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Identifying environmental drivers of greenhouse gas emissions under warming and reduced rainfall in boreal–temperate forests

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Abstract

1. Atmospheric concentrations of carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O) are predicted to increase as a consequence of fossil fuel emissions and the impact on biosphere–atmosphere interactions. Forest ecosystems in general, and forest soils in particular, can be sinks or sources for CO_2, CH_4, and N_2O. Environmental studies traditionally target soil temperature and moisture as the main predictors of soil greenhouse gas (GHG) flux from different ecosystems; however, these emissions are primarily biologically driven. Thus, little is known about the degree of regulation by soil biotic vs. abiotic factors on GHG emissions, particularly under predicted increase in global temperatures, and changes in intensity and frequency of precipitation events.
2. Here we measured net CO_2, CH_4 and N_2O fluxes after 5 years of experimental warming (+3.4°C), and 2 years of ≈45% summer rainfall reduction, in two forest sites in a boreal–temperate ecotone under different habitat conditions (closed or open canopy) in Minnesota, USA. We evaluated the importance of microbial gene abundance and climo-edaphic factors (soil texture, canopy, seasonality, climate, and soil physicochemical properties) driving GHG emissions.
3. We found that changes in CO_2 fluxes were predominantly determined abiotically by temperature and moisture, after accounting for bacterial abundance. Methane fluxes on the other hand, were determined both abiotically, by gas diffusivity (via soil texture) and microbially, by methanotroph *pmoA* gene abundance, whereas, N_2O emissions showed only a strong biotic regulation via ammonia-oxidizing bacteria *amoA* gene abundance. Warming did not significantly alter CO_2 and CH_4 fluxes after 5 years of manipulation, while N_2O emissions were greater with warming under open canopy.
4. Our findings provide evidence that soil GHG emissions result from multiple direct and indirect interactions of microbial and abiotic drivers. Overall, this study highlights the need to include both microbial and climo-edaphic properties in predictive models in order to provide improved mechanistic understanding for the development of future mitigation strategies.

KEYWORDS
bacteria, carbon dioxide, denitrifiers, methane, methanotrophs, nitrifiers, nitrous oxide, rainfall, warming

1 | INTRODUCTION

Soil greenhouse gas (GHG) fluxes are the result of biological processes leading to their production and/or consumption in terrestrial ecosystems. However, the majority of field-based studies focus on the importance of abiotic (soil physicochemical properties), rather than biotic (microbial communities) factors in driving carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O) fluxes. These studies have attributed flux responses to effects of soil oxygen levels, water content, pH, temperature and substrate availability on microbial community activity without a direct measure of how such abiotic determinants change microbial communities in ways that might alter GHG fluxes (Dalal & Allen, 2008; Hu, Chen, & He, 2015; Tate, 2015).

At the soil microsite level, CO_2, CH_4 and N_2O fluxes are primarily driven by microbial pathways, controlled at the gene and cellular level (Singh, Bardgett, Smith, & Reay, 2010); however, soil abiotic properties are capable of indirectly affecting flux rates into the atmosphere by regulating microbial abundance and activity, but also simultaneously, by affecting gas diffusion rates into the soil profile or to the atmosphere (Martins, Macdonald, Anderson, & Singh, 2016; Martins, Nazaries, Macdonald, Anderson, & Singh, 2015). At the landscape level, soil physicochemical properties are strongly affected by soil texture, climatic conditions, vegetation type and land-use. Therefore, lack of empirical evidence of the degree of regulation by biotic or abiotic factors from smaller to larger environmental scales still exists and needs to be clarified. This current limitation of our understanding on the role of soil microbes in controlling soil functioning directly impacts the prediction of the direction, magnitude and duration of GHG emissions (Tian et al., 2015, 2016).

Forests ecosystems are particularly important because they consume on average more CH_4 than all other terrestrial ecosystems (Luo, Kiese, Wolf, & Butterbach-Bahl, 2013; Tian et al., 2015) and are major contributors to carbon (C) storage in soil and aboveground vegetation (Le Quéré et al., 2015). In the case of CO_2, heterotrophic respiration (a broad microbial function—as defined in Schimel, Bennett, and Fierer (2005)) in forest soils is a major contributor to CO_2 efflux from these ecosystems, together with autotrophic root respiration (Hanson, Edwards, Garten, & Andrews, 2000; Subke, Inglima, & Francesca Cotrufo, 2006). In contrast to CO_2 production, specific microbial groups are responsible for CH_4 and N_2O production and consumption and thus these fluxes are considered specialized ecosystem processes (Schimel et al., 2005). Anaerobic methanogenic archaea carry out CH_4 production, whereas aerobic methanotrophic bacteria are responsible for CH_4 consumption (Nazaries, Murrell, Millard, Baggs, & Singh, 2013). The oxidation of atmospheric CH_4 by aerobic soils serves as a significant global CH_4 sink in terrestrial ecosystems (Dutaur & Verchot, 2007; IPCC, 2013). In the case of N_2O, multiple specialized microbial groups are responsible for N_2O production, namely (1) aerobic ammonia-oxidizing archaea (AOA) and ammonia-oxidizing bacteria (AOB) through nitrification-mediated pathways (ammonia oxidation and/or nitrifier denitrification); or (2) denitrifying microorganisms through the multistep process of heterotrophic denitrification (Hu et al., 2015). To date, this multistep reaction is also the only one known to be responsible for the sink of N_2O in the soil, carried out by specialized N_2O-reducing bacteria (Jones et al., 2014). In fact, there is evidence that 30%–80% of the N_2O produced from deeper soil layers may be reduced to N_2 before diffusion into the atmosphere (Clough, Sherlock, & Rolston, 2005).

By the late 21st century, global mean annual temperatures are predicted to increase between 1.2 and 4.8°C, with more uncertainty associated with how intensity and frequency of precipitation patterns will change (IPCC, 2013). However, despite recent advances, only a few manipulative long-term field studies in forest ecosystems have directly assessed the combined effects of warming and reduced summer rainfall on CO_2, CH_4 and N_2O emissions (Blankinship, Brown, Dijkstra, Allwright, & Hungate, 2010; Schindlbacher et al., 2012). Climate change may potentially alter the relative importance of biotic and abiotic factors in driving GHG emissions (e.g. shifting microbial abundance), however little is known about the impacts of climate change on GHG emissions via abiotic and biotic factors. Modelling studies suggest the interaction between warming and soil moisture are a significant determinant of ecosystem responses to the ongoing changing climate due to a regulation of biological responses (Kirschbaum, 2004; Niyogi & Xue, 2006; Zhou, Dickinson, Dai, & Dirmeyer, 2010). Studies including the combined effects of warming and changing rainfall patterns are therefore imperative because they are expected to occur simultaneously and thus lead to different effects on soil biotic and abiotic properties in comparison to their individual effects.

In this study, we investigated the long-term impact of warming (+3.4°C) and reduced summer rainfall manipulation (≈45% exclusion) on soil CO_2, CH_4 and N_2O emissions in a boreal-temperate ecotone, the Boreal Forest Warming at an Ecotone in Danger (B4WarmED), 5 years after the beginning of the experiment. We aimed to determine (1) whether these biogenic GHG fluxes were primarily explained by changes in soil physicochemical characteristics (abiotic) or by changes in microbial community abundances (biotic), regardless of whether these differences arose because of climate change treatments (i.e. experimental warming and rainfall manipulation), variation between sites (reflected in different soil texture), habitat (presence or absence of canopy) or seasonality (difference in ambient climate between monthly measurements); and (2) the long-term effects of climate treatments on CO_2, CH_4 and N_2O emissions responses. In addressing these aims we hypothesized first, that abiotic factors, such as soil temperature and/or moisture, would be the main drivers of CO_2, CH_4 and N_2O fluxes, by indirectly affecting microbial gene abundance and/or gas diffusion, irrespective of site, habitat, seasonal variation, warming and rainfall

manipulation. Second, we hypothesized that warming and reduced rainfall would individually increase CO_2 and N_2O emissions and CH_4 uptake by favouring aerobic conditions, whereas the two climate treatments combined would have an offsetting effect on GHG feedback responses.

2 | MATERIALS AND METHODS

2.1 | Field site description: The B4Warmed experiment

A free-air warming experiment was established at two field sites, in northern Minnesota, USA, in two different habitat conditions (closed canopy or open canopy) at each site. The B4WarmED experiment was established in 2008, with one site located in the Cloquet Forestry Center (CFC), in Cloquet (46°40′46″N, 92°31′12″W) and the second site located approximately 150 km further north, in the Hubachek Wilderness Research Center (HWRC), in Ely (47°56′46″N, 91°45′29″W) (Reich et al., 2015; Rich et al., 2015). Briefly, both sites were situated in 40–60-year-old mixed aspen-birch-fir forests scattered with pine, spruce and other species, representing the transition from temperate to boreal biomes. Both sites are located on coarse-textured upland soils classified as Inceptisols and Entisols (USDA soil taxonomy) for Cloquet and Ely, respectively.

The warming treatment (+3.4°C) comprised simultaneous warming of the plants and soil. Warming was implemented from early spring to late autumn each year, in open air, maintaining a fixed temperature differential of 3.4°C from ambient conditions, via feedback control at the plot scale. Within each plot, seedlings of 11 tree species were planted into existing herbaceous vegetation in a gridded design. In 2012, a reduced rainfall treatment was implemented in both sites by excluding ≈45% of rainfall to half of the plots under open canopy conditions throughout the summer period (June to September). A temporary water removal technique was applied, with individual rainfall events targeted only when the cumulative seasonal reduction fell below the 45% threshold. Climate of field sites and climate treatments manipulation description can be found in Appendix S1 in the Supporting information.

Within each canopy habitat in each site, three blocks were established for a total of 12 blocks (i.e. 6 in each site, 3 in each habitat). Each block contained two ambient temperature and two +3.4°C warmed plots. In 2013, in the open canopy treatment only, one plot from each of the two warming treatments (ambient, +3.4°C) in each block was randomly assigned to the rainfall removal treatment. Thus, the experiment comprised a total of 36 plots, 18 plots in each site. The treatments consisted of closed canopy + ambient temperature + ambient rainfall (CAA), closed canopy + warming + ambient rainfall (CWA), open canopy + ambient temperature + ambient rainfall (OAA), open canopy + warming + ambient rainfall (OWA), open canopy + ambient temperature + reduced rainfall (OAR) and open canopy + warming + reduced rainfall (OWR) (Figure S1). Thus, an incomplete factorial design was considered for the present study wherein each closed canopy block had two climate treatments (CAA, CWA), and each open canopy block had four climate treatments (OAA, OWA, OAR, OWR). Aboveground ambient temperature, natural rainfall and percentage of rainfall removal in 2013 can be found in Table 1.

2.2 | Greenhouse gas measurements

Greenhouse gas fluxes were measured once per month in each plot, from May to October 2013 using a static chamber technique (Venterea, Parkin, Cardenas, Petersen, & Pedersen, 2015). Measurements were only taken during the growing season (May to October) because climate treatments were not imposed during the snowfall season. One polyvinyl chloride (PVC) chamber base (diameter = 20 cm; height = 15 cm) was permanently inserted 8 cm into the soil in each plot 2–3 days before the first measurement. Chamber tops consisted of a PVC collar (height = 10 cm) with one end sealed, covered with reflective tape and sealed tightly to the base with a rubber band. Air samples (12 ml) were taken from the chamber (headspace volume = 5341 cm^3) 0, 30, 60 and 90 min after closure using a polypropylene syringe inserted through a butyl rubber septum in the chamber top. Gas samples were immediately injected into 9-ml glass vials sealed with butyl rubber septa (Grace Discovery sciences, USA). Measurements were taken between 1000 h and 1400 h to minimize diurnal variations. Gas samples were analysed for CO_2, CH_4 and N_2O concentrations within 1 week of collection using a headspace autosampler (Teledyne Tekmar, USA) connected to a 5,890 gas chromatograph (Agilent/Hewlett-Packard, USA) equipped with a thermal

TABLE 1 Mean aboveground ambient temperature (°C) from unwarmed plots, ambient rainfall and corresponding ≈45% rainfall removal (mm) from June to September period in 2013 for Cloquet and Ely sites. Total values refer to growing season mean ambient temperature and cumulative ambient and reduced rainfall

	Cloquet			Ely		
Month	Ambient temperature (°C)	Ambient precipitation (mm)	≈45% reduction (mm)	Ambient temperature (°C)	Ambient precipitation (mm)	≈45% reduction (mm)
May	8.7	260	260	10.2	171	171
June	15.2	263	145	15.9	128	70
July	18.6	41	23	18.9	111	61
August	18.8	79	43	18.5	122	67
September	14.0	55	30	14.3	58	32
October	5.6	144	144	5.6	68	68
Total	13.5	842	645	13.9	658	469

conductivity detector (TCD) for CO_2, a flame ionization detector (FID) for CH_4 and an electron capture detector (ECD) for N_2O. This system used three 1/8" stainless steel packed columns (ECD and TCD: Porapak Q; FID: Hayesep N) and each run was calibrated with analytical grade standards of CO_2, CH_4 and N_2O (Scott Specialty Gases, Plumsteadville, PA).

The choice of regression model for flux rate determination can be found in Appendix S1. From all measured fluxes, 97% of CO_2, 99% of CH_4 and 99.5% of N_2O fluxes were calculated following a linear model (Matthias, Yarger, & Weinbeck, 1978) with the remaining ones following a quadratic model (Wagner, Reicosky, & Alessi, 1997). Posteriorly, fluxes were scaled up to daily estimates and reported as CO_2 equivalents (mg CO_2 eq ha^{-1} d^{-1}) based on a 100-year time horizon (GWP: 1 for CO_2, 34 for CH_4 and 298 for N_2O, Myhre et al., 2013), which allowed for comparison between the three GHG flux responses. Analytical precision of gas chromatographic measurements and corresponding minimum detectable fluxes (MDFs) of CO_2, CH_4 and N_2O were determined following methodologies described in Parkin, Venterea, and Hargreaves (2012). All soil CO_2 fluxes measured were above the MDF, whereas 98% of CH_4 and 68% of N_2O fluxes were above the MDF. To avoid bias against low fluxes, fluxes below MDF were not discarded, but instead were considered "neutral" fluxes. Greenhouse gas fluxes reported as negative represent net sinks (flux from atmosphere to soil).

2.3 | Soil sampling and physicochemical analyses

Soil temperature and soil volumetric moisture were monitored continuously at each plot (Rich et al., 2015). Here, we report mean values during the 90 min of GHG collection. Soils were sampled for chemical and microbial analyses during two different time periods: at the beginning of the growing season (May), after snow melt, and at the end of the growing season (September), during litterfall and just before the early snowfall. Greater microbial activity was expected during these periods because of higher soil moisture and temperatures at the beginning of the growing season, and higher C inputs to the soil after litterfall and plant development at the end of the growing season (Lammel, Feigl, Cerri, & Nüsslein, 2015; Zinger, Shahnavaz, Baptist, Geremia, & Choler, 2009). One soil core (5 cm diameter, 0–20 cm depth) was collected in each plot and sieved through a 2 mm-mesh sieve after which gravimetric water content was measured. Soil was then transported on ice to the laboratory and stored at 4°C prior to chemical analyses (NH_4^+, NO_3^-, pH and total C and N). Subsamples for DNA extraction were stored at −80°C. The total C and N were analysed for September 2013 sampling only. Soil particle size and total C and N determination were conducted prior to the establishment of climate manipulation (2008) from 0–5, 5–10 and 10–20 cm depth. Full description of methodology used for soil physicochemical analyses can be found in Appendix S1.

2.4 | DNA extraction and quantitative PCR analysis

Total genomic DNA was extracted, using the MoBio PowerSoil DNA Isolation Kit (Mobio Laboratories, Carlsbad, USA) according to manufacturer instructions, with modification of the soil mass used (0.50 g) and the initial cell-lysis step, using a Mini-BeadBeater-8 (Biospec Products, USA) for 120 s. DNA samples were transported to Western Sydney University (Australia) on dry ice for further analysis. Quantification of the phylogenetic 16S *rRNA* gene was determined to assess total bacteria present in the soil using the primer pair Eub338f/Eub518r (Fierer, Jackson, Vilgalys, & Jackson, 2005). Quantification of the functional genes *pmoA* for methanotrophs, *amoA* for AOA and AOB and *nosZ* for N_2O-reducing bacteria were determined using the following primers, respectively: pmo189f/pmo650r (Bourne, McDonald, & Murrell, 2001), crenamoA23f/crenamoA616r (Tourna, Freitag, Nicol, & Prosser, 2008), amoA1f/amoA2r (Rotthauwe, Witzel, & Liesack, 1997) and nosZ2f/nosZr (Henry, Bru, Stres, Hallet, & Philippot, 2006). All primers were purchased from Integrated DNA Technologies, Australia. Full details of gene-specific qPCR primer sequences, thermal cycling programs, qPCR reactions and calibration curve production can be found in Martins et al. (2015). DNA extraction yields and PCR evaluation can be found in Appendix S1.

2.5 | Statistical analysis

Effects of warming, reduced rainfall, site and canopy on GHG fluxes and soil attributes across all time points were assessed by repeated measures linear mixed models analyses. Due to the incomplete factorial nature of this study, canopy condition and warming were analysed separately from warming and reduced rainfall, that is, because the combined climate treatments only took place under open canopy. Therefore, some treatments (OAA, OWA) are graphically repeated to aid visual interpretation of the results. As random effects, replicate plots were nested within time, and blocks were nested within treatments. A linear mixed model approach was followed for each individual time point to assess treatment effects for each monthly measurement, whereby blocks were nested within treatments as random effects. When necessary, data were transformed (logarithm or square root) to improve assumptions of normality and equality of variance. Treatment effects were considered to be statistically significant at $p < .05$ and marginally significant at $p < .1$ given the small number of treatment replicates ($n = 3$) (Drake et al., 2016; Oishi, Palmroth, Johnsen, McCarthy, & Oren, 2014). A Bonferroni post hoc test was used for multiple pairwise comparisons. All statistical analyses were performed with JMP v12.0.0 (SAS Institute).

2.5.1 | Structural equation modelling

Structural equation modelling (SEM, Grace, 2006) was used to build a system-level understanding and evaluate the multiple effects of climate change (warming, reduced rainfall and interaction of warming × reduced rainfall), canopy, soil texture (sand content only), and seasonality, acting via effects on soil temperature and moisture and microbial gene abundances (16S *rRNA* for CO_2, *pmoA* for CH_4 and *amoA* AOA/AOB and *nosZ* for N_2O), on GHG fluxes. Because microbial data were only available for two of the 6 monthly measurements, two different sets of models were evaluated, including:

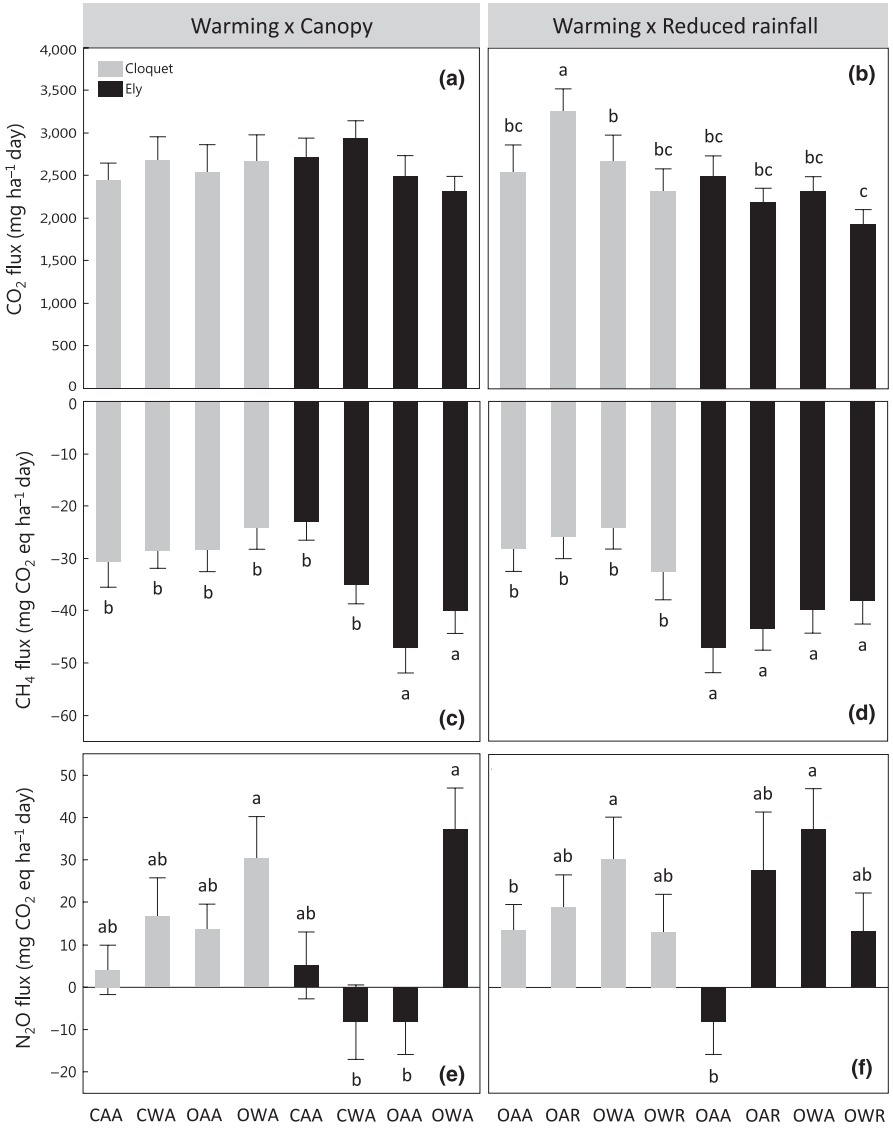

FIGURE 1 Effect of warming × canopy and warming × reduced rainfall on CO_2 (a and b), CH_4 (c and d) and N_2O (e and f) fluxes in Cloquet and Ely sites. Values represent *M* ± *SE* of all time points. Monthly measurements can be found in Figure S1. Statistically significant differences between treatments are represented by different lower-case letters (a–c). CAA, Closed canopy + Ambient temperature + Ambient rainfall; CWA, Closed canopy + Warming + Ambient rainfall; OAA, Open canopy + Ambient temperature + Ambient rainfall; OWA, Open canopy + Warming + Ambient rainfall; OAR, Open canopy + Ambient temperature + Reduced rainfall; OWR, Open canopy + Warming + Reduced rainfall

(1) only abiotic factors from all 6 monthly measurements (full growing season); and (2) both microbial and abiotic factors for a subset of the data (beginning and end of growing season). All SEM analyses were conducted using AMOS 20.0 (IBM SPSS, Chicago, IL, USA). A full description of the methodology applied for SEM can be found in Appendix S1.

3 | RESULTS

3.1 | Effectiveness of climate change treatments and soil site textural differences

Full description of the effectiveness of climate change treatments can be found in Appendix S1, based on variation of soil temperature and moisture throughout the growing season (Tables S1 and S2; Figure S2m–t) and soil site textural differences based on measurements before the climate treatments were initiated (2008; Table S2).

3.2 | Effect of experimental warming and rainfall manipulation, site, habitat and seasonality on GHG emissions and microbial gene abundances

3.2.1 | Carbon dioxide

Overall, the warming and canopy effects detected in CO_2 fluxes were only observed in certain months throughout the growing season (Figure 1a and Figure S2a–d). Even though CO_2 emissions were higher under warming at the start and end of the season (Figure S2b,d), they were lower during mid-summer in Ely, but only under open canopy conditions (Figure S2d). Following the ≈45% rainfall reduction in open canopy conditions, CO_2 fluxes increased by approximately 30% in comparison to ambient control treatments at Cloquet (p = .064; Table S1; Figures 1b and S2c). Overall, CO_2 fluxes were 21% higher in Cloquet than Ely under open canopy conditions (p = .007; Table S1). When warming was combined with reduced rainfall, CO_2 fluxes decreased by 23% in comparison to ambient controls but only in Ely (Figure 1b).

3.2.2 | Methane

Soils were a net sink for CH_4 throughout the growing season. Warming and rainfall manipulations did not significantly affect seasonally averaged CH_4 fluxes at either site. Only in August, the hottest month, significant warming effects (both individual warming and combined warming × reduced rainfall) were detected under open canopy conditions. Hence, a decrease in CH_4 uptake of approximately 59% and 43% in Cloquet and Ely, respectively, was observed in comparison to ambient controls (Figure S2g,h). Overall, CH_4 uptake was significantly higher in Ely, by 29% in comparison to Cloquet (Figure 1c,d; Table S1). In addition to promoting a higher sink, the open canopy in Ely also led to a 33% increase in CH_4 uptake in comparison to closed canopy conditions whereas in Cloquet no significant canopy differences were observed (p = .019; Table S1; Figure 1c).

3.2.3 | Nitrous oxide

Nitrous oxide fluxes were the highest under warming in open canopy conditions by approximately 123% in Cloquet and 556% in the Ely site, in comparison to ambient controls (p = .051; Table S1; Figure 1e,f). This trend was mostly observed from the start of growing season to mid-summer, with all climate treatments declining to neutral fluxes in August, and remaining low until the end of summer (Figure S2i–l). Following rainfall reduction under open canopy conditions, no significant differences were observed from ambient control treatments. However, N_2O fluxes under warming × reduced rainfall were less compared to individual warming effects (p = .012; Table S1; Figure 1f). In general, N_2O fluxes were low throughout the growing season and fluctuated between positive and negative fluxes, suggesting some soil N_2O uptake could be taking place at those times (Figure S2i–l).

3.2.4 | Microbial gene abundance

Soil bacteria, *pmoA*, and AOA *amoA* genes were found to be 30%, 23% and 30% less abundant, respectively, under warming (p = .072; p < .001; p = .059; Tables S1 and S2). *pmoA* was 57% more abundant in Ely in comparison to Cloquet (p = .043; Table S1). Ammonia-oxidizing bacteria on the other hand, were found to be the highest in Ely but lowest in Cloquet under warming, as well as under reduced rainfall (p = .025; Table S1). Overall, *nosZ* gene abundance was highest under reduced rainfall but lowest under warming × reduced rainfall (p = .082; Table S1).

3.3 | Direct and indirect effects of microbial gene abundances and abiotic properties on CO_2, CH_4 and N_2O emissions

3.3.1 | Carbon dioxide

Structural equation modelling explained 40% of the variance of CO_2 emissions and revealed that soil temperature and moisture, throughout a full growing season, had the strongest direct positive effects on measured fluxes (respectively, r = .51 and r = .49; Figure 2a). Warming also drove soil CO_2 flux indirectly via changes in soil temperature (increase) and moisture (decrease). Nonetheless, our SEM analysis showed bacterial gene abundance did not directly affect soil CO_2 emissions. Warming × reduced rainfall combined also supported an indirect negative effect on CO_2 fluxes via soil C. In fact, overall, total C (2013) was the highest under reduced rainfall and lowest in warming × reduced rainfall (p = .044; Table S2), whereas there were no significant differences between plots before the climate treatments were initiated (Table S2). In general, the total standardized effects from SEM also revealed that abiotic factors were major determinants in comparison to bacterial abundance in controlling CO_2 fluxes (Figure 3b; Appendix S1), with both models showing approximately an R^2 = .40, suggesting the additional biotic and abiotic parameters measured did not improve soil CO_2 emissions prediction (Figures 2 and 3).

3.3.2 | Methane

Structural equation modelling explained 20% of the variance of CH_4 fluxes and illustrated that from all climate treatments only warming had an effect on CH_4 fluxes (Figure 2b). Indirect negative effects of warming via soil temperature (thus, indirect positive effects on CH_4 uptake) contrasted the weaker positive direct effect of warming on CH_4 fluxes (thus, direct negative effects on CH_4 uptake), with no direct effects of soil moisture on CH_4 fluxes observed (Figure 2b). Sand content was also positively associated with *pmoA* gene abundance, however the latter did not directly affect soil CH_4 uptake (Figure 3b and Figure S3b; Appendix S1). The SEM analysis also revealed that sand content and soil temperature had the strongest negative direct effects on measured fluxes (thus, a positive direct effect on CH_4 uptake; r = −.37, r = −.30; Figure 2b), also illustrated by the standardized total effects (Figure 2e). Moreover, although a direct significant effect of *pmoA* gene abundance on CH_4 flux was not detected, the corresponding standardized total effects indicated that *pmoA* gene abundance had the second strongest negative total effects on emission (i.e. positive impact on CH_4 uptake) after sand content (Figure 3e). Thus, methanotroph abundance may be an important predictor of CH_4 flux.

3.3.3 | Nitrous oxide

In contrast to CO_2 and CH_4 fluxes, soil temperature and moisture did not have direct effects on N_2O emissions in any of the models considered (Figures 2c, 3c and Figure S3c). Nonetheless, warming had a direct positive effect on N_2O emissions, and similar to CO_2 emissions, warming × reduced rainfall had a negative direct effect on N_2O emissions (Figure 2c). At the start and end of the growing season, warming had a negative direct effect on AOA *amoA* gene abundance, as well as negative indirect effects on both AOA *amoA* and *nosZ* gene abundance via soil temperature (Figure 3c). Similar to CO_2 and CH_4 fluxes, warming × reduced rainfall had a direct negative effect on one of the microbial gene abundances (*nosZ*), soil C and pH (Figure 3c). Nonetheless, soil pH was close to neutral (≈6) in all soils (Table S2). Furthermore, soil C had a positive direct effect on AOB *amoA* gene abundance, and pH had a negative direct effect on AOA *amoA*. Of

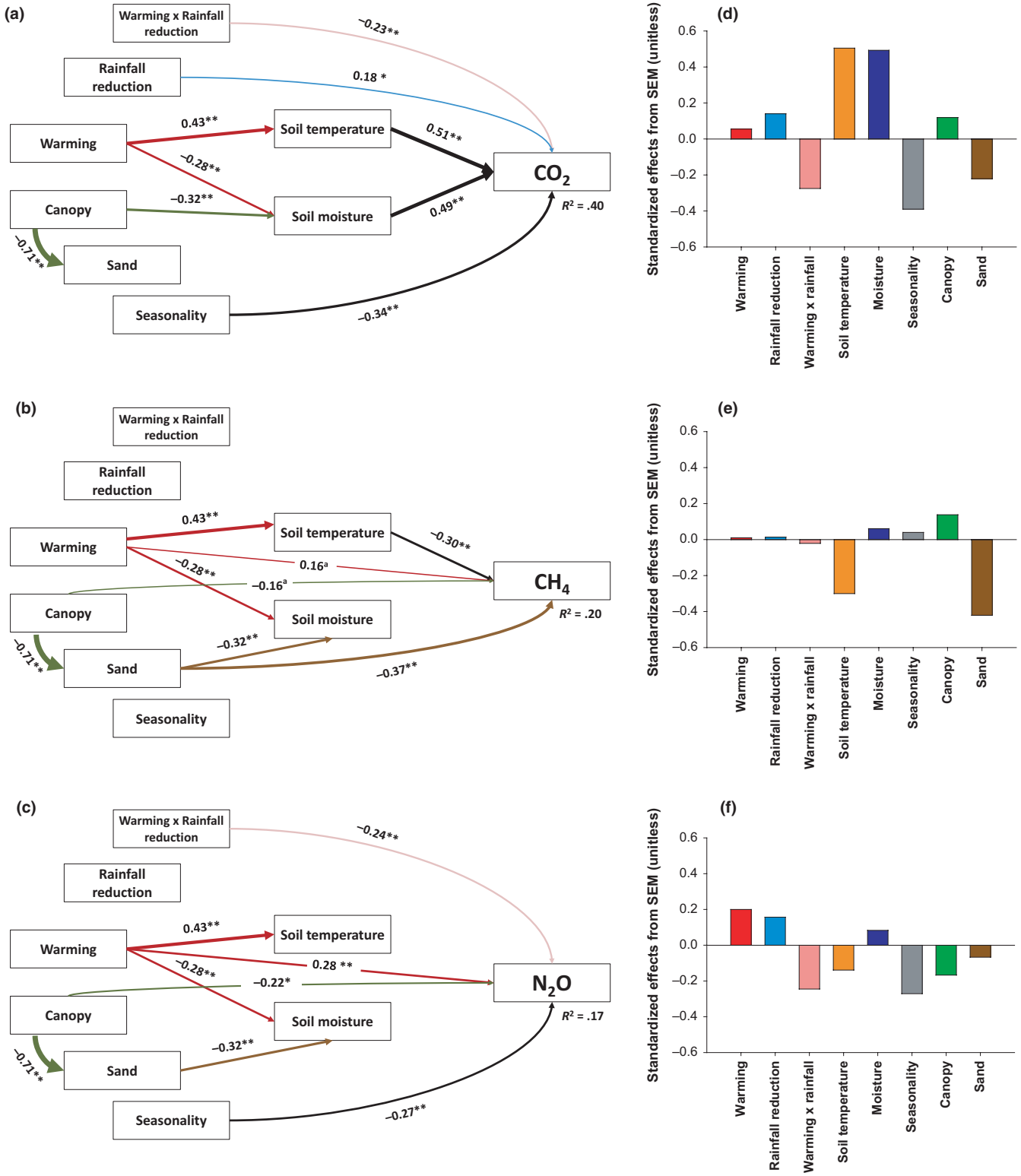

FIGURE 2 Structural equation models for (a) CO_2, (b) CH_4 and (c) N_2O emissions during the full growing season, based on the effects of climate treatments, canopy condition, sand content, seasonality and soil temperature and moisture. Numbers adjacent to arrows are standardized path coefficients, analogous to partial regression weights and indicative of the effect size of the relationship (a–c). Arrow width is proportional to the strength of path coefficients. R^2 indicates the proportion of variance explained. For all models: χ^2 values (χ^2 = 0.00; p = 1.00; df = 8), nonparametric bootstrap (p = 1.00) and $RMSEA$ = 0.00; p = 1.00. Significance levels are as follows: $^{a}p<.1$, $^{*}p < .05$ and $^{**}p < .01$. (d–f) represent the standardized total effects (direct plus indirect effects) derived from the corresponding structural equation model

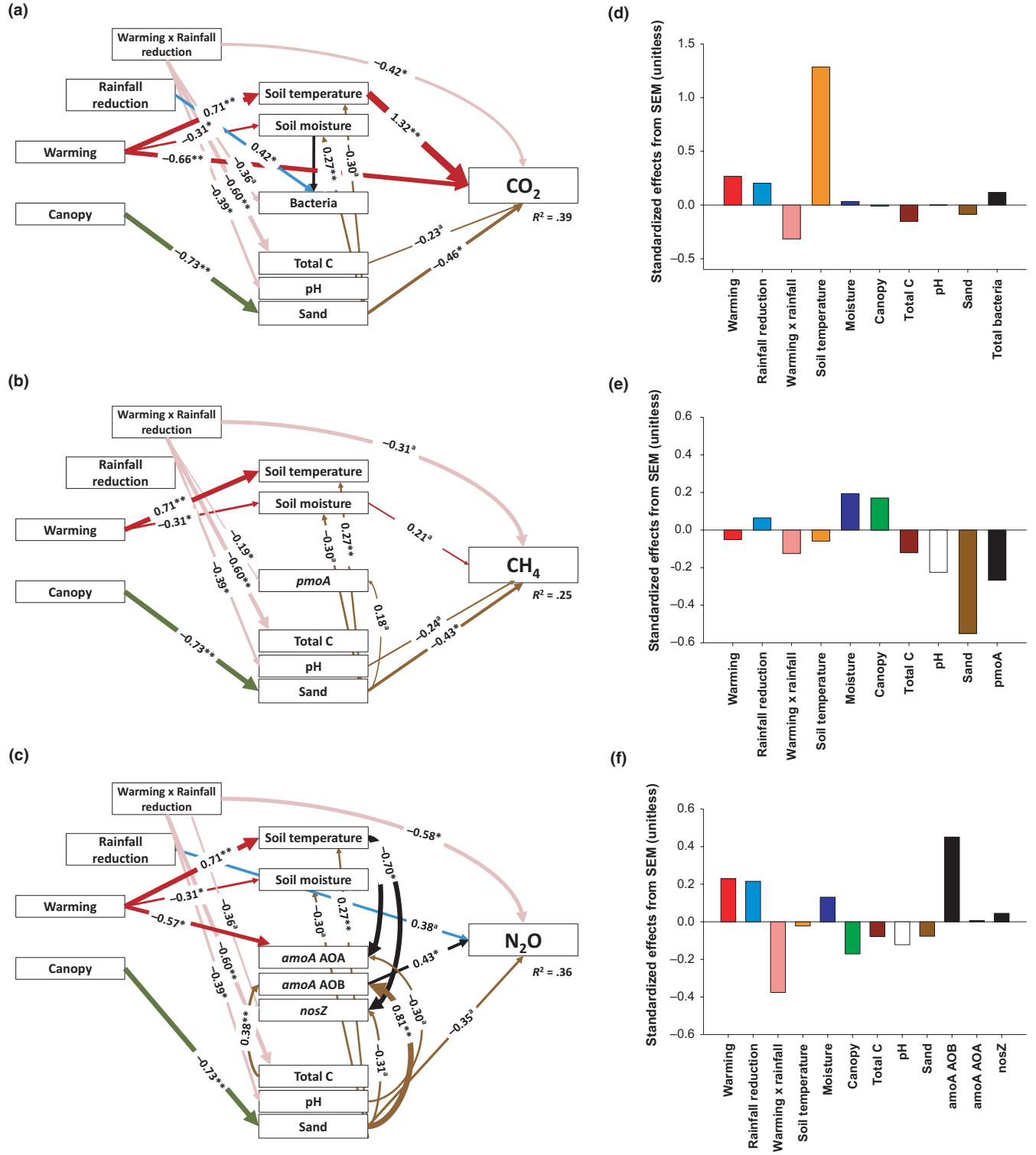

FIGURE 3 Selected structural equation models (from the start and end of growing season data only – see full model in Figure S3) for (a) CO_2, (b) CH_4 and (c) N_2O, based on the effects of climate treatments, canopy condition, sand content and selected soil attributes (abiotic properties and microbial gene abundances). Numbers adjacent to arrows are standardized path coefficients, analogous to partial regression weights and indicative of the effect size of the relationship (a–c). Arrow width is proportional to the strength of path coefficients. R^2 indicates the proportion of variance explained. For all models: χ^2 values ($\chi^2 = 0.00$; $p = 1.00$; $df = 8$), nonparametric bootstrap ($p = 1.00$) and *RMSEA* = 0.00; $p = 1.00$. Significance levels are as follows: $^{a}p < .1$, $^{*}p < .05$ and $^{**}p < .01$. (d–f) represent the standardized total effects (direct plus indirect effects) derived from the corresponding structural equation model

special interest, AOB *amoA* gene abundance was the variable having the highest positive total effects on N_2O, followed by negative total effects of warming × reduced rainfall and seasonality (Figure 3f; Appendix S1). Interestingly, when comparing both models, the variance explaining N_2O emissions in the second model (R^2 = .36) was over twice as much as in the first model (R^2 = .17), suggesting *amoA* AOB gene abundance was the most important measured predictor of N_2O emissions in this study (Figures 2f and 3f).

4 | DISCUSSION

After 5 years of manipulation, warming effects on soil CO_2 emissions were small and largely site- and season-dependent. Warming effects on CH_4 uptake were not detected except during the hottest summer month (i.e. August) when they decreased in comparison to ambient treatments. Our results also show that N_2O responded significantly to long-term (years) effects of warming, with a consistent increase in N_2O emissions at both sites under open canopy conditions. This is supported by recent modelling studies, showing the increase of atmospheric N_2O emissions registered in the last decades to be linked to increasing air temperatures, in addition to N deposition (Tian et al., 2015; Xu, Prentice, Spahni, & Niu, 2012). After 2 years of rainfall reduction, only CO_2 responded with higher emissions in one of the forest sites (Cloquet). This comes as a surprise, since field studies have shown lower CO_2 emissions under rainfall reduction, due to higher dependency of soil respiration to soil moisture (Borken, Savage, Davidson, & Trumbore, 2006; Muhr & Borken, 2009; Schindlbacher et al., 2012). However, since the standard diffusion coefficient for CO_2 is much lower in water than in air; even small changes in water content could affect the diffusion coefficient (Haynes, 2012). On the other hand, when warming was combined with reduced rainfall, both CO_2 and N_2O fluxes were less than when under their individual effects, similar to previous long-term field studies (Cantarel et al., 2012; Schindlbacher et al., 2012). Our results provide evidence that from all the variables measured, abiotic factors are the main determinants of soil CO_2 and CH_4 emissions at these geographically and texturally distinct sites; even though the former is a process carried out by many different soil microbes and plants, whereas the latter is carried out by specialized microbes. Moreover, microbial gene abundance played a more important role in regulating specialized functioning, with the presence of particular bacteria (methanotrophs and AOB, respectively) positively improving CH_4 and N_2O emissions prediction.

4.1 | Direct and indirect effects of environmental parameters regulating CO_2 emissions

Our SEM analysis provides evidence that the small net warming effects observed on soil CO_2 emissions are indirectly determined by changes in abiotic factors such as soil temperature and moisture due to long-term warming manipulation and not a reflection of bacterial abundance variation. The small change in magnitude of CO_2 fluxes under warming after 5 years of manipulation may therefore reflect an acclimation of soil respiration to higher temperatures. In the present field experiment, Eddy (2015) found that heterotrophic (root exclusion) respiration (which accounted for ~80% of soil respiration) was consistently higher under warming throughout 5 years of treatment, in contrast to total soil respiration which decreased after 3 years, suggesting likely acclimation of CO_2 efflux derived from autotrophic root respiration. Similarly, root metabolic rates have been found to decrease under warming, either due to physiological acclimation or induced soil moisture deficits (Burton, Melillo, & Frey, 2008; Wang et al., 2014).

Studies of combined climate conditions are particularly critical to fully understand the feedbacks between climate and the terrestrial biosphere since warming and reduced rainfall in combination should lead to stronger water deficits than warming alone. Our SEM models not only demonstrate a negative effect of those combined treatments on CO_2 emissions but also indirect effects via soil C reduction. Such negative impact could reflect a thermal adjustment of soil microorganisms to increasing temperatures but also a reduction of belowground C pools (Bradford, 2013; Bradford et al., 2008; Kirschbaum, 2004), particularly under increasing desiccation (Follett, Stewart, Pruessner, & Kimble, 2012; Zhang, Wylie, Ji, Gilmanov, & Tieszen, 2010), which in turn may reduce substrate availability for soil respiration.

We also illustrate with the SEM, that bacterial abundance is not a major predictor of soil CO_2 emissions at these sites, consistent with the fact that soil CO_2 emissions result from the activity of many different microbial taxa and root respiration. Schindlbacher et al. (2011), in a long-term field study, similarly showed that warming did not affect microbial biomass or microbial group abundances but did enhance microbial respiration. Supporting this idea, Delgado-Baquerizo, Grinyer, Reich, and Singh (2016), in a microcosm study, showed evidence that soil properties in general, and resource availability in particular, are more important than soil microbial communities in predicting soil respiration. Finally, our models also demonstrate that soil properties, particularly soil texture, directly regulates CO_2 emissions differences derived from site and canopy through moisture availability, since higher CO_2 emissions were observed in Cloquet and under closed canopy conditions, where sand content was lower. Less sand content would result in lower water loss due to less proportion of large pores, and thus increase the capacity for soils to hold moisture from the finer soil particles. Higher moisture should thus promote microbial and root activity.

4.2 | Direct and indirect effects of environmental parameters regulating CH_4 emissions

Our results demonstrate soil particle size (sand content) followed by *pmoA* gene abundance are the main regulators of CH_4 uptake in the boreal-temperate forest soils studied, suggesting gas diffusion and microbial community are the primary drivers of the CH_4 sink in temperate-boreal soils. Contrary to soil respiration, CH_4 consumption is a specialized process (Schimel et al., 2005) conducted by specific microbial communities, which explains the role of *pmoA* in controlling CH_4 emissions. Soils with coarse texture (more sand) will have more

rapid water drainage due to having a higher proportion of macropores and thus contribute to higher gas diffusion. Firstly, CH_4 diffusion rates from the atmosphere into the soil profile will be enhanced, and thus increase substrate availability for methanotrophy to occur. This may explain why we found a direct effect of sand content on methanotroph abundance, resulting in an increase of *pmoA* gene under those conditions. Secondly, higher air-filled porosity will increase oxygen diffusion into the soil microsites and as a result favour aerobic methanotroph activity (Conrad, 2005; Dijkstra et al., 2012; Le Mer & Roger, 2001). Accordingly, we found greater CH_4 uptake in the site with higher sand content (Ely), particularly under open canopy conditions, with SEM showing sand as the strongest environmental parameter predicting CH_4 fluxes. A meta-analysis by Dutaur and Verchot (2007) supports our findings, showing soil texture is a strong predictor of CH_4 uptake rates. Nonetheless, our models only explain approximately 25% of the variance found in CH_4 fluxes, suggesting other variables, such as methanotroph community composition and diversity were not accounted for.

Although CH_4 oxidation rates were not statistically altered after 5 years of climate manipulation, SEM analysis demonstrated warming has a positive impact on CH_4 uptake, by increasing the CH_4 sink when soil moisture is not limiting. However, when soil moisture decreases to levels below optimum methanotrophic activity, warming has the opposite effect, leading to a significant reduction of CH_4 uptake. Methane uptake increase due to warming occurred particularly in closed canopy in Ely (site near the colder range limit), which has 73% higher clay content. Blankinship et al. (2010), using a soil transfer approach to warmer latitudes (+1.8°C increase) showed contrasting results where warmer and drier ecosystems compared to colder ecosystems exhibited a reduction in CH_4 uptake with increasing temperatures. Soils with higher clay content tend to have higher water retention, constraining gas diffusion and hence CH_4 uptake (Dijkstra et al., 2012). This suggests under higher temperatures and optimum moisture levels, CH_4 oxidation sensitivity to soil temperature is enhanced, similar to CO_2 emissions. However, soil temperature may indirectly lead to inhibition of methane oxidation due to increased osmotic stress, as observed in the hottest summer month (Conrad, 1996; Jäckel, Schnell, & Conrad, 2001; Khalil & Baggs, 2005; Striegl, McConnaughey, Thorstenson, Weeks, & Woodward, 1992). The optimum soil water content is thus thought to reflect the balance between gas transport rates and microbial physiological water stress (Luo et al., 2013). Thus, under future warming, CH_4 uptake in boreal-temperate forests may be limited by methanotroph activity, similar to what is found in dry ecosystems (Dijkstra, Morgan, von Fischer, & Follett, 2011).

4.3 | Direct and indirect effects of environmental parameters regulating N_2O emissions

Our SEM approach show that higher N_2O emissions under warming in open canopy are explained by AOB over AOA, instead of abiotic parameters. Overall, AOA's preference for acidic soils (Gubry-Rangin et al., 2011; Hatzenpichler, 2012), could explain the present functional dominance of AOB over AOA, since our soils were close to neutrality (pH 6). In particular, such functional advantage under warming could be due to selection of specific AOB lineages by higher temperatures, since temperature has been shown to be an important driver of AOB distributions (Fierer, Carney, Horner-Devine, & Megonigal, 2009). Furthermore, SEM analysis also shows that the soils under open canopy, due to the presence of higher sand content, indirectly favored higher AOB abundance while simultaneously constraining *nosZ*. The stronger biotic regulation of increasing N_2O emissions under warming in open canopy conditions over abiotic factors can also be explained by the direct negative effect of soil temperature on both AOA *amoA* and *nosZ* gene abundances while no direct effect was observed between soil temperature and N_2O fluxes. Such disadvantage of N_2O-reducing bacteria over AOB may be related to soil oxic conditions, paramount for ammonia oxidizer and denitrifier activity which are, respectively, aerobic and anaerobic by nature. The soils under warming in open canopy conditions are suggested to support higher gas diffusivity since both coarse texture (higher sand content) and soil temperature are capable of promoting oxic conditions, which in turn may constrain *nosZ* gene abundance due to the anaerobic nature of N_2O-reducing bacteria. In fact, higher oxygen levels are known to reduce N_2O reductase activity, due to its high sensitivity to oxygen availability (Richardson, Felgate, Watmough, Thomson, & Baggs, 2009). Although N_2O reduction to N_2 via the denitrification pathway is thought to make a small contribution to global net N_2O emission balance (Syakila, Kroeze, & Slomp, 2010), N_2O-reducing bacteria can be important players at the soil microsite level by acting on the N_2O produced (Spiro, 2012). Conversely, the mechanism by which AOA is negatively impacted by warming (soil temperature) is less clear. Nonetheless, some studies have reported a negative response of AOA towards higher temperatures (Jung et al., 2011; Szukics et al., 2010).

The combined warming and reduced rainfall effects on N_2O emissions were less than individual effects of warming, suggesting an offset of N_2O production. This could be due to (1) indirect effects of soil C reduction observed under this treatment by reducing AOB metabolic activity and abundance as exemplified by our SEM model, and to (2) simultaneous inhibition of *nosZ* abundance due to higher oxygen levels under increasing water deficit. The results presented here are in agreement with a recent microcosm experiment highlighting the importance of the bacterial community vs. soil properties in driving N_2O emissions (Delgado-Baquerizo et al., 2016). Thus, similar to CH_4 consumption, and different from soil respiration, ammonia oxidation and heterotrophic denitrification are specialized microbial processes, which explains the critical role of functional genes in controlling N_2O emissions (Schimel et al., 2005).

5 | CONCLUSIONS

Altogether, our study shows that CO_2 flux (a broad ecosystem process) variation due to warming and reduced rainfall manipulation, site, habitat and seasonality were determined mostly by abiotic factors such as soil temperature and moisture and less by microbial abundance. On the contrary, the specialized process of CH_4 consumption

was mostly limited by gas diffusivity (via soil texture) and methanotroph gene abundance. Similarly, higher N_2O emissions observed under warming are suggested to be a response of AOB having an advantage over other microbial functional groups. Finally, an offset of CO_2 and N_2O feedback responses under combined climate treatments is suggested to be due to increased water deficit and thus loss of substrate availability to microbial communities. These results suggest that microbial abundance becomes more important in specialized ecosystem processes, particularly when performed by multiple specialized groups, such as in N_2O emissions; whereas when there is an ecological dominance of particular taxa over a process rate, such as CH_4 oxidation by methanotrophs, environmental factors increase in importance in predicting flux rates. Disentangling those mechanisms and the conditions under which they are likely to be dominant should be the focus for global change science today and in the near future.

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AUTHORS' CONTRIBUTIONS

C.S.C.M., L.N., C.A.M., I.C.A., P.B.R. and B.K.S. conceived the ideas and designed methodology; C.S.C.M., L.N. collected the data with S.E.H. and R.T.V. support; C.S.C.M. and M.D.B. analysed the data with input from B.K.S. and P.B.R.; C.S.C.M. led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

DATA ACCESSIBILITY

Data available from the Dryad Digital Repository https://doi.org/10.5061/dryad.41n6g (Martins et al., 2017).

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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