not significantly differ between the three topographical positions for any season (Table 3), although total C was significantly greater at the footslope than at the shoulder and backslope in summer.

3.2. Spatio-temporal variation of denitrification rates and denitrifier and nitrifier abundance

Average DEA rates ranged from 40 to 900 times greater than the BD rates (Table 4). When topographical positions were averaged across season, significant differences were observed for both DEA and BD rates across the shoulder and backslope, with rates greatest in summer and lowest in winter (Figure 1a). The average DEA rate was approximately 14-fold and 7-fold greater in the summer than in winter with (Figure 1a) and without inclusion of footslope (Figure 1b), respectively. When averaged across season, the DEA rates were generally greater at the backslope position than at the shoulder whereas BD rates, which excluded the summer samples, were similar between the two topographic positions (Figure 3a).

Statistical comparisons for DEA and BD rates at the larger landscape scale which included the footslope were prohibited by an interaction between season and topography. For season and topographical comparisons, the DEA rates at the footslope were significantly greater than at the shoulder in autumn and were also significantly greater at the footslope than at the backslope and shoulder in winter (Table 4). The BD rates were significantly greater at the footslope than at the shoulder and backslope in autumn. The DEA and BD rates were significantly correlated to each other across all seasons ($r = 0.64$, $P < 0.0001$ and $r = 0.67$, $P < 0.0001$ with and without footslope inclusion, respectively).

Seasonal differences were observed for both the denitrifier and nitrifier populations across the shoulder and backslope, and similar trends were observed when the footslope was included in analysis (Figure 2a-d). At both landscape scales, the *nirS* gene abundance was at least an order of magnitude greater than *nirK* in every season (Figure 2a,b). Across the shoulder and backslope, both *nirK* and *nirS* were significantly lower in autumn than the other seasons with the greatest *nirK* and *nirS* quantities recovered in summer (Table 4). With the footslope included, both denitrifier populations were still lowest in the autumn and *nirS* was still greatest in the summer, but the *nirK* population became greatest in the winter. Denitrifier specific activity was generally greater in autumn and summer than in winter and spring at both landscape scales (Figure 2e,f).

Significant seasonal differences were not observed in AOA and AOB population densities across the shoulder and backslope (Figure 2c). The AOB abundance was consistently greater than AOA abundance in every season, with at least a 3-fold difference in seasonal means at both landscape scales (Figure 2c,d).

Significant topographical differences were not observed in the denitrifier and nitrifier abundance between the shoulder and backslope across all seasons (Figure 3b,c). However, significant differences were observed between the footslope and the two upper topographical positions for the denitrifier and nitrifier population densities in certain seasons (Table 4). The winter footslope *nirK+nirS* population was significantly greater than either the winter shoulder or backslope population and significantly correlated to total C with and without the footslope inclusion ($r = 0.28$, $P = 0.02$ and $r = 0.50$, $P = 0.0003$, respectively). Similarly, the AOA+AOB population was significantly greater at the footslope than at the shoulder in autumn, and was significantly greater at the footslope than the backslope in winter (Table 4).

3.3. Predictors of denitrification rates

Significant predictors of DEA rates ($P < 0.05$) across all seasons were identified using SMR at two different landscape scales: shoulder and backslope; and shoulder, backslope and footslope (Table 5). Generating the model with the greatest predictive power (largest R^2), gravimetric soil water content was the primary explanatory variable of DEA rates regardless of whether the footslope was included or not. In both cases the correlation of DEA to water content was negative ($r = -0.62$, $P < 0.0001$ with footslope inclusion, $r = -0.82$, $P < 0.0001$ without footslope inclusion). The *nirS* abundance was a significant explanatory variable in the greatest R^2 model at the broader landscape scale, but was not a significant explanatory variable in the greatest R^2 model across the shoulder and backslope.

A second model, referred to as the proposed drivers model, was developed using only the variables of soluble NPOC, total soluble N, and denitrifier abundance. These variables were significant drivers when gravimetric water content was not included as an explanatory variable.

In the proposed drivers model, soluble NPOC became the primary explanatory variable at both landscape scales (Table 5). The proposed drivers model did not greatly differ in R^2 from the corresponding greatest- R^2 model. For both of the proposed drivers models, *nirS* abundance was a significant variable ($R^2 = 0.16$ and $R^2 = 0.07$, with and without footslope inclusion, respectively). Of the nitrifier and denitrifier abundances, *nirS* was the only significant explanatory variable included in the SMR models developed for DEA. Across the shoulder and backslope, the abundance of *nirS* was significantly correlated to DEA rates ($r = 0.46$, $P = 0.001$) but *nirK* abundance was not significantly correlated to DEA rates ($r = 0.27$, $P = 0.06$). With footslope samples included in the analysis, DEA rates were significantly correlated to both *nirS* and *nirK* abundance ($r = 0.43$, $P = 0.0002$ and $r = 0.35$, $P = 0.003$, respectively). Measurements of nitrifier abundance did not correlate significantly to DEA or BD rates at either landscape scale.

Significant predictors for BD were also identified using SMR ($P < 0.05$; Table 6). The BD rates were analyzed at the two landscape scales, but unlike the SMR of DEA rates, summer samples were excluded because the BD protocol measured a dry soil under anaerobic conditions, which is considered an unnatural condition that would not occur in the field. Only the greatest- R^2 model had significant variables at the shoulder and backslope scale, which was a single variable model of $\text{NO}_3\text{-N}$ explaining 22 % of variance of BD rates ($R^2 = 0.22$). At the greater landscape scale, the model developed for the greatest- R^2 included total soluble N and *nirK+nirS* abundance, which were also the proposed drivers. The two variables explained 48 % ($R^2 = 0.48$) of the variance of BD rates. The correlation between BD rates and *nirK+nirS* abundance was negative ($r = -0.24$, $P = 0.09$, summer samples excluded). Soil $\text{NH}_4\text{-N}$ concentration was also strongly correlated to BD rates at the greater landscape scale, and as a single variable SMR model of BD rates $\text{NH}_4\text{-N}$ explained 39 % of variance ($R^2 = 0.39$; data not shown).

4. Discussion

This study evaluates the relative utility of biological and environmental predictors of denitrification rates along a hillslope in a topographically sloping agricultural landscape. Previous studies of denitrification rates at the field scale do not consistently find biological predictors to be significant, as some studies find no correlation between measured biological predictors and denitrification rates (Enwall et al., 2005; Dandie et al., 2008; Le Roux et al., 2013). Our goal was to determine whether nitrifier or denitrifier abundance would be useful predictors for field scale denitrification rates if seasonal and topographical variation was captured. We also sought to analyze seasonal and topographical trends of the soil environment, denitrifier and nitrifier communities, and denitrification activity for the area of study.

4.1. Spatio-temporal variation of denitrification rates, denitrifier and nitrifier communities, and relevant soil characteristics

Most of the measured soil and biological characteristics did not differ significantly between the two main topographical positions located 40 to 80 m apart; however, several differences were observed between the upper two sites and the footslope, which also varied in crop management strategy. Differences in the *nirS* gene by slope was observed by Dandie et al. (2011), although *nirK* was not sensitive to spatial differences. Although the microbial community sizes in the current study were similar between the two main topographical positions, the DEA rates were significantly greater at the backslope than the shoulder. Similarly, van Kessel et al. (1993) observed lower topographical features having greater DEA rates and the spatial relationship being consistent throughout the year.

The greatest DEA rates were observed in the driest samples of the summer season at both landscape scales and correspondingly, DEA rates were negatively correlated to gravimetric water content. The summer soil was wetted with the C and N solution at the start of the DEA incubation but was not brought up to field-capacity water content prior to the incubation. The elevated denitrification rates of the summer samples may have been in part due to the wetting of dry soil in the DEA assay, as the rewetting of soil has been associated with a rapid increase in microbial activity (Orchard and Cook, 1983); however, this cannot explain that BD rates were also significantly greater in summer samples across the shoulder and backslope, since no solution was added to the soil for the BD incubation. Also greatest in summer soils were denitrifier community size and denitrifier specific activity, indicating that enhanced summer DEA rates may also be attributable to denitrifier population growth and increased vigor of the denitrifier community. Similar increases in the abundance of denitrifier abundance in summer compared to spring or fall samplings of agricultural soils has been observed based on quantification of *nosZ*, *cnorB_B* and *cnorB_P* genes (Dandie et al., 2008), although *nirK* abundance was greatest in the spring. The significant correlation of the denitrifiers (based on *nirK+nirS*) to total C in the current study suggests that denitrifier population growth and activity in summer soils may be related to increased concentrations of soil soluble NPOC and total C. Both C forms are generally correlated with other available nutrients and microbial activity in soil (Doran, 1987). In contrast, inorganic N was least in summer, suggesting that it may have a lesser role than C in influencing denitrification rates or that turnover may be rapid enough that inorganic N concentration is a poor indication of substrate availability.

Other seasonal trends in denitrification rates are likely due to the influence of soil characteristics. Denitrification rates were lowest in winter, as was denitrification specific activity. The lower

denitrification rates and denitrification specific activity may be due to less soluble NPOC and total soluble N in the winter, although soil inorganic N was greater in winter than in spring and summer. Denitrifier and nitrifier populations are known to be sensitive to pH (Čuhel et al., 2010; Gubry-Rangin et al., 2011; Hu et al., 2013). In autumn, when the soil pH was the greatest in our study, an incongruence between denitrification rates and denitrifier abundance occurred. This disconnect between activity and community size may be the result of certain, sensitive denitrifiers not measured in this study being adversely affected by a greater pH. For example, fungal denitrifiers were not accounted for with the specific nitrite reductase primers utilized.

The same seasonal trend in abundance was observed for both *nirK* and *nirS* populations across the shoulder and backslope, from greatest to lowest: summer, winter, spring and autumn. With the footslope included *nirK* peaks in the winter instead of the summer, but otherwise the two populations follow the same trend as seen at the smaller landscape scale. In contrast, Dandie et al. (2008) found that seasonal trends differed among denitrifier populations studied (specifically, *nirK*, *nosZ*, *cnorB_B*, and *cnorB_P*). The seasonal trends of nitrifier AOA and AOB abundance were also similar to each other, as both populations were greater in the autumn and winter compared to the spring and summer at both landscape scales. Although seasonal differences were not significant for AOA and AOB populations in our study, Taylor et al. (2012) found significant seasonal differences for AOA and AOB population size in soils cropped to wheat in western Oregon with the lowest abundances occurring in January. The similarity in the seasonal trends for each of the two populations of nitrifiers and denitrifiers suggests that each are influenced by the same seasonal factors, despite large genetic and evolutionary differences.

4.2. Niche differentiation of the denitrifier and nitrifier communities

In our study, *nirS* abundance was typically an order of magnitude greater than *nirK* abundance, regardless of season or topographical position. Other studies find different relationships between the relative abundance of *nirS* and *nirK*, yet the factors that cause one group to become predominant over the other remain unclear. Greater *nirS* abundance compared to *nirK* abundance have also been observed in boreal water-logged soils (Petersen et al., 2012), unfertilized fallow soils (Hallin et al., 2009), and overwintering pasture soils (Chroňáková et al., 2009). The range of *nirK* abundance in our study was lower than that observed in other agricultural studies (Dandie et al., 2008; Yoshida et al., 2009; Chen et al., 2010; Attard et al., 2011; Dandie et al., 2011), while the range of *nirS* abundance was comparable to other agricultural studies including values observed in a rice paddy soil (Chen et al., 2010).

The relative size of the two nitrifier groups also exhibited a consistent pattern across the field of study, with AOB abundance consistently greater than AOA abundance across each season and topographical position. The moderately acidic soils in our study may have contributed to the dominance of AOB over AOA, as other studies have found acidic soil pH to negatively impact AOA abundance disproportionately to AOB abundance (Hallin et al., 2009; Bru et al., 2011). However, Nicol et al. (2008) found the opposite trend, with AOA abundance increasing and AOB abundance decreasing with decreasing soil pH, so it is inconclusive whether soil acidity was responsible for the dominance of AOA abundance in this study. The range of AOB and AOA population size was similar to that observed by Hallin et al. (2009) in soils of similar pH from a 50-year-old fertilization experiment in Ultuna, Sweden.

4.3. Influence and relative importance of denitrification biological predictors

Studies differ as to whether a significant correlation exists between denitrifier abundance and denitrification rates. However, biological variables may be a useful predictor of denitrification rates when the relative influence of different environmental variables becomes complicated from spatio-temporal variation. In this study, addition of the footslope position to the shoulder and backslope increased both the variation in field scale and crop management strategy.

At the larger landscape scale, a greater number of significant correlations and predictive relationships were observed between denitrification population size and denitrification rates. For example, the *nirK* abundance correlated to DEA rates only when footslope samples are included in the analysis. Further, the additional landscape variability almost doubled the predictive power of the *nirS* abundance in the proposed driver SMR model (increase in R^2 of 0.07 to 0.16 from 2- to 3-landscape positions) and was only included as a significant variable in the greatest R^2 model when the footslope was included. Similarly, for BD rates a biological measurement (the *nirK+nirS* abundance) was a significant explanatory variable only at the larger landscape scale. Regardless of scale, nitrifier population size was not a significant variable in any SMR model.

Despite the improvement of the predictive ability of biological measurements at larger landscape scales, it is important to note their limitations within the scope of our study. In all of the SMR models, denitrifier population size was never a primary explanatory variable, and instead gravimetric water content, soluble NPOC, soluble total N or $\text{NO}_3\text{-N}$, or $\text{NH}_4\text{-N}$ explained more variance of DEA and BD rates. Our study indicates that measurements of soil moisture and C and N availability are stronger predictors of denitrification rates than measurements of denitrifier population size. Similarly, Attard et al. (2011) found soil measurements to be better predictors of denitrification rates than denitrifier abundance, as the authors found *nirK* abundance to be a significant explanatory variable of potential denitrification rates if it was added first to a multiple

regression analysis, but *nirK* abundance became insignificant as an explanatory variable if soil environmental measurements such as water-filled pore space, soil organic C, or NO₃-N, were added to the model first. In addition, Jusselme et al. (2016) studied the temporal changes in DEA for two locations in subalpine grasslands. Denitrifier abundance only explained 1.6 % or 17.5 % of DEA variations for a single location, but explained 21 % of variation across both locations. In contrast, Petersen et al. (2012) found *nosZ* abundance to be the primary predictor of potential denitrification rates by both stepwise multivariate regression and path analysis, with *nirK+nirS* abundance the primary predictor of *nosZ* abundance according to their path analysis. Potential denitrification rates varied over 200-fold among soils in Petersen et al. (2012) – much greater than the over 60-fold variation measured across all samples in our study, which is similar in range to that found in Attard et al. (2011).

For our study, the variables of soluble NPOC, total soluble N and *nirS* abundance were identified as the best set of predictors in the proposed driver model for DEA rates, consistently producing robust predictive models at both landscape scales. Although greater landscape variation improved the R^2 values of the *nirS* gene abundance and total soluble N, the predictive power of soluble NPOC was greatly reduced by greater landscape variability therefore reducing the overall power of the proposed driver model. In contrast, the greatest- R^2 model of BD rates was improved by greater landscape variation and demonstrated that soil N and denitrifier abundance are also important predictors in substrate limiting conditions.

Conclusion

Our study indicated that the abundances of the denitrifier and nitrifier groups studied differ seasonally and exhibit similar seasonal trends within their functional groups, indicating that these

functional groups respond similarly to seasonal environmental changes despite major genetic differences. Seasonality was observed for the specific activity of denitrifiers, indicating that the vigor of the denitrifier community changes throughout the year and/or the measured *nirS* and *nirK* abundance does not fully account for the active denitrifier community. We hypothesized that denitrifier population size would be a significant predictor of denitrification rates at the field scale when seasonal and topographical variation was included. Although we found denitrifier abundance to be a significant explanatory variable of denitrification rates, especially at our broader landscape scale, denitrifier abundance was not as useful a predictor as measurements of soil chemical and environmental characteristics. Measurements of soil moisture, C and N availability are likely the most suitable predictors of denitrification rates. However, our study gives evidence that greater landscape variation results in more observed significant correlations and predictive relationships between denitrifier abundance and denitrification rates, indicating that biological measurements of the denitrifier community are likely important and useful predictors of denitrification rates at larger scales.

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Figure 1. Denitrification enzyme activity (DEA) and basal denitrification (BD) rates by season for backslope and shoulder (a) and for backslope, shoulder and footslope (b). The footslope is excluded in panel a because it was under a different crop (winter wheat) than the backslope and shoulder (spring wheat); it is included in panel b to portray greater landscape variation. Values given are arithmetic mean ($n = 12$ for panel a, $n = 18$ for panel b). Error bars are SE. Data sharing the same letter and letter case are not statistically different in each analysis ($P < 0.05$). Note the difference scale for each measurement. DEA and BD were not analyzed for significant statistical differences in panel b due to an interaction between season and topography.

Figure 2. Seasonal variation of *nirK* and *nirS* abundance (panels a,b), ammonia-oxidizing bacteria (AOB) and archaea (AOA) abundance (c,d), and denitrifier specific activity (e,f) for backslope and shoulder (a,c,e) and for backslope, shoulder and footslope (b,d,f). The footslope is excluded in panels a, c and e because it is under a different crop (winter wheat) than the backslope and shoulder (spring wheat); it is included in panels b,d, and f to portray greater landscape variation. Values given are arithmetic mean ($n = 12$ for panels a and e, $n = 6-12$ for panel c, $n = 18$ for panels b and f, $n = 12-18$ for panel d). Data sharing the same letter and letter case in each panel are not statistically different ($P < 0.05$). Error bars are SE. Measurements of *nirK*, *nirS*, AOB and AOA were not analyzed for significant statistical differences in panels b and d due to an interaction between season and topography.

Figure 3. Topographical variation of denitrification enzyme activity (DEA) and basal denitrification (BD) rates (panel a), *nirK* and *nirS* abundance (b), ammonia-oxidizing bacteria (AOB) and archaea (AOA) abundance (c), and denitrifier specific activity (d). The footslope is excluded from statistical analysis because the footslope was under a different crop (winter wheat) than the backslope and shoulder (spring wheat). The BD rates exclude summer values because the summer values measure an unnatural soil condition of anaerobic dry soil. Values given are arithmetic mean ($n = 24$ for panels a, b, and d; $n = 18-23$ for panel c). Data sharing the same letter case in each panel (uppercase for DEA in panel a, *nirK* in panel b, and AOB in panel c; lowercase for BD in panel a, *nirS* in panel b, AOA in panel c and denitrifier specific activity in panel d) are not statistically different ($P < 0.05$). Error bars are SE.

Table 1. Seasonal variation of selected soil characteristics averaged across the shoulder and backslope positions.

Season	Gravimetric water content	Soluble NPOC	Total soluble N	NO ₃ - N ^a	NH ₄ - N	Total N	Total C	pH	Electrical conductivity
	(%)	(mg kg ⁻¹)	(mg kg ⁻¹)	(mg kg ⁻¹)	(mg kg ⁻¹)	(%)	(%)		(dS m ⁻¹)
Autumn	28.9 ± 1.0 b	45 ± 4 b	11.9 ± 2.5 ab	0.5 ± 0.3	3.4 ± 0.9 a	0.19 ± 0.01 a	2.3 ± 0.2 b	5.9 ± 0.2 a	0.20 ± 0.02 a
Winter	33.8 ± 1.0 a	33 ± 4 c	6.2 ± 0.8 b	1.4 ± 0.1	1.0 ± 0.1 a	0.19 ± 0.01 a	2.5 ± 0.1 ab	5.3 ± 0.2 b	0.18 ± 0.02 a
Spring	23.0 ± 1.5 c	40 ± 6 bc	19.8 ± 5.9 ab	0.1 ± 0.0	0.3 ± 0.2 b	0.18 ± 0.01 a	2.4 ± 0.1 ab	5.2 ± 0.1 b	0.19 ± 0.03 a
Summer	3.3 ± 0.1 d	204 ± 64 a	15.2 ± 1.5 a	0.0 ± 0.0	0.0 ± 0.0 c	0.18 ± 0.00 a	2.9 ± 0.1 a	5.3 ± 0.1 b	0.19 ± 0.02 a

^aThe NO₃-N concentration was not analyzed for significant statistical differences due to an interaction between season and topographical position.

Values given are arithmetic mean ± standard error ($n = 7-12$). Values sharing the same letter in the column are not significantly different at $P < 0.05$. Soluble NPOC = Soluble non-purgeable organic C.

Table 2. Seasonal variation of selected soil characteristics averaged across the shoulder, backslope and footslope positions.

Season	Gravimetric water content	Soluble NPOC	Total soluble N	NO ₃ - N	NH ₄ - N	Total N ^a	Total C	pH	Electrical conductivity
	(%)	(mg kg ⁻¹)	(mg kg ⁻¹)	(mg kg ⁻¹)	(mg kg ⁻¹)	(%)	(%)		(dS m ⁻¹)
Autumn	30.6 ± 1.0	41 ± 3	26.9 ± 6.0	4.4 ± 1.1	6.7 ± 1.2	0.19 ± 0.01 a	2.3 ± 0.1	6.0 ± 0.1	0.20 ± 0.03
Winter	35.6 ± 1.3	32 ± 3	6.9 ± 0.6	6.8 ± 0.5	1.3 ± 0.1	0.19 ± 0.01 a	2.5 ± 0.1	5.3 ± 0.1	0.17 ± 0.02
Spring	22.0 ± 1.2	41 ± 5	14.5 ± 4.3	0.4 ± 0.1	0.3 ± 0.2	0.19 ± 0.01 a	2.6 ± 0.1	5.2 ± 0.1	0.16 ± 0.02
Summer	3.4 ± 0.2	194 ± 42	16.8 ± 1.5	0.1 ± 0.0	0.1 ± 0.0	0.19 ± 0.01 a	3.3 ± 0.2	5.2 ± 0.1	0.20 ± 0.01

^aValues sharing the same letter in the column are not significantly different at $P < 0.05$.

Values given are arithmetic mean ± standard error ($n = 13-18$). With the exception of total N, an interaction between season and topography or insufficient normalization after transformation ($P < 0.05$, Anderson-Darling test) prevented statistical comparison. Footslope samples were included in these averages to provide additional landscape variation. The footslope was under a different crop in addition to being an additional topographical position. Soluble NPOC = Soluble non-purgeable organic C.

Season and topographic position	Gravimetric water content (%)	Soluble NPOC (mg kg ⁻¹)	Total soluble N (mg kg ⁻¹)	NO ₃ ⁻ N (mg kg ⁻¹)	NH ₄ ⁻ N ^a (mg kg ⁻¹)	Total Inorganic N ^b (mg kg ⁻¹)	Total N (%)	Total C (%)	pH ^c	Electrical conductivity ^a (dS m ⁻¹)
Autumn										
Shoulder	29.5 ± 1.6 bc	44 ± 4 ab	11.6 ± 3.4 bc	0.1 ± 0.0 bc	2.8 ± 0.9	3.7 ± 1.1 b	0.20 ± 0.02 a	2.5 ± 0.2 b	6.1 ± 0.3 bc	0.22 ± 0.04
Backslope	28.3 ± 1.3 bc	47 ± 6 ab	12.2 ± 4.0 bc	1.2 ± 0.5 a	4.2 ± 1.7	6.2 ± 2.6 ab	0.17 ± 0.01 a	2.2 ± 0.2 b	5.6 ± 0.1 bc	0.18 ± 0.02
Footslope	34.1 ± 1.1 ab	32 ± 5 b	56.8 ± 8.0 a	1.5 ± 0.3 a	8.5 ± 1.3	10.0 ± 1.3 a	0.19 ± 0.01 a	2.1 ± 0.1 b	6.2 ± 0.1 c	0.40 ± 0.01
Winter										
Shoulder	33.8 ± 1.5 ab	35 ± 5 ab	6.3 ± 1.4 bc	1.4 ± 0.2 a	0.9 ± 0.1	2.3 ± 0.1 ab	0.19 ± 0.01 a	2.5 ± 0.1 b	5.5 ± 0.4 abc	0.21 ± 0.03

Backslope	33.8 ± 1.4 ab	31 ± 6 b	6.1 ± 0.8 bc	1.3 ± 0.2 a	1.2 ± 0.1	2.5 ± 0.3 ab	0.19 ± 0.01 a	2.5 ± 0.2 b	5.1 ± 0.1 abc	0.15 ± 0.03
Footslope	39.2 ± 3.0 a	30 ± 4 b	8.2 ± 0.7 bc	1.8 ± 0.2 a	1.0 ± 0.2	2.8 ± 0.3 ab	0.19 ± 0.01 a	2.4 ± 0.2 b	5.3 ± 0.1 abc	0.16 ± 0.03
Spring										
Shoulder	25.4 ± 1.1 cd	30 ± 4 b	15.0 ± 4.4 bc	0.1 ± 0.0 b	0.2 ± 0.1	0.3 ± 0.2 c	0.18 ± 0.01 a	2.5 ± 0.1 b	5.3 ± 0.1 abc	0.19 ± 0.03
Backslope	20.7 ± 2.4 d	51 ± 11 ab	24.5 ± 11.1 b	0.1 ± 0.0 b	0.5 ± 0.4	0.6 ± 0.5 c	0.18 ± 0.01 a	2.4 ± 0.2 b	5.1 ± 0.1 a	0.19 ± 0.05
Footslope	19.8 ± 1.8 d	43 ± 6 ab	3.9 ± 0.9 c	0.0 ± 0.0 d	0.1 ± 0.0	0.1 ± 0.0 d	0.21 ± 0.01 a	3.1 ± 0.2 ab	5.2 ± 0.2 ab	0.10 ± 0.01
Summer										
Shoulder	3.4 ± 0.2 e	261 ± 18 a	17.0 ± 2.7 bc	0.0 ± 0.0 cd	0.0 ± 0.0	0.0 ± 0.0 d	0.18 ± 0.01 a	2.8 ± 0.2 b	5.3 ± 0.1 abc	0.22 ± 0.02
Backslope	3.1 ± 0.2 e	147 ± 12 a	13.5 ± 1.2 bc	0.0 ± 0.0 d	0.0 ± 0.0	0.0 ± 0.0 d	0.18 ± 0.01 a	3.0 ± 0.2 b	5.3 ± 0.0 abc	0.16 ± 0.01
Footslope	3.8 ± 0.5 e	174 ± 13 a	19.8 ± 2.9 b	0.0 ± 0.0 cd	0.1 ± 0.0	0.1 ± 0.0 d	0.22 ± 0.01 a	4.0 ± 0.4 a	5.0 ± 0.1 a	0.22 ± 0.03

Table 3. Seasonal and topographical variation of selected soil characteristics.

^aThe NH₄-N concentration and electrical conductivity were not analyzed for significant statistical differences because these data sets were not normalized sufficiently ($P < 0.05$, Anderson-Darling test) by transformation.

^bTotal inorganic N is the sum of NH₄-N and NO₃-N concentration.

^cSignificance letters for pH were calculated from concentration of hydronium ion.

Values given are arithmetic mean $\pm$ standard error ($n = 3-6$). Values sharing the same letter within each column are not significantly different at $P < 0.05$. Soluble NPOC = Soluble non-purgeable organic C.

Table 4. Seasonal and topographical variation of denitrification rates, denitrifier specific activity, denitrifier and nitrifier population size.

Season and topographic position	BD ng N ₂ O-N h ⁻¹ g ⁻¹	DEA ng N ₂ O-N h ⁻¹ g ⁻¹	<i>nirK</i> 10 ⁶ copies g ⁻¹	<i>nirS</i> 10 ⁶ copies g ⁻¹	<i>nirK+nirS</i> 10 ⁶ copies g ⁻¹	AOB 10 ⁶ copies g ⁻¹	AOA 10 ⁶ copies g ⁻¹	AOA+AOB 10 ⁶ copies g ⁻¹
Autumn								
Shoulder	0.7 ± 0.3 bcd	210 ± 40 de	0.5 ± 0.1 cd	6.5 ± 0.8 cd	7.1 ± 0.9 cd	0.6 ± 0.1 c	0.5 ± 0.3 ab	1.1 ± 0.2 cd
Backslope	1.5 ± 0.9 bcd	230 ± 20 cde	0.5 ± 0.1 d	4.8 ± 2.3 d	5.3 ± 2.5 d	2.0 ± 0.8 abc	BDL ^a	BDL ^a
Footslope	12.0 ± 5.7 a	500 ± 80 bc	1.2 ± 0.2 cd	16.1 ± 3.0 cd	17.3 ± 3.2 cd	5.6 ± 1.4 ab	0.9 ± 0.4 a	6.5 ± 1.3 ab
Winter								
Shoulder	0.1 ± 0.1 d	70 ± 20 f	0.8 ± 0.2 cd	10.6 ± 3.4 cd	11.4 ± 3.6 cd	3.5 ± 1.4 abc	1.2 ± 0.7 ab	6.0 ± 2.7 abc
Backslope	0.0 ± 0.0 d	100 ± 20 ef	1.2 ± 0.2 cd	17.5 ± 2.9 bcd	18.7 ± 3.0 bcd	1.3 ± 0.2 bc	0.1 ± 0.0 b	1.4 ± 0.2 cd
Footslope	0.0 ± 0.0 d	330 ± 80 cd	3.6 ± 0.7 a	36.5 ± 7.7 a	40.1 ± 8.4 a	6.9 ± 1.1 a	0.7 ± 0.3 a	7.6 ± 1.4 a
Spring								
Shoulder	3.2 ± 1.88 abc	130 ± 20 def	0.9 ± 0.2 cd	10.9 ± 1.4 cd	11.8 ± 1.6 cd	1.3 ± 0.5 c	0.1 ± 0.0 ab	1.3 ± 0.6 cd
Backslope	0.4 ± 0.19 cd	150 ± 20 de	0.9 ± 0.1 cd	7.9 ± 1.1 cd	8.8 ± 1.1 cd	1.4 ± 0.3 c	0.2 ± 0.1 ab	1.5 ± 0.3 cd
Footslope	0.2 ± 0.11 cd	180 ± 30 de	1.4 ± 0.2 c	13.3 ± 2.7 cd	14.7 ± 2.9 cd	0.7 ± 0.1 c	0.2 ± 0.1 ab	0.8 ± 0.2 d
Summer								
Shoulder	3.6 ± 0.55 ab	1080 ± 130 ab	1.0 ± 0.1 cd	19.5 ± 1.7 bcd	20.5 ± 1.7 bcd	1.4 ± 0.2 abc	0.6 ± 0.4 ab	2.0 ± 0.4 bcd
Backslope	3.2 ± 0.42 ab	1270 ± 50 a	1.5 ± 0.2 bc	22.8 ± 3.0 abc	24.3 ± 3.1 abc	1.3 ± 0.4 bc	0.1 ± 0.0 ab	1.5 ± 0.4 cd

Footslope	3.8 ± 0.40	1240 ±	3.0 ± 0.5	32.1 ±	35.1 ±	1.3 ± 0.4	0.2 ± 0.0	1.5 ± 0.4
	ab	140 a	ab	4.7 ab	5.1 ab	abc	ab	cd

^aFive out of six replicates were below detection level (BDL).

Values given are arithmetic mean ± standard error ($n = 4-6$). Values sharing the same letter in the column are not significantly different at $P < 0.05$. BD=basal denitrification, DEA=denitrification enzyme activity, AOB=ammonia-oxidizing bacteria, AOA=ammonia-oxidizing archaea.

Table 5. Stepwise multivariate regression of denitrification enzyme activity (DEA) rates across all seasons, with and without inclusion of footslope samples.

Shoulder and backslope			Shoulder, backslope and footslope		
Explanatory	<i>P</i>	<i>R</i> ²	Explanatory	<i>P</i>	<i>R</i> ²
Greatest <i>R</i>² (<i>n</i> = 47)			Greatest <i>R</i>² (<i>n</i> = 72)		
Gravimetric water	<.0001	0.68	Gravimetric water	<0.0001	0.39
Total C	0.0010	0.07	Total soluble N	<0.0001	0.14
pH ^b	0.0040	0.05	<i>nirS</i> abundance	0.0002	0.09
Total Model		0.79	Total Model		0.61
Proposed Drivers (<i>n</i> =			Proposed Drivers (<i>n</i> =		
Soluble NPOC	<.0001	0.59	Soluble NPOC	<0.0001	0.22
<i>nirS</i> abundance	0.0056	0.07	<i>nirS</i> abundance	<0.0001	0.16
Total soluble N	0.0148	0.05	Total soluble N	<0.0001	0.15
Total Model		0.70	Total Model		0.53

^aVariables are listed in the same order as entered into the model. The variable with the greatest *F*-statistic value was added at each step.

^bThe variable of pH was included as concentration of hydronium ion.

The first model is the greatest *R*² model developed from significant variables (*P* < 0.05). A second model was generated that included the proposed drivers of soluble NPOC, total soluble N and denitrifier abundance, where and if significant (*P* < 0.05). The SMR models were generated at two different landscape scales: shoulder and backslope; and shoulder, backslope and footslope. Soluble NPOC = Soluble non-purgeable organic C.

Table 6. Stepwise multivariate regression of basal denitrification (BD) rates.

Shoulder and backslope			Shoulder, backslope and footslope		
Explanatory	<i>P</i>	<i>R</i>²	Explanatory	<i>P</i>	<i>R</i>²
Greatest <i>R</i>² (<i>n</i> = 31)			Greatest <i>R</i>²/Proposed Drivers (<i>n</i> = 54)		
NO ₃ -N	0.0077	0.22	Total soluble N	<0.0001	0.43
			<i>nirK+nirS</i> abundance	0.0373	0.05
Total Model		0.22	Total Model		0.48

^aVariables are listed in the same order as entered into the model. The variable with the greatest *F*-statistic value was added at each step.

Summer samples were excluded from the stepwise multivariate regression model of BD rates because they were unrepresentative of naturally occurring soil conditions (dry soil under anaerobic conditions). The SMR models were generated at two different landscape scales: shoulder and backslope; and shoulder, backslope and footslope. The shoulder and backslope SMR model using proposed drivers only (soluble NPOC, total soluble N and denitrifier abundance) did not have any variables significant enough to be included ($P < 0.05$). The shoulder, backslope and footslope SMR model shown is the greatest R^2 model developed from significant variables ($P < 0.05$), which also is composed entirely of the proposed drivers.