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A High Arctic soil ecosystem resists long-term environmental manipulations

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Abstract

We evaluated above- and belowground ecosystem changes in a 16 year, combined fertilization and warming experiment in a High Arctic tundra deciduous shrub heath (Alexandra Fiord, Ellesmere Island, NU, Canada). Soil emissions of the three key greenhouse gases (GHGs) (carbon dioxide, methane, and nitrous oxide) were measured in mid-July 2009 using soil respiration chambers attached to a FTIR system. Soil chemical and biochemical properties including Q_{10} values for CO_2, CH_4, and N_2O, Bacteria and Archaea assemblage composition, and the diversity and prevalence of key nitrogen cycling genes including bacterial *amoA*, crenarchaeal *amoA*, and *nosZ* were measured. Warming and fertilization caused strong increases in plant community cover and height but had limited effects on GHG fluxes and no substantial effect on soil chemistry or biochemistry. Similarly, there was a surprising lack of directional shifts in the soil microbial community as a whole or any change at all in microbial functional groups associated with CH_4 consumption or N_2O cycling in any treatment. Thus, it appears that while warming and increased nutrient availability have strongly affected the plant community over the last 16 years, the belowground ecosystem has not yet responded. This resistance of the soil ecosystem has resulted in limited changes in GHG fluxes in response to the experimental treatments.

Keywords: arctic tundra plant community, carbon dioxide, GHG flux, methane, nitrogen cycling, nitrous oxide, soil microbial community

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Introduction

Most global climate models indicate that climate warming will be of greater magnitude at high latitudes and especially in the Arctic (Moritz *et al*., 2002; ACIA, 2005). Climate warming and air pollution in the High Arctic are expected to bring increased atmospheric nitrogen deposition, phosphate availability, and precipitation in addition to the primary changes of increased surface air temperatures and corresponding increase in soil temperatures and lengthening of the melt season (ACIA, 2005). The increased temperature, moisture, and nutrients are expected to cause a wide array of changes in the plant and soil communities including increases in vegetation height and cover, soil respiration, and rates of N-fixation (Henry & Svoboda, 1986; Arft *et al*., 1999; Rustad *et al*., 2001; Dormann & Woodin, 2002; Walker *et al*., 2006; Arens *et al*., 2008; Hudson & Henry, 2010; Hill & Henry, 2011), though effects of these changes on net ecosystem CO_2 exchange can be variable (Illeris *et al*., 2004; Oberbauer *et al*., 2007; Arens *et al*., 2008).

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In High Arctic tundra ecosystems much less is known about the combined effects on the flux of other key greenhouse gases (GHGs) such as CH_4 and N_2O. The effects of climate warming on CH_4 and N_2O emissions will likely be mediated by responses of the soil microbial community to climate-driven changes in resource supply and environmental conditions, yet only a very few studies have examined the long-term effects of ecosystem manipulation on High Arctic soil microbial communities (Deslippe *et al*., 2005; Fujimura *et al*., 2007; Walker *et al*., 2008) and no studies have examined microbial changes associated with GHG emissions in these systems.

While the CO_2 dynamics are fairly well understood, less is known about CH_4 dynamics and very little is known about nitrous oxide (N_2O) production in High Arctic terrestrial systems other than that N_2O flux rates are higher than expected, ranging between 1 and 290 $\mu g\, N_2O\, m^{-2}\, h^{-1}$ (Christensen *et al*., 1999; Ma *et al*., 2007; Dalal & Allen, 2008; Elberling *et al*., 2010). N_2O is a potent GHG with 298 times the global warming potential per molecule of CO_2 (Forster *et al*., 2007), and the dominant ecological mechanisms producing N_2O differ between Arctic and temperate systems. In the High

Arctic, N_2O appears to be primarily produced through the activity of prokaryotic nitrification–denitrification and fungal denitrification pathways (Ma *et al*., 2007; Ma *et al*., 2008; Siciliano *et al*., 2009) while bacterial denitrification is the dominant mechanism producing N_2O in temperate systems (Dalal & Allen, 2008). Similarly, questions remain about the dynamics of methane production in dry and mesic High Arctic tundra systems. Such systems are generally considered to be net methane sinks with methane production occurring in anoxic layers close to the permafrost table and consumption throughout the soil profile, while wetland sites are normally observed to be methane sources (Whalen & Reeburgh, 1990; Bartlett *et al*., 1992; Le Mer & Roger, 2001; Wagner *et al*., 2003; Elberling *et al*., 2008). The relationship between nitrogen fertilization and methane oxidation is complex in subarctic and temperate soils with low levels of fertilization reducing nutrient limitation and thus increasing oxidation but with a switch to inhibition at high levels of fertilization (Adamsen & King, 1993; Neff *et al*., 1994; Tlustos *et al*., 1998; Christensen *et al*., 1999; Le Mer & Roger, 2001). Little is known about the relationship between increased nitrogen availability and methane oxidation in High Arctic tundra soils.

In this paper, we examine the plant and soil bacterial community changes and GHG production in an ongoing 15-year-old warming, fertilization, and irrigation experiment in a High Arctic tundra ecosystem. Long-term ecosystem manipulation experiments are critical to understand the relationships between soil microbial communities and GHG dynamics because in Arctic systems, changes in soil microbial community structure can take more than 10 years to manifest themselves (Rinnan *et al*., 2007; Walker *et al*., 2008). We measured a diverse array of ecosystem properties including CO_2, CH_4, and N_2O fluxes, available soil nutrients, soil biochemistry, as well as the size and richness of the overall bacterial community by 16S rRNA and functional genes involved in the nitrogen cycle that lead to N_2O emissions. Given that changes in plant community biomass and stature are known to have occurred in this experiment, we wished to evaluate (1) whether there were any accompanying changes in the belowground ecosystem and (2) whether those changes had any consequences for soil GHG emissions.

Materials and methods

Study site and experimental design

A fertilization and warming experiment was established in 1995 at a coastal lowland on Alexandra Fiord (78° 53′N, 75° 46′W), Ellesmere Island, Nunavut, Canada. The experimental site is in a dwarf shrub tundra plant community dominated by the deciduous dwarf shrub *Vaccinium uliginosum* and a mixture of the evergreen dwarf shrub *Cassiope tetragona* and the graminoids *Carex misandra* and *Luzula arctica* (Muc *et al*., 1989). The site is on an alluvial bench approximately 5 m from and 0.5 m above a glacial meltwater stream. The soils are static cryosols underlain by permafrost with a maximum active layer of ~50 cm in early August (Muc *et al*., 1994).

The experiment included three treatments: passive warming, fertilizer addition, and water addition. The warming and fertilizer treatments were applied in a factorial design with warmed and control plots crossed with three levels of fertilization (0, 10, and 50 $g\,m^{-2}\,yr^{-1}$). Passive warming treatments were imposed using open top chambers (OTCs) following the International Tundra Experiment (ITEX) protocols (Walker, 1996). The chambers (hexagons of inclined panels of transparent fiberglass, 0.5 m in height enclosing 1.8 m^2) remain in place year-round and warm the near-surface air temperature on average between 1 and 2 °C during the growing season (Marion *et al*., 1997; Hudson & Henry, 2010). Fertilization treatments were applied annually in mid to late July by dissolving 20 : 20 : 20 NPK fertilizer in ca. 10 L of water from the nearby glacial meltwater stream. The control fertilizer treatments also received the same amount of water, thus two additional treatments (with and without passive warming) were included that received no water addition. Glacial melt water from a nearby stream was used for irrigation. Large fertilizer addition rates were used to ensure sufficient nitrogen was available to the plants (rather than immobilized by microorganisms) to result in a significant vegetation response within 3–10 years of experimental establishment (Jonasson *et al*., 1996). Five replicates of each treatment combination were established for a total of 40 samples. As the original experimental design had relatively few replicates, an additional 40 control samples that were not established as part of the initial experiment were also sampled. Sampling additional controls allowed us to assess the full range of variation expected within each treatment to ensure that the five samples within the other treatments accurately captured the mean response. The additional samples were interspersed among the other plots in the experiment, but at least 2 m from any existing plot.

We measured a wide range of ecosystem properties in each replicate of this experiment in the field and laboratory. Detailed protocols for each property including an assessment of temporal variation in GHG production are included in the supporting information. Field measurements taken *in situ* included vegetation characteristics (height and total plant cover including litter) following ITEX protocols (Walker, 1996) and the flux of three GHGs (CO_2, CH_4, and N_2O) measured using a Fourier Transform Infrared trace gas analyzer (FTIR-TGA) (Gasmet DX-4015, Gasmet, Helsinki, Finland) linked to an opaque LI-COR long-term monitoring chamber (supporting information). Soil-surface temperature and moisture was recorded with a 5.2 cm long ProCheck digital sensor inserted vertically into the soil (Decagon Devices, Pullman, WA, USA). A small soil sample was collected from each replicate and subsequently analyzed for gravimetric soil moisture content, and plant-available nutrients including nitrate (NO_3^-), ammonium (NH_4^+), and orthophosphate (PO_4^{3-}),

and nonpurgeable organic carbon and dissolved organic nitrogen. Activation energies (E_a or Q_{10}) for the production of CO_2, CH_4, and N_2O were determined under optimal substrate conditions to assess the microbial physiologies of a given process independent of soil conditions that may also modulate the overall activity of a soil process (methods are described in more detail in supporting information).

DNA was extracted from the soil samples and amplified using a range of bacterial and archaeal primers including 16S rDNA and *nosZ*, *pmoA* and bacterial and archaeal *amoA* primers (Table S1) Denaturing gradient gel electrophoresis (DGGE) was performed on the PCR products to examine how the experimental treatments influenced microbial community structure. The prevalence of the key functional genes linked to nitrogen cycling including bacterial *amoA*, crenarchaeal *amoA*, and *nosZ* (Table S1) in each soil sample were estimated using quantitative real-time PCR. We wished to include the methane oxidizing gene *pmoA* but were unable to amplify it from any samples (Table S1).

Statistical analysis

Analyses of gas fluxes, vegetation and soil characteristics, microbial diversity, activation energies, and functional gene prevalence were carried out using general linear models fitted using the glm function in the R 2.10.0 package (R Development Core Team, 2010). The 40 additional control points were included in the gas flux analyses for a total sample size of 80, while all other analyses were conducted only on the 40 samples included in the original experiment. A model selection approach where nonsignificant model terms were removed (Crawley, 2007) was used to determine the significance of experimental treatments and interactions. In each case, an initial (maximal) model was fitted with warming, fertilization, and irrigation as main effects and warming × fertilization and warming × water interaction terms. The incomplete factorial design precluded additional interaction terms. The field GHG flux models also included soil temperature readings taken at the time of flux measurement, allowing the separation of the effects of short-term changes in soil conditions on gas flux from the long-term effects of the soil treatments. These terms were not included in the models examining vegetation properties or soil physical microbial community properties as these variables were not expected to be driven by short-term fluctuations in soil conditions. The deviance explained by the best model should not be significantly different ($P > 0.05$) from the deviance explained by the maximal model. The drop1 function was used to select unimportant model terms for removal. In cases where the three fertilizer treatment levels were significant, factor level reduction was used to test for differences between treatments. A model simplification approach was used in this study because the small number of replicates (5) in the experiment limits the power to test for multiple main effects and interactions, and the removal of nonsignificant terms increases the power of tests for the remaining model terms. One-sample *t*-tests were used to demonstrate that the retained model parameters were significantly different from zero.

Results

Long-term changes in vegetation parameters were observed in this experiment, but there were few main effects and no interactive effects on GHG fluxes and soil microbial community properties (Fig. 1; Table S2). Vegetation height increased by 45.5% in the high fertilization treatment compared with the control and low treatments ($t = 2.415$, $P = 0.021$), but was not affected by either irrigation or warming (maximal vs. best model $F_{6,39} = 1.709$, $P = 0.151$). Vegetation cover increased by 21.9% with warming ($t = 2.427$, $P = 0.020$) and by 34.1% with fertilization (low and high fertilizer vs. none $t = 3.576$, $P < 0.001$; maximal vs. best model $F_{5,39} = 0.653$, $P = 0.661$).

Carbon dioxide emissions were positively associated with soil temperature measured at the time of sampling ($t = 2.273$, $P = 0.026$), but were not affected by the long-term increase in average temperature caused by the warming treatment. The water addition treatment increased CO_2 fluxes by 31.0% ($t = 2.082$, $P = 0.041$), however, CO_2 flux was not affected by soil moisture measured at the time of sampling or the fertilization treatment (maximal vs. best model: $F_{7,79} = 0.163$, $P = 0.335$). The low fertilizer treatment was on average a net methane source while the control and high fertilizer treatments were sinks ($t = 3.568$, $P = 0.001$), but there were no other significant treatment or covariate effects on methane flux (maximal vs. best model: $F_{8,79} = 1.364$, $P = 0.228$). The only effect on N_2O fluxes was a reduction ($t = -2.092$, $P = 0.040$) in the low fertilizer treatment compared with the control and high fertilizer treatments (maximal vs. best model: $F_{8,79} = 0.849$, $P = 0.564$). However, this effect is likely unimportant as it was driven by a single data point in the low fertilizer treatment with high N_2O consumption ($-14.41\ \mu mol\ m^{-2}\ s^{-1}$; maximal vs. null model without outlier: $F_{9,79} = 1.011$, $P = 0.440$). In summary, there were no interactions affecting GHG fluxes, we did not observe any covariate or additive effects of these treatments, and there were few significant correlations (Table S2) between GHG fluxes and any measured soil properties.

Despite the minimal changes due to the experimental treatments, this High Arctic ecosystem was a potentially important GHG source. Overall GHG CO_2 equivalents across all treatments were 1.63 ± 1.31 SD $\mu mol\ m^{-2}\ s^{-1}$ with CO_2 contributing 84.3% and N_2O contributing 15.9% to the total flux. On the whole, the site was a slight methane sink ($-0.0039 \pm 0.0186\ \mu mol\ m^{-2}\ s^{-1}$).

There were no changes in the concentrations of soil organic carbon, dissolved organic nitrogen, available NH_4^+, or NO_3^- (supporting information; Fig. 1), but there was a significant warming × water interaction influencing orthophosphate ($F_{1,36} = 4.418$; $P = 0.043$), where available PO_4 increased 104.2% with warming

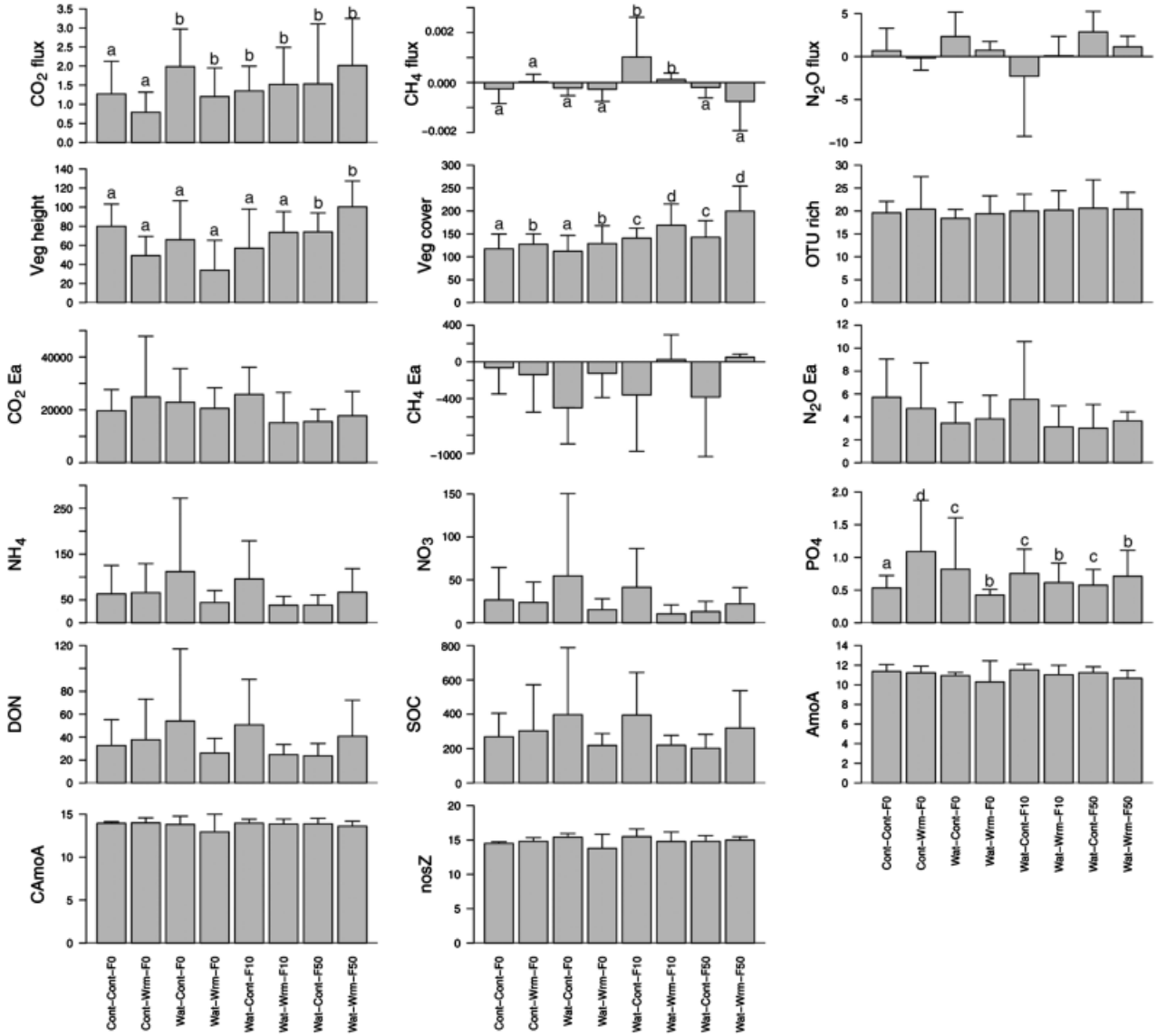

Fig. 1 Means of CO_2, CH_4, and N_2O fluxes (nmol m^{-2} s^{-1}), vegetation height (mm), live vegetation and litter cover (# hits), soil microbial diversity (# 16s RNA bands), activation energy (kJ mol^{-1}) for CO_2, CH_4 and N_2O, concentrations (mg kg^{-1}) of NH_4, NO_3, PO_4, dissolved organic nitrogen (DON), and soil organic carbon (SOC), and the abundances of *amoA*, *CrenamoA*, and *nosZ* (ln copy numbers g^{-1} soil) in each treatment combination. The treatments are: Cont-Cont-F0 = water control, zero fertilizer, warming control; Cont-Wrm-F0 = water control, zero fertilizer, warmed; Wat-Cont-F0 = water, zero fertilizer, warming control; Wat-Wrm-F0 = water, zero fertilizer, warmed; Wat-Cont-F10 = water, low fertilizer, warming control; Wat-Wrm-F10 = water, low fertilizer, warmed; Wat-Cont-F50 = water, high fertilizer, warming control; Wat-Wrm-F50 = water, high fertilizer, warmed. Error bars are one standard deviation. Letters indicate significant differences between treatment combinations. Plots with no letters had no significant differences between treatments.

in plots that did not receive water, but decreased 18.5% with warming in plots receiving water (maximal vs. best model $F_{4,39} = 0.436$, $P = 0.782$). Similarly, there were no effects of experimental treatments or interactions on apparent activation energies for CO_2, CH_4 or N_2O, or on the prevalence of functional gene markers for denitrification (*nosZ*) or nitrification (*amoA* or *CrenamoA*) (Fig. 1; supporting information). We were unable to detect methanotroph marker genes (*pmoA*) in any sample.

Despite the increases in vegetation coverage and height in some of the treatments, there were few effects of the experimental treatments on the soil bacterial community. DGGE of 16S rRNA genes indicated no significant differences in the number of dominant taxa between treatments (maximal vs. null model

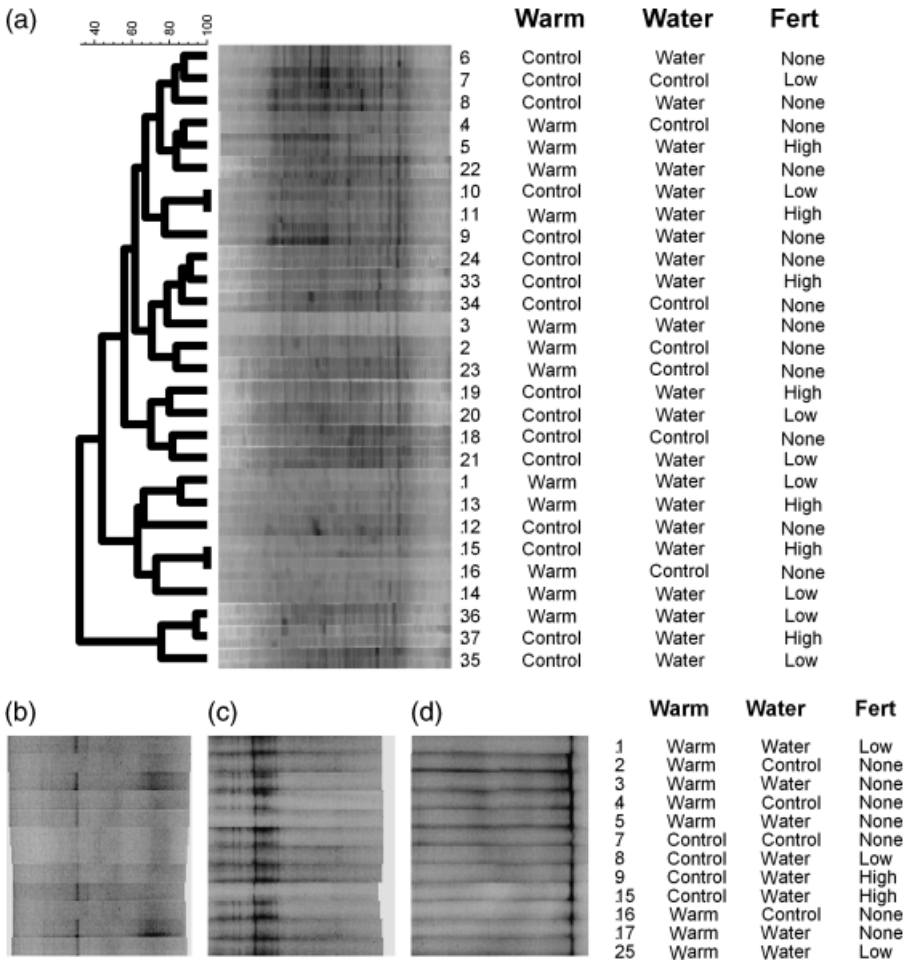

Fig. 2 Diversity of microbes in a subset of experimental soils based on (a) 16S rRNA, (b) bacterial ammonia monoxygenase (*amoA*), (c) crenarchaeal *amoA*, and (d) nitrous oxide reductase (*nosZ*) denaturing gradient gel electrophoresis. Plot numbers and treatments are indicated. Cluster analysis based on the presence/absence of bands (Dice similarities) is included for 16S rRNA gene patterns only. Not all plots are shown for functional gene analysis; however, other samples provided similar results.

$F_{7,39} = 0.106$, $P = 0.997$; Fig. 2a). While there was a moderate amount of variability in the assemblage structure between treatments, this variation was not correlated with any treatment or combination of treatments and thus is interpreted as sample to sample variation. There were no detectable differences in the diversities of functional genes (bacterial and crenarchaeal amoA and nosZ), indicating that the assemblages were highly stable across treatments (Fig. 2b–d).

Discussion

Very limited effects on soil community composition and function and GHG fluxes were observed following 15 years of warming, fertilization, and irrigation. Environmental variables predicted to be associated with climate warming, i.e. increased air and soil temperatures and nutrient availability, had only minimal impacts on GHG emissions from the soil of this High Arctic plant community. These results are surprising given the large magnitude of the fertilization treatments, and demonstrate that there is a great deal of resistance in the soil microbial communities of this High Arctic ecosystem. Given expected levels of warming and nutrient deposition, the full effects of climate change may take many decades to become apparent in the soil of this community. Such resistance to change may be a general property of Arctic soil microbial communities. Turnover rates of Arctic microbial communities can be very slow as a similar warming and fertilization experiment took more than 10 years to affect a subarctic tundra soil microbial community close to the tree line (Rinnan *et al*., 2007; Rinnan *et al*., 2008). Not all elements of the soil community display such resistance, however, as

fertilization of another community at our site stimulated nitrogen fixation between 19 and 35 days after application (Deslippe *et al*., 2005). Fertilization had no effect on the diversity of the *nif*H (dinitrogenase reductase) community, however, warming did shift the structure of the *nif*H community, indicating there was likely a seasonal succession in the diazotroph community (Deslippe *et al*., 2005).

We found a high diversity of bacteria in the soil samples, as has been seen previously in Arctic soil samples (Nemergut *et al*., 2005; Neufeld & Mohn, 2005; Walker *et al*., 2008; Chu *et al*., 2011). The significant increases in vegetation and litter cover and height with warming and fertilization observed in both this and many other long-term Arctic vegetation studies (Henry *et al*., 1986; Arft *et al*., 1999; Rustad *et al*., 2001; Dormann & Woodin, 2002; Walker *et al*., 2006; Arens *et al*., 2008) may also contribute to the lag in the soil community response by shading and insulating the soil from the increased air temperatures in the OTCs (Walker *et al*., 2003); however, soil temperatures generally have been found to be warmer in OTCs even under vegetation and litter (Marion *et al*., 1997). The increased vegetation cover should also be associated with increased fine root mass (Rinnan *et al*., 2008; Hill & Henry, 2011). Thus, shifts in the soil microbial community may occur in the rhizosphere, but might be undetectable in bulk soil samples. For example, 5–7 years of experimental warming in other plant communities at Alexandra Fiord increased the density of certain genotypes within the fungal community associated with roots of *Salix arctica* but had not affected the diversity of genotypes (Fujimura *et al*., 2007). Finally, a higher resolution richness analysis, rather than the relatively coarse DGGE technique used here, may be able to detect a significant change in the microbial community (Walker *et al*., 2008). Given, however, that nonmolecular approaches in our study indicate that there is relatively little impact of warming and fertilization on GHG fluxes or soil chemistry and biochemistry, any subtle changes in the soil microbial community that have occurred are unlikely to impact GHG emissions. Thus, we conclude that while the aboveground community has responded to warming and fertilization over the last 15 years, the belowground community has not yet responded to these manipulations.

While there were very limited impacts of the experimental treatments on GHG fluxes, our study demonstrates that this High Arctic ecosystem is a substantial emitter of N_2O relative to the CO_2 emissions. The environmental controls on N_2O production are complex, with Arctic N_2O emissions thought to arise largely through prokaryotic nitrification–denitrification and fungal denitrification pathways (Ma *et al*., 2007; Ma *et al*., 2008; Siciliano *et al*., 2009). The ammonia oxidizer and denitrifier populations in these High Arctic soil ecosystems were abundant and similar in size to other temperate soils (Henry *et al*., 2006; Leininger *et al*., 2006; He *et al*., 2007; Nicol *et al*., 2008; Shen *et al*., 2008). In general, the ammonia oxidizer archaeal population size was larger than their bacterial counterpart which is in agreement with previous studies (Nemergut *et al*., 2005). The *Vaccinium* heath observed in this experiment was a slight net CH_4 sink, as has been found at other sites with similar vegetation types (Le Mer & Roger, 2001; Elberling *et al*., 2008). We were unable to detect methanotrophs in our soil samples, suggesting a very limited ability for these soils to consume CH_4, although this negative result may also be an artifact of poor coverage of methane oxidation gene diversity by currently available PCR primers.

The significant water treatment effect on CO_2 fluxes indicated that small additions of water late in the growing season can affect tundra soil processes. Short-term increases in plant root and soil respiration can follow experimental irrigation in Arctic tundra (Illeris *et al*., 2003), but the effects of irrigation on soil water content are generally small relative to natural variation in soil moisture (Sullivan *et al*., 2008). The strong effect from a relatively small water addition (equivalent to a single rainfall event of 5 mm) in the mid to latter part of the growing season is likely due to the location of the study site on well drained soils that depend on precipitation once snow melt has occurred.

This is the first study to simultaneously examine the influence of experimental warming, fertilization and irrigation on both soil bacterial community composition and GHG flux in a High Arctic tundra ecosystem. As in other experiments (Henry *et al*., 1986; Arft *et al*., 1999; Rustad *et al*., 2001; Dormann & Woodin, 2002; Walker *et al*., 2006; Arens *et al*., 2008), plants responded strongly to the experimental treatments but there were surprisingly few changes in GHG fluxes or soil community properties. While this study and others (Walker *et al*., 2008; Hudson & Henry, 2010) suggest that some High Arctic tundra ecosystems are resistant to a warming climate, presumably this inertia is maintained, i.e. while the communities are slow to change, any changes that do occur will be correspondingly resistant to further change. Finally, ours was the first study to examine the potential effects of climate warming on N_2O production in a High Arctic tundra community. While we found little effect from the experimental treatments on N_2O, the large contribution of N_2O to the net GHG equivalent production in this system suggests tundra communities have the potential to be substantial sources of N_2O in a warming climate.

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Supporting Information

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

Figure S1. Relationships between box flux (static chamber) and active chamber estimates of CO_2, CH_4, and N_2O fluxes. The line is a regression line between the calibration box and the measured chamber flux.

Figure S2. Annual and diurnal variability of greenhouse gas flux at Alexandra Fiord. A) Annual variation in greenhouse gas flux from the fertilizer experiment. Only non-fertilized plots $n = 5$ (both control and warmed) were measured in both 2009 and 2010. Bars represent the average of 10 measurements (control and warmed are reported together) with error bars representing the standard error of the estimate. B) Diurnal variation of CO_2 and N_2O in a *Salix arctica* (Arctic Willow) dominated ecosystem. Symbols indicate the average of four replicate plots measured simultaneously with error bars representing the standard error of the mean. Gas flux was measured daily roughly every 20 min for a two day period for a total of 125 measurements.

Table S1. Primer information.

Table S2. Plant community characteristics, GHG fluxes, and soil chemical and microbial properties measured in each experimental treatment. Warm indicates an OTC, Water added water, and F0, 10, and 50 indicate the amount of fertilizer added ($g\,m^{-2}$). Values are means $\pm$ 1 standard deviation. $n = 5$ for all values except the Control treatment for CO_2, CH_4, and N_2O where $n = 45$.

Table S3. Matrix of correlations between CO_2 N_2O, and CH_4 fluxes ($\mu mol\,m^{-2}\,s^{-1}$), live vegetation height (mm) and cover (number of hits), soil characteristics including soil organic carbon (SOC), NH_4, NO_3, PO_4, and dissolved organic nitrogen (DON) concentrations ($mg\,kg^{-1}$), activation energies (Ea) of CO_2 N_2O, and CH_4 production, and prevalence (copy# g^{-1}) of *CrenamoA*, *amoA, and nosZ*. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

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