environmental microbiology

Environmental Microbiology (2012) **14**(9), 2601–2613 doi:10.1111/j.1462-2920.2012.02814.x

Spatially tripartite interactions of denitrifiers in arctic ecosystems: activities, functional groups and soil resources

Samiran Banerjee and Steven D. Siciliano*
Department of Soil Science, University of Saskatchewan, Saskatoon, Canada S7N 5A8.

Summary

Soil denitrification is one of the most significant contributors to global nitrous oxide (N_2O) emissions, and spatial patterns of denitrifying communities and their functions may reveal the factors that drive denitrification potential and functional consortia. Although denitrifier spatial patterns have been studied extensively in most soil ecosystems, little is known about these processes in arctic soils. This study aimed to unravel the spatial relationships among denitrifier abundance, denitrification potential and soil resources in 279 soil samples collected from three Canadian arctic ecosystems encompassing 7° in latitude and 27° in longitude. The abundance of *nirS* (10^6–10^8 copies g^{-1} dry soil), *nirK* (10^3-10^7 copies g^{-1} dry soil) and *nosZ* (10^6–10^7 copies g^{-1} dry soil) genes in these soils is in the similar range as non-arctic soil ecosystems. Potential denitrification in Organic Cryosols (1034 ng N_2O-N g^{-1} soil) was 5–11 times higher than Static/Turbic Cryosols and the overall denitrification potential in Cryosols was also comparable to other ecosystems. We found denitrifier functional groups and potential denitrification were highly spatially dependent within a scale of 5 m. Functional groups and soil resources were significantly ($P < 0.01$) correlated to potential denitrifier activities and the correlations were stronger in Organic Cryosols. Soil moisture, organic carbon and nitrogen content were the predominant controls with *nirK* abundance also linked to potential denitrification. This study suggests that the dominant control on arctic ecosystem-level denitrification potential is moisture and organic carbon. Further, microbial abundance controls on ecosystem level activity while undoubtedly present, are masked in the nutrient-poor arctic environment by soil resource control on denitrifier ecosystem level activity.

Received 28 June, 2011; revised 11 May, 2012; accepted 14 May, 2012. *For correspondence. E-mail steven.siciliano@usask.ca; Tel. (+1) 306 966 4035; Fax (+1) 306 966 6881.

Introduction

Nitrous oxide is a potent greenhouse gas with a global warming potential 298 times higher than CO_2 (IPCC, 2007). Soils play a key role in N_2O dynamics by contributing up to 90% of the world's total N_2O emissions and in soil nearly 70% of N_2O emissions originate from microbial transformations such as nitrification and denitrification (Mosier *et al.*, 1998). Although limited information is available about N_2O fluxes in arctic ecosystems, a recent review suggests that arctic soils contribute 0.49 kg ha^{-1} annually to world N_2O release, which is similar to emissions from temperate grassland, boreal forest, or desert ecosystems (Dalal and Allen, 2008). Arctic ecosystems comprise about 13% of the Earth's and 40% of Canada's total land area; these ecosystems contain 25% of the world's total soil organic matter pool (Bockheim and Tarnocai, 1998; Tarnocai *et al.*, 2009) and could release enormous amounts of greenhouse gases to the atmosphere. Arctic soils are predominantly in the Cryosolic Order (Soil Classification Working Group, 1998), soils having one or more *cryic* horizons (from Greek *kryos*, cold, ice; a perennially frozen soil horizon) within 2 m from the soil surface for 2 or more years in succession. Cryosols can be divided into three great groups: Static Cryosols, Turbic Cryosols and Organic Cryosols, with Static and Turbic referring to the degree of cryoturbation (Bockheim and Tarnocai, 1998). Although both Static and Turbic Cryosols have developed on mineral deposits, Turbic Cryosols are largely affected by cryoturbation. Turbic Cryosols display unique cryopedogenic features and consist of permafrost within 2 m of soil surface. Mixed or disturbed horizons and displaced soil materials are the diagnostic features of this great group (Tarnocai and Bockheim, 2011). Static Cryosols have formed mainly in coarse-textured parent materials with permafrost layer within 1 m of the soil surface. Existence of patterned ground and small cryopedogenic structures such as banded fabrics and silt caps are common in these soils albeit cryoturbation is typically absent (Tarnocai and Bockheim, 2011). Organic Cryosols have formed on

organic parent materials with more than 17% organic matter content by weight and the organic horizon is greater than 40 cm thick (Soil Classification Working Group, 1998). These soils comprise humic (well-decomposed), mesic (moderately decomposed) and fibric (undecomposed) peat materials (Tarnocai and Bockheim, 2011).

Denitrification is a key regulator of soil inorganic N concentration and N losses from ecosystems via nitrate (NO_3^-) leaching and N_2O emissions. Denitrifier communities regulate N_2O production and consumption and as such, the abundance of denitrifier functional groups can serve as an essential basis for understanding N_2O dynamics in soils (Morales *et al*., 2010). Denitrification has been well-recognized for more than a century (Voorhees, 1902) and the denitrifier abundance and potential denitrification have been measured in various soil ecosystems including agricultural (Miller *et al*., 2008; Hallin *et al*., 2009; Philippot *et al*., 2009; Enwall *et al*., 2010; Morales *et al*., 2010), grassland (Miller *et al*., 2009; Keil *et al*., 2011), riparian (Rich and Myrold, 2004; Dandie *et al*., 2011) and forest (Bohlen *et al*., 2001; Levy-Booth and Winder, 2010) soils. However, we still know little about denitrifier abundance across Cryosols types and how they are linked to potential denitrifier activities and soil attributes in Canadian arctic ecosystems. The key determinants of denitrification in soil, known as proximal controls, are pH, oxygen, moisture, nitrate (NO_3^-) and organic carbon availability (Knowles, 1982; Wallenstein *et al*., 2006). These proximal factors influence soil denitrification primarily by controlling the denitrifier physiology but also by shaping abundance and composition of denitrifiers. Therefore, unravelling the relationships among denitrifier abundance, potential activities and soil attributes is central to our understanding of soil denitrification and N_2O dynamics.

Microbial spatial variability remained largely unexplored until the end of the 1980s (Parkin, 1987; Robertson *et al*., 1988). As a result of the intrinsic heterogeneity of soil resources, microbial abundance also varies spatially across multiple scales (Parkin, 1993). Microbial spatial dependency, in which similarity between samples declines with increasing inter-sample distance, occurs in agricultural (Bru *et al*., 2011), grassland (Nicol *et al*., 2003) and forest (Saetre and Baath, 2000) soils. Spatial heterogeneity in denitrifier communities and potential activities has also been examined in managed ecosystems (Parkin, 1987; Robertson *et al*., 1988; Philippot *et al*., 2009; Enwall *et al*., 2010). Arctic soils are highly heterogeneous (Banerjee *et al*., 2011a) and recent reports demonstrated high spatial autocorrelation and scale dependence of microbial communities in permafrost soil ecosystems (Banerjee *et al*., 2011b; Banerjee and Siciliano, 2012). However, to our knowledge, no study has elucidated spatial patterns of denitrifier abundance and activity in arctic soils. As described by Enwall and colleagues (2010), the spatial link between ecosystem properties and the microbial communities allows us to interpret how field-scale processes influence microbial communities. Thus, for arctic ecosystems, undergoing climate change and with the potential to release large quantities of greenhouse gases, the link between microbial spatial structure and soil resources will allow us to downscale climate change models to soil microbial systems.

This study aimed to characterize the spatial associations among denitrifier functional groups, potential denitrification and soil properties in three Canadian high arctic ecosystems. The three research sites selected in this study encompass 7° in latitude and 27° in longitude of the Canadian arctic. The sites comprise a polar oasis (Truelove Lowland), a typical Static/Turbic Cryosolic ecosystem (Simpson Lake) and an Organic Cryosolic ecosystem (Ross Point), and hence represent a large portion of the diversity of Canadian permafrost ecosystems. Specifically we tested the following hypotheses: (i) potential denitrification activity is higher in Organic Cryosols than Static or Turbic Cryosols; (ii) denitrifier abundance and functional potential are spatially dependent within a scale of 10 m in Cryosolic ecosystems; (iii) the relationships between denitrifier abundance, potential activities and soil attributes are more consistent in Organic Cryosols. Despite having high TOC content, Ross Point soils also have high moisture and NO_3^- content, and this uniqueness distinguishes these soils from Truelove Lowland and Simpson Lake (Banerjee *et al*., 2011a). Our first hypothesis was based on the higher functional potential of Organic Cryosols. In these ecosystems, soil properties and microbial abundance are spatially structured within 10 m and the largest spatial range is found in Ross Point soils (Banerjee *et al*., 2011a,b). Owing to this spatial nature of these ecohabitats, denitrifier abundance and denitrification processes may also exhibit similar patterns, which was the foundation of our second hypothesis. The Turbic Cryosols at Truelove Lowland and Simpson Lake will have different intrinsic cryopedogenic processes compared with Ross Point, which likely influences the correlation patterns in Truelove Lowland and Simpson Lake. Our third hypothesis was based on the lack of heterogeneity in Organic Cryosols of Ross Point.

Results

Denitrifier potential activity and functional guilds in arctic soils

Potential denitrification activities varied significantly ($P < 0.05$) among three Cryosolic ecosystems with greatest activity at Ross Point, the Organic Cryosols (Fig. 1A). Ross Point soils contain higher moisture, organic matter

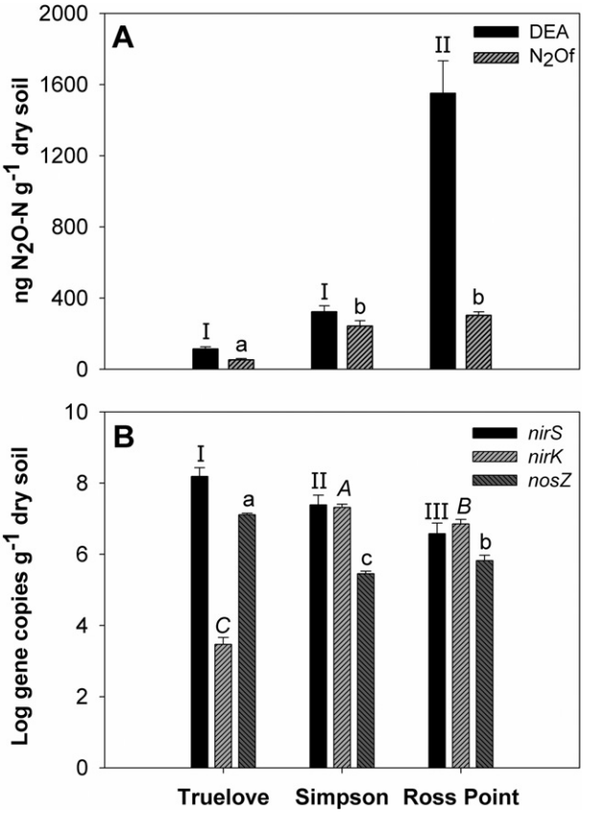

Fig. 1. A. Denitrifying enzyme (DEA) and N_2O reductase (N_2Of) activity in three contrasting arctic soil ecosystems: Truelove Lowland, Simpson Lake and Ross Point.
B. Abundance of denitrifying functional genes (*nirS*, *nirK* and *nosZ*) in three arctic ecosystems. Different letters/symbols indicate statistical different at $P \leq 0.05$.

and nitrogen content than the other two sites (see Table S1). Soil moisture content at Ross Point was about 3 times higher than the other two arctic ecosystems. The lowest NO_3^- content was observed in Truelove Lowland soils (0.89 μg g^{-1} dry soil) whereas Simpson Lake had the lowest pH among the study sites. The NO_3^- and TN content at Ross Point were also as much as 3 times higher than Truelove Lowland and Simpson Lake. Expectedly, the Organic Cryosols of Ross Point was 5–13 times more enriched in TOC than Static/Turbic Cryosols. Owing to the higher moisture and nutrient content, the overall denitrification potential (DEA) in Ross Point soils (1034 ng N_2O-N g^{-1} dry soil) was 5–11 times higher than Simpson Lake (230 ng N_2O-N g^{-1} dry soil) and Truelove Lowland (92 ng N_2O-N g^{-1} dry soil) soils, which directly supports our first hypothesis that Organic Cryosols will have higher denitrifier potential activity. However, net N_2O formation (N_2Of) did not differ between Ross Point (206 ng N_2O-N g^{-1} dry soil) and Simpson Lake (177 ng N_2O-N g^{-1} dry soil), which had 5 times higher potential than Truelove Lowland soils (44 ng N_2O-N g^{-1} dry soil). The value of rN_2O at these three arctic sites ranged between 0.35 and 0.86 with the lowest rN_2O recorded in Ross Point soils (see Table S2). Similar to potential denitrification, functional gene abundance also varied significantly (Duncan Test; $P < 0.05$) among all three sites (Fig 1B). Highest abundance of *nirS* and *nosZ* was found in Truelove Lowland whereas Simpson Lake had the highest abundance of *nirK*. Abundance of *nirK* functional groups in Truelove Lowland soils was substantially lower than other denitrifier groups at all three sites.

Spatial patterns of potential denitrification and functional guilds

Spatial analysis revealed that denitrifier functional gene abundance in various Cryosols is spatially autocorrelated within a spatial scale of 5 m, (Fig. 2). The value of spatial dependence (SPD) ranges between 0.5 and 0.999, which demonstrates high spatial variance and low nugget (unexplained and stochastic variance) effects. In Truelove Lowland, denitrifiers exhibited different spatial behaviour with *nirS* gene being spatially independent, *nosZ* moderately dependent, and *nirK* highly dependent. Overall, gene abundance in Ross Point was highly spatially structured within a range between 1.8 m and 3.4 m. Similar to functional groups, potential denitrifier activities also operated within 5 m spatial scale at all three study sites (Fig. 3). Although the scale of spatial autocorrelation was small in Simpson Lake (~ 0.5 m), dependence was extremely high (SPD > 0.95). Ross Point had the largest scale (~ 4.6 m) of autocorrelation.

Factors driving denitrifier potential activity in arctic soils

Potential denitrifier activities, functional guilds and soil resources were significantly correlated ($P < 0.05$) with each other across the Canadian arctic but association among soil resources and denitrifier potential activity were considerably stronger than denitrifier abundance (see Table S3). Overall, the highest and most consistent correlations were observed in the Organic Cryosols of Ross Point, supporting the third hypothesis of this study. Since soil moisture and total organic carbon content were identified as the key driving factors, their relationships with denitrifier functions were further elucidated by regression analysis. Although significant, the correlations between potential denitrifier activities and gene abundances were comparatively weaker than soil resources and as such, they were not included in the regression analysis. Associations between TOC, moisture and denitrifier functions are highly significant ($P < 0.0001$) across the Cryosolic ecosystems (Fig. 4). In general, denitrifier potential activity is high in soils with both high TOC and moisture content. However, the nature (demonstrated by the

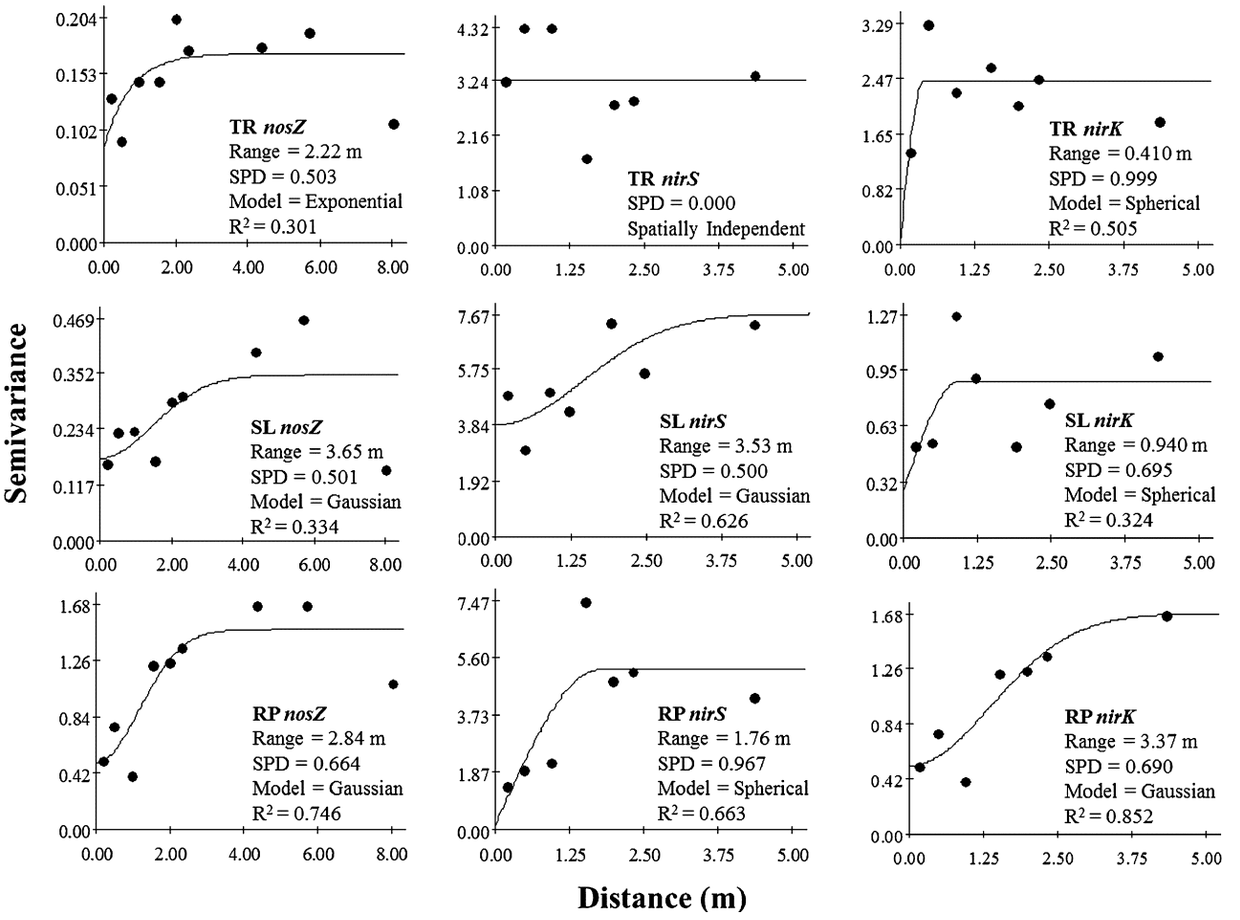

Fig. 2. Semivariograms showing spatial structure of denitrifier functional genes (*nosZ*, *nirS* and *nirK*) in three arctic soil ecosystems: Truelove Lowland (TR), Simpson Lake (SL) and Ross Point (RP). Range indicates the area of spatial dependence (SPD). Spatial dependence (SPD) was calculated as: SPD = $C/(C + C_0)$, where C is the structural variance, C_0 is the nugget, and $C + C_0$ is the sill. Values of SPD vary from 0 (no spatial dependence) to 1 (high spatial dependence). Various models (Gaussian, spherical and exponential) were fitted (solid line) to each semivariogram. Spatial dependency was considered from fine (10 cm) to large scale (300 m); the semivariograms are shown up to specific lag distance for clarity of spatial patterns near origin. Each point in the semivariogram represents the mean semivariance (dissimilarity) for a single lag class, which is a group of pairs separated by a specific lag distance.

response surface) of these associations varies considerably among three sites. Although the strength of correlation did not differ markedly between DEA and N_2Of and among the research sites, correlation was slightly stronger for DEA at Ross Point.

Discussion

Arctic soil ecosystems are often assumed as 'functionally inert' due to their slow turnover rate and thus very few studies have measured DEA in arctic soils (Chapin, 1996; Bjork *et al.*, 2007). Our results suggest that these ecosystems possess strong denitrification potential and this is particularly true for Organic Cryosols. The denitrifier potential activities in Organic Cryosols are in the same range as non-arctic soil ecosystems such as agricultural (Cavigelli and Robertson, 2000; Enwall *et al.*, 2010), grassland (Miller *et al.*, 2009; Philippot *et al.*, 2009; Cuhel *et al.*, 2010), and riparian and creek (Rich and Myrold, 2004), whereas DEA and N_2Of activities in Truelove Lowland and Simpson Lake were lower than agricultural or pristine soil ecosystems. The DEA in Ross Point (Organic Cryosols) is in the similar range as in heath snowbed whereas DEA in Truelove Lowland and Simpson Lake was comparable to dry heath soils measured in Northern Sweden (Bjork *et al.*, 2007). In Truelove Lowland soils, Chapin (1996) found that denitrification activity is primarily driven by moisture and nitrate content; however, denitrification is comparatively higher in drier hummocks, which differs from the results presented in this study. Denitrification activity is typically higher in soils with high moisture and nitrate content (Knowles, 1982; Wal-

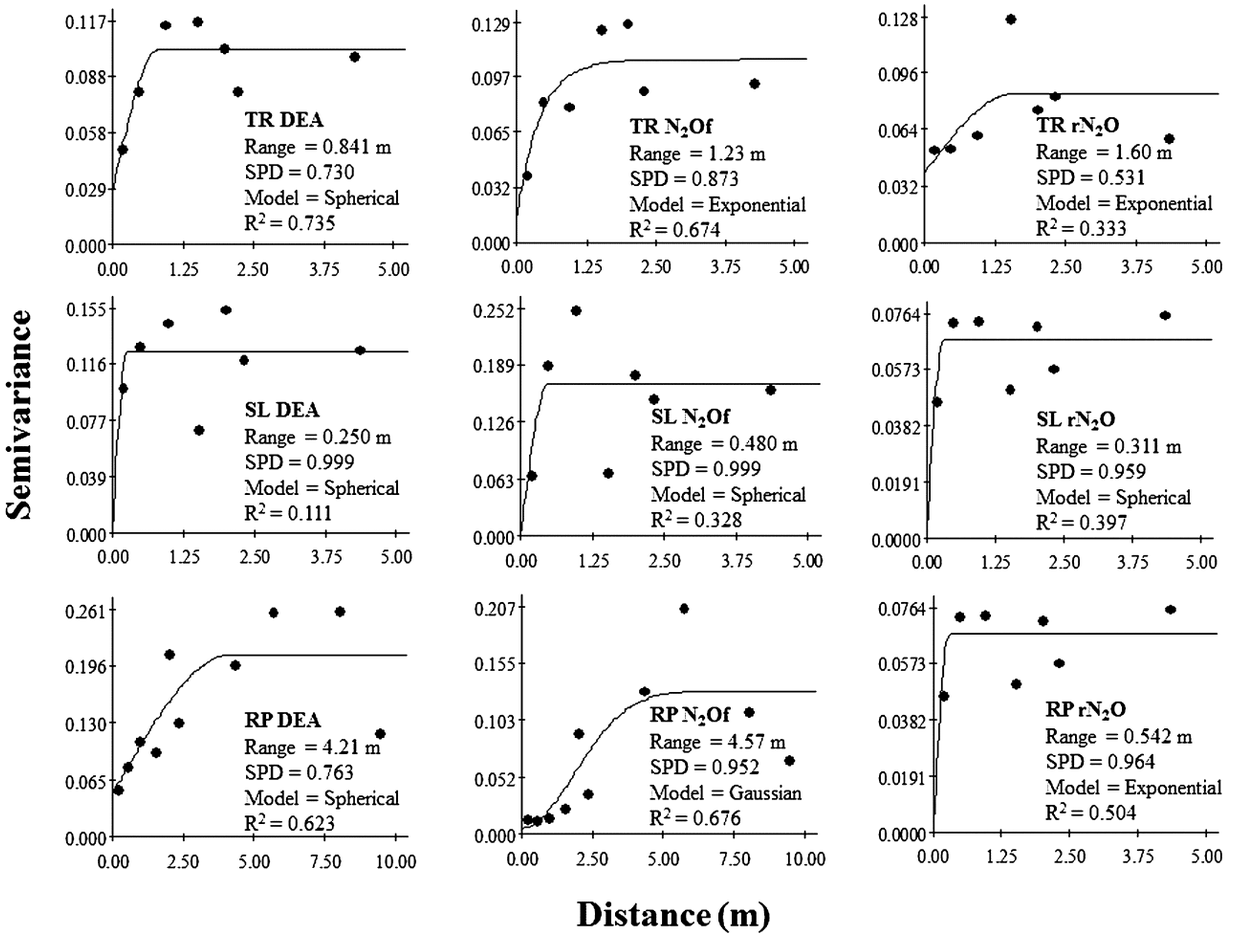

Fig. 3. Semivariograms exhibiting spatial structure of denitrifier activity (DEA, N_2Of and the ration of N_2Of to DEA i.e. rN_2O) in three arctic soil ecosystems: Truelove Lowland (TR), Simpson Lake (SL) and Ross Point (RP). Range indicates the area of spatial dependence (SPD). Spatial dependence (SPD) was calculated as: $SPD = C/(C + C_0)$, where C is the structural variance, C_0 is the nugget, and $C + C_0$ is the sill. Values of SPD vary from 0 (no spatial dependence) to 1 (high spatial dependence). Various models (Gaussian, spherical and exponential) were fitted (solid line) to each semivariogram. Spatial dependency was considered from fine (10 cm) to large scale (300 m); the semivariograms are shown up to specific lag distance for clarity of spatial patterns near origin. Each point in the semivariogram represents the mean semivariance (dissimilarity) for a single lag class, which is a group of pairs separated by a specific lag distance.

lenstein *et al.*, 2006) and as such, high DEA activity in Organic Cryosols is expected. Ross Point soils have considerably high moisture, carbon, and nitrogen content and these nutrient-rich soils can be extremely dynamic. Higher nutrient and moisture content of Organic Cryosols in Ross Point is also reflected in the functional processes, such that the functional potential of these soils is considerably greater than Static/Turbic Cryosolic ecosystems of Truelove Lowland and Simpson Lake. This is also consistent with the findings of Banerjee and Siciliano (2012) who demonstrated significantly higher ammonia oxidation potential in Ross Point than the other two sites.

The abundance of denitrifying bacteria is usually assessed by quantifying functional genes i.e., genes encoding enzymes responsible for different steps of the denitrification pathway. The functional genes involved in denitrification are *narG*, *nirS*, *nirK*, *norB* and *nosZ* (Philippot, 2002). The *narG* gene can be found in both denitrifying and non-denitrifying organisms, and thus is not a suitable marker. Moreover, primers for *norB* are mainly designed for a single organism. Many *nirS* and *nirK* primers will also amplify sequences from non-denitrifying microorganisms and further, are not necessarily linked to N_2O emissions. However, given the difficulties associated with *norB* primers, *nirS* and *nirK* genes remain popular choices and have regularly been used for quantification of denitrifier abundance. In spite of the cryopedogenic processes in arctic, the overall abundance is also similar to other ecosystems such as grassland (Miller *et al.*, 2009; Keil *et al.*, 2011), agricultural (Miller *et al.*, 2008; Hallin *et al.*, 2009; Enwall *et al.*, 2010) and forest (Levy-Booth and Winder, 2010). The abundance of deni-

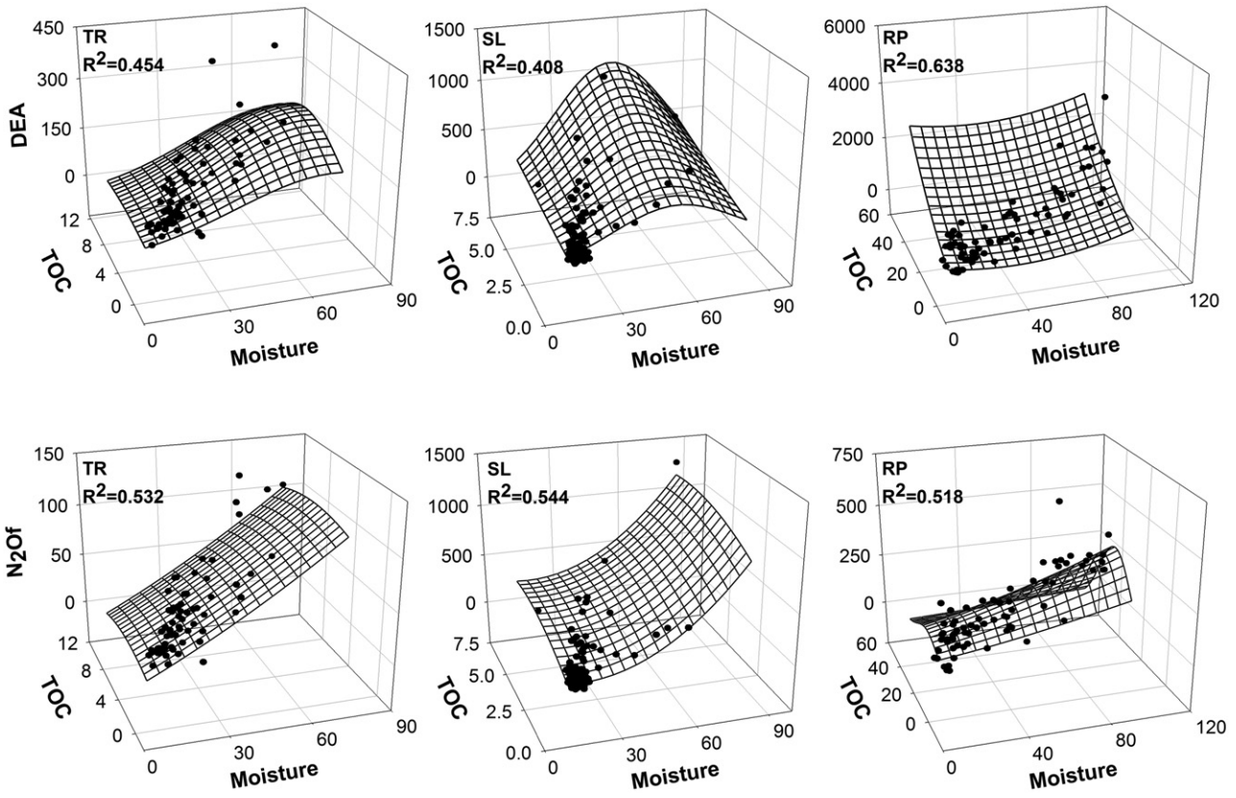

Fig. 4. Relationships among denitrifier potential activity, soil moisture and total organic carbon (TOC) content at three arctic ecosystems: Truelove Lowland (TR), Simpson Lake (SL) and Ross Point (RP). Multiple regression analysis was performed between soil resources and overall denitrification potential (DEA) and net N_2O formation (N_2Of). The pattern and strength of the associations are shown by response surface i.e. grid lines and R^2 respectively.

trifier functional genes in this study is similar to previous reports from arctic soils (Siciliano *et al*., 2009; Lamb *et al*., 2011). However, previous studies investigating denitrifier abundance in arctic soils focussed on a single research site and did not comprehensively elucidate abundance patterns in different types of Cryosols. Our results demonstrate that distribution of denitrifier functional groups varies significantly among the sites; and this difference was also observed when gene abundances were calculated per ng DNA (data not shown). In general, Truelove Lowland had high denitrifier abundance which is in agreement with the findings of Banerjee and colleagues (2011b) who found that overall bacterial biomass at Truelove Lowland (2.9×10^{10} copies g^{-1} dry soil) was considerably higher than at Simpson Lake (4.2×10^{8} copies g^{-1} dry soil) or Ross Point (1.5×10^{9} copies g^{-1} dry soil). Interestingly, Truelove soils had highest abundance of *nirS* and lowest abundance of *nirK* functional groups whereas Ross Point had similar abundance of all denitrifier groups. It should be noted that Truelove Lowland is strongly nitrogen limited with lowest nitrate content whereas Ross Point soils had highest nitrate content. It may indicate a possible niche-differentiation of nitrite reducing denitrifier groups in Static Cryosols due to nitrogen limitation whereas these groups are all equally present in nutrient rich Organic Cryosols. However, the patterns of abundance of nitrite reducing (*nirK* and *nirS*) and nitrous oxide reducing (*nosZ*) functional groups differed between at the three study sites. Others have also found that these groups differ in their distribution pattern in Cryosols, although these other reports were in glacier forefield soils which are considerably harsher environments than the one's studied here (Kandeler *et al*., 2006; Brankatschk *et al*., 2011). The *nosZ* primers selected in this study have been successfully used to examine *nosZ* communities in many soil microbiological studies (Rösch *et al*., 2002; Rich *et al*., 2003; Liu *et al*., 2010; Ma *et al*., 2011). However, the *nosZ* amplicons (700 bp) generated by these primers are longer than 500 bp and may not be ideal for qPCR work. Therefore, we corroborated *nosZ* abundance using primers designed by Henry and colleagues (2006) that yield comparatively short amplicons (267 bp). Both primer sets produced similar copy numbers with an r^2 value of 0.98 (Fig. S1).

The rN_2O (the ratio of N_2Of to DEA) indicates the rate of N_2O accumulation; it can be used to explain the balance

between N_2O formation and reduction. Low rN_2O indicates that high N_2O consumption by N_2O reductase whereas higher values indicate that relatively little N_2O reduction is occurring. Similar to Rich and Myrold (2004), we found rN_2O varied among the ecohabitats. Lower rN_2O is linked to greater soil carbon content while higher rN_2O is linked to lower pH (Firestone *et al*., 1980; Rich and Myrold, 2004). Because rN_2O is influenced by soil properties, it explains the lack of the strict congruence between gene abundance and activity. Ross Point, with the highest organic carbon had a much lower rN_2O compared with Truelove Lowland despite similar *nosZ* abundance; Truelove soils have one-fifth less organic carbon content than Ross Point. Similarly, the high rN_2O in Simpson Lake is linked to the differences in pH among the sites. The soils with high DEA activity did not necessarily have high N_2Of activity, which is consistent with the results of Philippot and colleagues (2011).

Scale of spatial patterns has significant implications for pedological and ecological sampling strategies and in this aspect geostatistics is highly useful. Geostatistics is a powerful statistical tool for elucidating microbial spatial variability. By focusing on the dissimilarity between data pairs as a function of distance (semivariance) and modelling spatial dependency, geostatistics estimates uncertainty and interpolate values at unsampled areas (Franklin and Mills, 2007). Overall, we found that denitrifier functional groups and potential denitrification in Cryosolic ecohabitats are highly spatially structured and their spatial dependence is in the same range as agricultural (Enwall *et al*., 2010) and grassland (Philippot *et al*., 2009; Keil *et al*., 2011) soils. Range of a semivariogram is a unique and key attribute, which indicates the zone of spatial dependence of a property. Inherent landscape patterns, soil processes and vegetation together regulate the spatial extent of autocorrelation. The spatial range of denitrifier functional guilds and activities were substantially smaller than what has been previously reported in agricultural ($>$ 200 m; Enwall *et al*., 2010) and grassland ($\sim$ 60 m; Keil *et al*., 2011) ecosystems. Arctic soils are usually less homogeneous than agricultural or grassland soils because micro-relief and microclimatic conditions contribute to considerable small-scale spatial heterogeneity that arises due to cryoturbation, irregular horizons and accumulation of organic and inorganic matter on the permafrost table (Bliss, 1977; Lev and King, 1999; Wagner, 2008). Cryoturbation, soil movement as a consequence of frost action, is a predominant cryopedogenic process occurring in permafrost ecosystems (Bockheim and Tarnocai, 1998) and is frequently accompanied by gelifluction, fissuring, frost stirring, mounding, patterned ground such as polygons, earth hummocks, nets, stripes, steps, sorted and non-sorted circles (Wagner, 2008). Thus, Cryosolic ecosystems are one of the most spatially heterogeneous soil habitats. Together, these mechanisms largely alter the spatial nature of soil properties, which is likely why the spatial scale in these arctic ecosystems is so much lower than that seen in temperate ecosystems. Supporting this idea that cryoturbation leads to lower spatial scales, the largest range of spatial dependence was found in Ross Point, an Organic Cryosols. Organic Cryosols are generally more homogenous and more consistent than Static or Turbic Cryosols and thus spatial similarity extends over longer distance. Nonetheless, a distinct similarity in spatial structure of denitrifier functional guilds and potential denitrification activity was detected in these soils. This suggests that in these ecosystems, DNA based estimations of gene abundances may reflect the functional potential of arctic denitrifier communities as has been seen in agricultural soils (Morales *et al*., 2010).

Denitrifier functional genes have been frequently linked to potential denitrifier activities, however, relatively weak and sometimes no correlations have been previously reported among soil resources and denitrifier abundance (Cuhel *et al*., 2010; Enwall *et al*., 2010). In this study, we found that denitrifier abundance was significantly correlated to the all tested soil attributes, particularly in the Organic Cryosols at Ross Point. Overall, the strong associations among denitrifier abundance, potential denitrification and soil resources found in present study may indicate that arctic ecosystems are confronted with severe nutrient-limitation and thus soil resources have a more readily detectable impact on microbial distribution and activities. The nature of this soil resource control on microbial activity differed between the sites, likely because the TOC and moisture contents differed significantly across the sites. For example, the relationship patterns of DEA and N_2Of, differ between Simpson Lake and Ross Point, suggesting niche-partitioning of denitrifier and N_2O reducing communities as reported previously in non-arctic ecosystems (Cuhel *et al*., 2010; Enwall *et al*., 2010). Consistently higher correlations in Organic Cryosols may have been caused by its relatively greater spatial homogeneity, which was also noted previously (Banerjee *et al*., 2011b).

A recent report found denitrifier functional gene abundance as a key factor determining denitrification-mediated N_2O emissions from agricultural soils (Morales *et al*., 2010). In contrast, this current study demonstrated that soil moisture and TOC content are the major factors shaping N_2O production/consumption in arctic soils although and other attributes such as pH, TN, NO_3^- and functional gene abundance are also correlated to denitrification potential but at weaker levels. Although strong nutrient limitation in the arctic may be a possible reason for this direct impact, the importance of soil resources as the primary drivers of potential denitrifier activities is also true for other ecosystems (Attard *et al*., 2011). The reason

for this difference between studies is likely linked to the identity of the denitrifier community at each site. That is, environmental factors exert their effects on denitrification through the denitrifying community (Wallenstein *et al*., 2006) and potential denitrification is often only associated with one of the functional guilds such as *nirS* (Cuhel *et al*., 2010), *nirK* (Attard *et al*., 2011) or *nosZ* (Philippot *et al*., 2009). Thus, the nature of the community at the site will modulate how the soil resource → gene abundance → ecosystem activity path is expressed. In our study, we postulate that because soil resources were so limited in these arctic ecosystems, their influence on ecosystem activity masked gene abundance effects. In contrast, gene abundance links to ecosystem activity in agricultural ecosystems with their eutrophic environment would likely mask soil resource modulations. Previous studies reported strong association between denitrifier abundance and organic carbon pool (Henry *et al*., 2004; Kandeler *et al*., 2006). Thus, this study suggests that the dominant control on ecosystem level denitrifier activity is moisture and organic carbon. Further, microbial abundance controls on ecosystem level activity while undoubtedly present are over-ridden in the nutrient poor arctic environment.

It should be noted that the assay of denitrification potential measures the maximum functional potential of denitrifying communities in absence of typical environmental constraints. While soil pH was not changed and the temperature of soil slurry was kept at ~ 20°C, it is possible that actual field conditions may have been slightly different. For example, the soil temperature may have been lower than 20°C even when the air temperature is 24°C. Moreover, the addition of glucose for enhancing microbial activity does not necessarily resemble typical soil conditions, such that it is possible that creating these optimum conditions may have allowed the selection of certain non-denitrifying groups that are not primarily involved *in situ* denitrification activities in Cryosolic ecosystems. While these limitations hinder linking functional groups with *in situ* denitrification activities, the denitrification potential assay employed in this study aimed to provide an overall idea of denitrification activities in arctic soils. Thus, the assay presented in this study reports overall activities and not the rate of soil denitrification per hour.

In this study, we evaluated 279 soil samples collected from three research sites encompassing 7° in latitude and 27° in longitude of the Canadian arctic. Arctic Cryosolic ecosystems are highly vulnerable to global warming and have been predicted to undergo greatest degree of changes. An increase of 3–4°C in mean annual air temperature by 2020 and 5–10°C by 2050, and as a consequence change in the balance between gains and losses of N have also been projected (Christensen, 1999; Tarnocai, 2006; IPCC, 2007). We showed that Cryosolic ecosystems have high denitrification potential and denitrifier abundance, similar to tropical and temperate ecosystems. Soil resources and the abundance of the denitrifier functional gene, *nirK*, were significantly correlated to N_2O production/consumption. Soil moisture, organic carbon and nitrogen contents are the key factors that drive denitrification in Cryosols. Denitrifier abundance and activities are spatially well-structured and the spatial dependence of these parameters comprised within 5 m scale due to cryopedogenic processes. Thus, experimental designs on denitrification in arctic regions should likely have field replicates greater than 5 m apart to insure independence. Future work will evaluate if the soil and gene parameters identified here can predict in situ, i.e. field, denitrification activity. Overall, the high N_2O emission potential of arctic soil ecosystems highlights that these soils could emit significant amount of N_2O gas upon favourable conditions, and this could have important implications in the light of the pervasive global climate change.

Experimental procedures

Research sites

Three arctic sites were selected for this study: Truelove Lowland, Simpson Lake and Ross Point (see Fig. S2). Truelove Lowland (75°40′N, 84°35′W) has been described by Lev and King (1999), Ma and colleagues (2007) and Siciliano and colleagues (2009). It is a coastal lowland covering an area of 43 km^2 on the north-eastern coast of Devon Island. The site is surrounded to the west and north by Jones Sound and two long cliffs to the east and south. The mean annual air temperature is about −16°C with the highest recorded daily temperature of 21°C in July and −45°C as the lowest daily temperatures (Bliss, 1977; King, 1969; Lev and King, 1999). The total annual precipitation is about 185 mm with 36 mm as rain. The topography of Truelove Lowland is distinguished by a series of raised beach crest ridge, lower foreslope and wet sedge meadow with Regosolic Static Cryosols, Brunisolic Eutric Turbic Cryosols and Gleysolic Turbic Cryosols respectively. The lower foreslope is dominated by lichen (*Alectoria* sp.) and upland sedges (*Eriophorum* sp.); as the name indicates, the wet sedge meadow is dominated by sedges (*Carex membranacea* and *Carex stans*) and mosses. Simpson Lake (68°35′N, 91°57′W) is situated in the middle of the Boothia Peninsula, approximately 80 kilometres west of Kugaaruk, Nunavut. Historical data from Kugaaruk suggests the temperature in winter (December–January) varies between −23° to −37°C and in summer (July–August) between 4°C to 24°C (Environment Canada, 2011). The total annual precipitation is about 260 mm with 110 mm as rain. The experimental site is dominated by lichen and dry heath vegetation. The upperslope positions are comprised of Static Cryosols whereas lower slope positions are Turbic Cryosol. Ross Point (68°31′N, 111°10′W) is situated in the southern part of Victoria Island. This site is about 150 km west of the town of Cambridge Bay. Historical weather data from the last 30

years in Cambridge Bay suggests the temperature in summer (July–August) ranges between 4°C to 23°C and in winter (December–January) between –25°C to –36°C (Environment Canada, 2011). The mean annual precipitation is about 138 mm with 69 mm as rain. The soil at this research site was predominantly Organic Cryosols with a relatively high degree of base saturation (indicated by pH) and thick (> 15 cm) organic (peaty) horizons.

Transect design

At each site, soil samples were collected along three parallel transects (300 m each) at 31 points (0, 0.1, 0.2, 0.5,1, 2, 5, 10, 20, 50, 100, 100.1, 100.2, 100.5, 101, 102, 105, 110, 120, 150, 200, 200.1, 200.2, 200.5, 201, 202, 205, 210, 220, 250, 300 m). The transects were separated from each other by 2 m. The variable-lag-distance transect approach was employed to simultaneously capture the fine, medium and large-scale spatial patterns of denitrifier communities in Cryosolic ecosystems (Banerjee *et al*., 2011a). Soil samples of approximately 250 g were collected at 1–10 cm depth using a hand trowel and sieved with a 4.75 mm sieve. Between samples, sieves and hand trowels were sterilized with 95% ethanol and dried before use. As facilities for performing biochemical and molecular analyses were not available on site, the soil samples were frozen at –20°C and were shipped to the laboratory at the University of Saskatchewan.

Soil analyses

Soil gravimetric water content (θ_g) was estimated by measuring the weight loss of 5 g soil samples after they were dried for 24 h at 105°C and the water content expressed as percentage of dry soil mass (Gardner, 1986). Soil pH was determined using 5 g soil in a 1:1 soil : water (deionized) mixture with an Accumet pH meter (Accumet 925, Fischer Scientific, Massachusetts, USA). Total organic carbon (TOC) was determined by combustion at 840°C using the Leco CR-12 Carbon Analyzer (LECO Corporation, St. Joseph, Michigan, USA) (Wang and Anderson, 1998). The determined quantity was expressed as a percentage of soil mass. Soil total nitrogen (% mass; TN) was determined by dry combustion using a Leco CNS-2000 elemental analyser (Wright and Bailey, 2001). For exchangeable nitrate (NO_3^-) content, soil subsamples were shaken with 0.5 M K_2SO_4 (1:10 soil: K_2SO_4) for 1 h and filtered using Whatman 90 μm filter papers (Maidstone, Kent, England). A 3 ml aliquot of extract was analysed using a SmartChem™ 200 discrete chemistry analyser using the manufacturer-provided methods (Westco Scientific Instruments, Brookfield, CT). It should be noted that the samples for chemical analyses were stored at –20°C and microbial cells may have potentially thawed or lysed during sample processing. The extra NO_3^- released from microbial cells may have contributed to slightly higher NO_3^- content.

Denitrification enzyme activity (DEA) and nitrous oxide reductase (N_2Of) assay

The assay is designed to measure the overall denitrification potential (DEA) and net N_2O formation (N_2Of) in soil by alleviating environmental constraints (Cavigelli and Robertson, 2000; Ma *et al*., 2011). The DEA and N_2Of were measured according to Ma and colleagues (2011). Briefly, soil slurries were prepared in a 70 ml crimp-sealed serum bottle by mixing 10 g soil and 10 ml of a test solution containing 10 mM glucose, and 5 mM KNO_3. The serum-bottles were flushed three times with high purity (99.995%) helium (Praxair, Danbury, CT). For DEA, 10% (v/v) acetylene was added into the slurries. Slurries were shaken (100 r.p.m.) at room temperature (~ 20°C) for 90 min. The ratio of N_2Of to DEA (denoted as rN_2O) was calculated. A 15 ml gas sample was collected from the headspace of the slurry using a 20 cc disposable syringe equipped with a 25-gauge needle and injected into a pre-evacuated Exetainer® vial (Labco Ltd, UK). Concentrations of N_2O in the headspace gas were estimated by a gas chromatograph equipped with an electron capture detector (Yates *et al*., 2006).

Typically, soil denitrification assay is measured at least two time points (Rich and Myrold, 2004; Philippot *et al*., 2009). However, due to large number of samples, denitrification potential was measured at one time point (90 min). This time point was selected on the basis of a time-course experiment. Ten randomly selected soil samples from three sites were incubated (with and without acetylene) according to the aforementioned method and gas samples were collected after 30, 60, 90 and 120 min. The 90 min time point was found in the linear range and thus chosen as the measurement time. There was no considerable difference in the lag phases of the three research sites. It should be noted that the soil samples were collected during 4th week of July when these research sites regularly experience temperature as high as 24°C. Furthermore, soil biological assays in previous studies conducted on these soils also used 20°C as the incubation temperature (Bliss, 1977; Banerjee and Siciliano, 2012). Therefore, the incubation temperature selected for the DEA assay in this study can fairly represent the midday temperature during growing season. A single incubation temperature (20°C) was selected for consistent measurement of soil samples collected from all three sites. Soils at these sites in particular Simpson Lake varied considerably and thus the pH of the soil slurry was not adjusted. Overall, the incubation temperature and pH selected for this assay can be representative of the field conditions in these arctic sites.

DNA extraction and quantification of denitrifier abundance

DNA extraction from soils was done according to the method described by Griffiths and colleagues (2000) with the modifications that DNA samples were precipitated in polyethylene glycol overnight and RNase was not added after extraction. To assess RNA contamination, DNA samples were examined using agarose gel electrophoresis and RNase treatment. No RNA contamination was found in samples. The concentration of purified DNA was determined by spectrophotometer (Ultrospec 2000 ultraviolet (UV)/visible spectrophotometer, Pharmacia Biotech, Cambridge, UK). The number of bacterial *nirK*, *nirS* and *nosZ* gene copies present in the soil DNA extracts was determined by performing quantitative real-time PCR (qPCR) using the Qiagen QuantiTect™ SYBR® Green PCR Master Mix real-time PCR kit (Qiagen, Ontario, Canada)

and an ABI 7500 real-time PCR machine (Applied Biosystems, Foster City, CA). Each 20 μl reaction contained 10 μl of master mix, 10 pmol of the appropriate forward and reverse primers (see Table S4), 6 μl sterilized milli-Q water, and 2 μl template DNA (1:10 diluted). Two no-template controls (NTCs) were run for each qPCR assay. As concentration of DNA varied between soil samples, the number of gene copies present in each sample was calculated by determining the concentration of DNA in the extract (Ma *et al*., 2007). Copy number per gram of dry soil was calculated on the basis of dilution factor and soil water content. Using the same primers, standard curves for *nirS* and *nosZ* were produced using purified PCR products obtained from the reference strain *Pseudomonas stutzeri* (ATTC 14405) and the *nirK* standard curve was prepared from purified PCR products obtained from the reference strain *Achromobacter cycloclastes* ATCC 21921. Only the standard curves linear over five orders of magnitude and r^2 value of 0.99 or higher were selected and standards were run on each individual qPCR plate. For all genes, the efficiency of the reaction was between 80% and 100% (based on the slopes of the standard curves). Consistency of Y-intercept values was checked between different qPCR runs and the specificity of the amplified products was assessed by melting curve analysis. Amplification inhibition effects were assessed by assessing gene abundance on three different dilutions of representative samples and selecting the dilution that minimizes the inhibition (Dumonceaux *et al*., 2006). A comparison of *nosZ* abundance estimated by primers designed by Kloos and colleagues (2001) and Henry and colleagues (2006) was performed for 10 randomly selected soil samples. For a direct comparison, copy numbers were calculated per nanogram of DNA (see Fig. S1).

Statistical analysis

To assess the relationships among soil moisture, TOC and denitrifier functions multiple linear regression analysis was performed using SigmaPlot 10.0 software (Systat Software, San Jose, CA). The spatial heterogeneity and spatial relationships were estimated using geostatistical analyses. The degree of spatial continuity was assessed by analysing the dissimilarity between two observations as a function of the separation distance or lag distance. This dissimilarity is measured through calculating semivariance, $\gamma(h)$, half of the average squared difference between the components of a data pair:

$$\gamma(h) = \frac{1}{2N(h)} \sum_{\alpha=1}^{N(h)} [z(x_k) - z(x_k + h)]^2$$

where $z(x_k)$ is the property, $z(x_k + h)$ is the value at h lag distance and $N(h)$ is the number of data pairs for a given distance (Goovaerts, 1998). The key features of a semivariogram (a plot of semivariance and lag distance) are range, sill and nugget. The range of a semivariogram indicates the zone of spatial dependency, i.e. the lag distance at which the semivariance value becomes highest whereas sill is the maximum variability attained by the variable. Nugget variance is the random variability due to experimental error. The spatial dependence (SPD) was calculated as: $\text{SPD} = C/(C + C_0)$, where C is the structural variance, C_0 is the nugget, and $C + C_0$ is the sill. Values of SPD vary from 0 (no spatial dependence) to 1 (high spatial dependence). All semivariograms were computed with a minimum of 30 sample pairs per lag class (Journel and Huijbregts, 1978). Gaussian, spherical, exponential models were fitted to the semivariograms using least-squares method in GS+ version 9 (Gamma Design Software, Plainwell, MI, USA).

Acknowledgements

The study was supported by Climate Change Impacts on Canadian Arctic Tundra (CiCAT) and International Polar Year (IPY). Logistical support was provided by Polar Continental Shelf Project with field assistance provided by A. Schafer and S. Ferguson. We thank the editor and three anonymous reviewers for their constructive comments and insightful suggestions. We also thank Martin Brummell for his helpful comments.

References

Attard, E., Recous, S., Chabbi, A., De Berranger, C., Guillaumaud, N., Labreuche, J., *et al.* (2011) Soil environmental conditions rather than denitrifier abundance and diversity drive potential denitrification after changes in land uses. *Glob Change Biol* **17:** 1975–1989.

Banerjee, S., and Siciliano, S.D. (2012) Factors driving potential ammonia oxidation in Canadian arctic ecosystems: does spatial scale matter? *Appl Environ Microbiol* **78:** 346–353.

Banerjee, S., Bedard-Haughn, A., Si, B.C., and Siciliano, S.D. (2011a) Soil spatial dependence in three arctic ecosystems. *Soil Sci Soc Am J* **75:** 591–594.

Banerjee, S., Si, B.C., and Siciliano, S.D. (2011b) Evidence of high microbial abundance and spatial dependency in three arctic soil ecosystems. *Soil Sci Soc Am J* **75:** 2227–2232.

Bjork, R.G., Majdi, H., Klemedtsson, L., Lewis-Jonsson, L., and Molau, U. (2007) Long-term warming effects on root morphology, root mass distribution, and microbial activity in two dry tundra plant communities in northern Sweden. *New Phytol* **176:** 862–873.

Bliss, L.C. (1977) *Truelove Lowland, Devon Island, Canada: A High Arctic Ecosystem*. Edmonton, Canada: University of Alberta Press.

Bockheim, J.G., and Tarnocai, C. (1998) Recognition of cryoturbation for classifying permafrost-affected soils. *Geoderma* **81:** 281–293.

Bohlen, P.J., Groffman, P.M., Driscoll, C.T., Fahey, T.J., and Siccama, T.G. (2001) Plant-soil-microbial interactions in a northern hardwood forest. *Ecology* **82:** 965–978.

Braker, G., Fesefeldt, A., and Witzel, K.P. (1998) Development of PCR primer systems for amplification of nitrite reductase gene (*nirK* and *nirS*) to detect denitrifying bacteria in environmental samples. *Appl Environ Microbiol* **64:** 3769–3775.

Brankatschk, R., Towe, S., Kleineidam, K., Schloter, M., and Zeyer, J. (2011) Abundances and potential activities of nitrogen cycling microbial communities along a chronosequence of a glacier forefield. *ISME J* **5:** 1025–1037.

Bru, D., Ramette, A., Saby, N.P.A., Dequidt, S., Ranjard, L., Jolivet, C., *et al*. (2011) Determinants of the distribution of nitrogen-cycling microbial communities at the landscape scale. *ISME J* **5:** 532–542.

Cavigelli, M.A., and Robertson, G.P. (2000) The functional significance of denitrifier community composition in a terrestrial ecosystem. *Ecology* **81:** 1402–1414.

Chapin, D.M. (1996) Nitrogen mineralization, nitrification, and denitrification in a high arctic lowland ecosystem, Devon Island, NWT, Canada. *Arctic Alpine Res* **28:** 85–92.

Christensen, T.R. (1999) Potential and actual trace gas fluxes in Arctic terrestrial ecosystems. *Polar Res* **18:** 199–206.

Cuhel, J., Simek, M., Laughlin, R.J., Bru, D., Cheneby, D., Watson, C.J., and Philippot, L. (2010) Insights into the effect of soil pH on N2O and N-2 emissions and denitrifier community size and activity. *Appl Environ Microbiol* **76:** 1870–1878.

Dalal, R.C., and Allen, D.E. (2008) Greenhouse gas fluxes from natural ecosystems. *Aust J Bot* **56:** 369–407.

Dandie, C.E., Wertz, S., Leclair, C.L., Goyer, C., Burton, D.L., Patten, C.L., *et al*. (2011) Abundance, diversity and functional gene expression of denitrifier communities in adjacent riparian and agricultural zones. *FEMS Microbiol Ecol* **77:** 69–82.

Dumonceaux, T.J., Hill, J.E., Briggs, S.A., Amoako, K.K., Hemmingsen, S.M., and Van Kessel, A.G. (2006) Enumeration of specific bacterial populations in complex intestinal communities using quantitative PCR based on the chaperonin-60 target. *J Microbiol Methods* **64:** 46–62.

Environment Canada. Weather data. Environment Canada, Fredericton, New Brunswick, Canada. http://www.weatheroffice.gc.ca. Accessed 14 October 2011.

Enwall, K., Throback, I.N., Stenberg, M., Soderstrom, M., and Hallin, S. (2010) Soil resources influence spatial patterns of denitrifying communities at scales compatible with land management. *Appl Environ Microbiol* **76:** 2243–2250.

Firestone, M.K., Firestone, R.B., and Tiedje, J.M. (1980) Nitrous oxide from soil denitrification: factors controlling its biological production. *Science* **208:** 749–751.

Franklin, R., and Mills, A. (2007) Statistical analysis of spatial structure in microbial communities. In *The Spatial Distribution of Microbes in the Environment*. Franklin, R., and Mills, A. (eds). Dordrecht, Netherlands: Springer, pp. 31–60.

Gardner, W.H. (1986) Water content. In *Methods of Soil Analysis. Part 1. Agronomy*, 2nd edn. Klute, A. (ed.). Madison, WI, USA: American Society of Agronomy, pp. 493–544.

Goovaerts, P. (1998) Geostatistical tools for characterizing the spatial variability of microbiological and physicochemical soil properties. *Biol Fertil Soil* **27:** 315–334.

Griffiths, R.I., Whiteley, A.S., O'Donnell, A.G., and Bailey, M.J. (2000) Rapid method for coextraction of DNA and RNA from natural environments for analysis of ribosomal DNA- and rRNA-based microbial community composition. *Appl Environ Microbiol* **66:** 5488–5491.

Hallin, H., and Lindgren, P.E. (1999) PCR detection of genes encoding nitrite reductase in denitrifying bacteria. *Appl Environ Microbiol* **64:** 1652–1657.

Hallin, S., Jones, C.M., Schloter, M., and Philippot, L. (2009) Relationship between N-cycling communities and ecosystem functioning in a 50-year-old fertilization experiment. *ISME J* **3:** 597–605.

Henry, S., Baudoin, E., Lopez-Gutierrez, J.C., Martin-Laurent, F., Brauman, A., and Philippot, L. (2004) Quantification of denitrifying bacteria in soils by *nirK* gene targeted real-time PCR. *J Microbiol Methods* **59:** 327–335.

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. *Appl Environ Microbiol* **72:** 5181–5189.

Intergovernmental Panel on Climate Change (2007) In Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to Fourth Assessment Report of the Intergovernmental Panel on Climate Change [S. Solomon, D. Qin, M. Manning (eds.)].

Journel, A.G., and Huijbregts, C.J. (1978) *Mining Geostatistics*. London, UK: Academic Press.

Kandeler, E., Deiglmayr, K., Tscherko, D., Bru, D., and Philippot, L. (2006) Abundance of *narG, nirS, nirK*, and *nosZ* genes of denitrifying bacteria during primary successions of a glacier foreland. *Appl Environ Microbiol* **72:** 5957–5962.

Keil, D., Meyer, A., Berner, D., Poll, C., Schutzenmeister, A., Piepho, H.P., *et al*. (2011) Influence of land-use intensity on the spatial distribution of N-cycling microorganisms in grassland soils. *FEMS Microbiol Ecol* **77:** 95–106.

King, R.H. (1969) Periglaciation on Devon Island, NWT. Doctoral Dissertation. Canada: Dept. of Geography, Univ. of Saskatchewan.

Kloos, K., Mergel, A., Rosch, C., and Bothe, H. (2001) Denitrification within the genus Azospirillum and other associative bacteria. *Aust J Plant Physiol* **28:** 991–998.

Knowles, R. (1982) Denitrification. *Microbiol Rev* **46:** 43–70.

Lamb, E.G., Han, S., Lanoil, B.D., Henry, G.H.R., Brummell, M.E., Banerjee, S., *et al*. (2011) A high Arctic soil ecosystem resists long-term environmental manipulations. *Glob Change Biol* **17:** 3187–3194.

Lev, A., and King, R.H. (1999) Spatial variation of soil development in a high Arctic soil landscape: Truelove Lowland, Devon Island, Nunavut, Canada. *Permafrost Periglac* **10:** 289–307.

Levy-Booth, D.J., and Winder, R.S. (2010) Quantification of nitrogen reductase and nitrite reductase genes in soil of thinned and clear-cut Douglas-Fir stands by using real-Time PCR. *Appl Environ Microbiol* **76:** 7116–7125.

Liu, B., Morkved, P.T., Frostegrad, A., and Bakken, L.R. (2010) Denitrification gene pools, transcription and kinetics of NO, N2O, and N2 production as affected by soil pH. *FEMS Microbiol Ecol* **72:** 407–417.

Ma, W.K., Schautz, A., Fishback, L.A.E., Bedard-Haughn, A., Farrell, R.E., and Siciliano, S.D. (2007) Assessing the potential of ammonia oxidizing bacteria to produce nitrous oxide in soils of a high arctic lowland ecosystem on Devon Island, Canada. *Soil Biol Biochem* **39:** 2001–2013.

Ma, W.K., Bedard-Haughn, A., Siciliano, S.D., and Farrell, R.E. (2008) Relationship between nitrifier and denitrifier community composition abundance in predicting nitrous oxide emissions from ephemeral wetland soils. *Soil Biol Biochem* **40:** 1114–1123.

Ma, W.K., Farrell, R.E., and Siciliano, S.D. (2011) Nitrous oxide emissions from ephemeral wetland soils are correlated with microbial community composition. *Front Microbiol* **2:** 1–11.

Miller, M.N., Zebarth, B.J., Dandie, C.E., Burton, D.L., Goyer, C., and Trevors, J.T. (2008) Crop residue influence on denitrification, N2O emissions and denitrifier community abundance in soil. *Soil Biol Biochem* **40:** 2553–2562.

Miller, M.N., Zebarth, B.J., Dandie, C.E., Burton, D.L., Goyer, C., and Trevors, J.T. (2009) Denitrifier community dynamics in soil aggregates under permanent grassland and arable cropping systems. *Soil Sci Soc Am J* **73:** 1843–1851.

Morales, S.E., Cosart, T., and Holben, W.E. (2010) Bacterial gene abundances as indicators of greenhouse gas emission in soils. *ISME J* **4:** 799–808.

Mosier, A., Kroeze, C., Nevison, C., Oenema, O., Seitzinger, S., and van Cleemput, O. (1998) Closing the global N2O budget: nitrous oxide emissions through the agricultural nitrogen cycle – OECD/IPCC/IEA phase II development of IPCC guidelines for National Greenhouse Gas Inventory Methodology. *Nutr Cycl Agroecosyst* **52:** 225–248.

Nicol, G.W., Glover, L.A., and Prosser, J.I. (2003) Spatial analysis of archaeal community structure in grassland soil. *Appl Environ Microbiol* **69:** 7420–7429.

Parkin, T.B. (1987) Soil microsites as source of denitrification variability. *Soil Sci Soc Am J* **51:** 1194–1199.

Parkin, T.B. (1993) Spatial variability of microbial processes in soil- a review. *J Environ Qual* **22:** 409–417.

Philippot, L. (2002) Denitrifying genes in bacterial and Archaeal genomes. *Biochim Biophys Acta* **1577:** 355–376.

Philippot, L., Cuhel, J., Saby, N.P.A., Cheneby, D., Chronakova, A., Bru, D., *et al.* (2009) Mapping field-scale spatial patterns of size and activity of the denitrifier community. *Environ Microbiol* **11:** 1518–1526.

Philippot, L., Andert, J., Jones, C.M., Bru, D., and Hallin, S. (2011) Importance of denitrifiers lacking the genes encoding the nitrous oxide reductase for N2O emissions from soil. *Glob Change Biol* **17:** 1497–1504.

Rich, J.J., and Myrold, D.D. (2004) Community composition and activities of denitrifying bacteria from adjacent agricultural soil, riparian soil, and creek sediment in Oregon, USA. *Soil Biol Biochem* **36:** 1431–1441.

Rich, J.J., Heichen, R.S., Bottomley, P.J., Cromack, K., and Myrold, D.D. (2003) Community composition and functioning of denitrifying bacteria from adjacent meadow and forest soils. *Appl Environ Microbiol* **69:** 5974–5982.

Robertson, G.P., Hutson, M.A., Evans, F.C., and Tiedje, J.M. (1988) Spatial variability in a successional plant community: patterns of nitrogen variability. *Ecology* **69:** 1517–1524.

Rösch, C., Mergel, A., and Bothe, H. (2002) Biodiversity of denitrifying and distribution-fixing bacteria in an acid forest soil. *Appl Environ Microbiol* **68:** 3818–3829.

Saetre, P., and Baath, E. (2000) Spatial variation and patterns of soil microbial community structure in a mixed spruce-birch stand. *Soil Biol Biochem* **32:** 909–917.

Siciliano, S.D., Ma, W.K., Ferguson, S., and Farrell, R.E. (2009) Nitrifier dominance of arctic soil nitrous oxide emissions arises due to fungal competition with denitrifiers for nitrate. *Soil Biol Biochem* **41:** 1104–1110.

Soil Classification Working Group (1998) *The Canadian System of Soil Classification*, 3rd edn. Ottawa, Canada: NRC Research Press.

Tarnocai, C. (2006) The effect of climate change on carbon in Canadian peatlands. *Global Planet Change* **53:** 222–232.

Tarnocai, C., and Bockheim, J.G. (2011) Cryosolic soils of Canada: genesis, distribution, and classification. *Can J Soil Sci* **91:** 749–762.

Tarnocai, C., Canadell, J.G., Schuur, E.A.G., Kuhry, P., Mazhitova, G., and Zimov, S. (2009) Soil organic carbon pools in the northern circumpolar permafrost region. *Global Biogeochem Cycles* **23:** GB2023.

Voorhees, E.B. (1902) Studies in denitrification. *J Am Chem Soc* **24:** 785–804.

Wagner, D. (2008) Microbial communities and processes in Arctic permafrost environment. In *Microbiology of Extreme Soils*, Vol. 13. Dion, P., and Nautiyal, C.S. (eds). New York, USA: Springer, pp. 133–154.

Wallenstein, M.D., Myrold, D.D., Firestone, M., and Voytek, M. (2006) Environmental controls on denitrifying communities and denitrification rates: insights from molecular methods. *Ecol Appl* **16:** 2143–2152.

Wang, D.L., and Anderson, D.W. (1998) Direct measurement of organic carbon content in soils by the Leco CR-12 carbon analyzer. *Commun Soil Sci Plan* **29:** 15–21.

Wright, A.F., and Bailey, J.S. (2001) Organic carbon, total carbon, and total nitrogen determinations in soils of variable calcium carbonate contents using a Leco CN-2000 dry combustion analyzer. *Commun Soil Sci Plan* **32:** 3243–3258.

Yates, T.T., Si, B.C., Farrell, R.E., and Pennock, D.J. (2006) Probability distribution and spatial dependence of nitrous oxide emission: temporal change in hummocky terrain. *Soil Sci Soc Am J* **70:** 753–762.

Supporting information

Additional Supporting Information may be found in the online version of this article:

Fig. S1. Regression plot of *nosZ* copy numbers estimated by Kloos and colleagues (2001) and Henry and colleagues (2006) primers.

Fig. S2. Geographic location of three experimental sites in circumpolar arctic region: Truelove Lowland (75°40′N, 84°35′W), Simpson Lake (68°35′N, 91°57′W) and Ross Point (68°31′N, 111°10′W). Adapted from the Toolik-Arctic Geobotanical Atlas (http://www.arcticatlas.org; Alaska Geobotany Center).

Table S1. Soil properties at three sites (from Banerjee *et al.*, 2011a). Mean values ($n = 93$) of different variables for three sites. Standard errors are shown in parentheses.

Table S2. The rate of N_2O production (rN_2O) and Spearman Rank correlations between rN_2O and other variables at three arctic ecosystems. The rN_2O was calculated as the ratio of N_2Of to DEA (i.e. $rN_2O = N_2Of/DEA$) (Rich and Myrold, 2004; Ma *et al.*, 2008).

Table S3. Spearman Rank correlation coefficients among denitrifier abundance, denitrifier potential activities and soil properties.
Table S4. Primers and thermal cycling conditions used for the quantification of denitrifier functional genes.

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.