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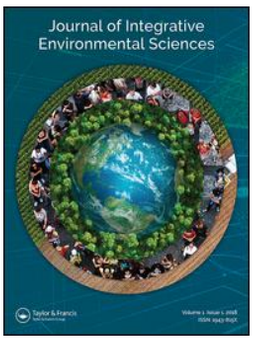

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No effect of warming and watering on soil nitrous oxide fluxes in a temperate sitka spruce forest ecosystem

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ABSTRACT

Soil fluxes of nitrous oxide (N_2O) play an important role in the global greenhouse gas budget. However, the response of soil N_2O emissions to climate change in temperate forest plantations is not yet well understood. In this study, we assessed the responses of soil N_2O fluxes to experimental warming with or without water addition, using a replicated *in situ* heating (~2°C above ambient) and water addition (170 mm) experiment in a temperate Sitka spruce plantation forest over the period 2014–2016. We found that seasonal fluxes of N_2O during the year were highly variable, ranging from net uptake to net emissions. Seasonal variations in soil N_2O fluxes were not correlated with either soil temperature or soil moisture. In addition, none of the individual warming/watering treatments, or their interactions, had significant effects on soil N_2O fluxes and N-related soil properties. Overall, our results suggest that despite future increases in temperature, soil N_2O emission may remain largely unchanged in many temperate forest ecosystems that are often N-limited.

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KEYWORDS
Climate change; rainfall manipulation; greenhouse gas; warming; sitka spruce

1 Introduction

For the past two centuries anthropogenic activities, such as increased fossil fuel combustion, deforestation, and land use change, have resulted in increased atmospheric concentrations of greenhouse gases (GHG), including carbon dioxide (CO_2), methane (CH_4), nitrous oxide (N_2O), ozone (O_3), and chlorofluorocarbons (CFCs) (IPCC 2013). The available evidence indicates that this will result in an increase in global air temperatures by 1.1–6.4°C by the end of this century, together with altered precipitation frequency and intensity at both regional and global scales (IPCC 2013). The mean precipitation is generally thought to increase in tropical regions and at high latitudes, but decrease in the subtropics (IPCC 2013); therefore, ecosystems in temperate regions may be subject to increases in precipitation in the future. Inevitably, in the absence of any input of water, an increase in soil temperature will result in a decrease in soil water availability. Any future changes in temperature are, therefore, inextricably linked with changes in water availability, although

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these are not always considered together and it is unclear what the combined effects of climate warming and alterations in precipitation will have on ecosystem processes and GHG emissions.

Whilst there have been a number of studies examining the effects of warming and altered precipitation on soil CO_2 fluxes (Loik et al. 2004; Angert et al. 2005; Breshears et al. 2005; Ciais et al. 2005; Liu et al. 2009; Wang et al. 2014; Bond-Lamberty et al. 2018; Zou et al. 2018), less is known about their impact on soil N_2O fluxes. Due to its large global warming potential (GWP, each kg of N_2O has the potential to contribute about 300 times as much to the greenhouse effect as each kg of CO_2), the increase in atmospheric concentrations of N_2O may have a stronger impact on climate change over the next century. Whilst the emissions of N_2O have increased in the last few decades, this was largely due to increased fertilizer use in agriculture (Skiba and Smith 2000), suggesting that this has been driven by increased soil N availability. In natural or unfertilized ecosystems that are often deficient in N other factors such as soil temperature and/or soil water availability, could be more important.

The production of N_2O is driven by microbial metabolism through the processes of nitrification (oxidation of NH_4^+ to NO_3^- via NO_2^-) and denitrification (reduction of NO_3^- to N_2O and N_2) (Robertson and Groffman 2006), which are largely thought to be regulated by inorganic N content, soil organic matter, soil temperature and soil moisture (Skiba and Smith 2000). Changes in temperature and moisture availability have strong impacts on microbial activity and hence N_2O emissions, but this can be highly dependent on substrate availability (Chapuis-lardy et al. 2007; Abdalla et al. 2010; Muñoz et al. 2010; Manzoni et al. 2012). Previous experiments have shown variable warming-related N_2O responses, ranging from positive (Liu et al. 2015; Griffis et al. 2017), to negative (Smith et al. 1998; Hall et al. 2012; Liu et al. 2016), or even no effect (Xu et al. 2004; Dijkstra et al. 2012; Unteregelsbacher et al. 2013).

The extent of N_2O emissions from forest soils is confounded by large spatiotemporal variations (Kesik et al. 2006; Butterbach-Bahl et al. 2013; Benanti et al. 2014), though they have sometimes been considered to be an important source of N_2O to the atmosphere, together with arable soils (Butterbach-Bahl et al. 1997; Pilegaard et al. 2006). A recent study of 13 different coniferous and deciduous forests showed that, in general, forests from Sweden to Italy and Spain on mineral soils that were previously unfertilized and uncultivated had small emissions of N_2O that were, on average, only 0.5 kg N_2O ha^{-1} yr^{-1}. However, higher N_2O fluxes of 14.9 kg N_2O ha^{-1} yr^{-1} and 13.3 kg N_2O ha^{-1} yr^{-1} (up to 31.4 kg N_2O ha^{-1} yr^{-1}) have been found for forest soils that had been cultivated and fertilized prior to afforestation (Gundersen et al. 2012). Due to the uncertain magnitude of N_2O emissions, further studies are required to fully understand the mechanisms that control N-transformations in forest soils.

Sitka spruce [*Picea sitchensis* (Bong.) Carr.] is the main species used for afforestation and reforestation activities in Ireland and the focus of GHG offsetting activities through enhanced C sequestration (DAFF 2007). Clearly, significant N_2O emissions from forest ecosystems could compromise the GHG mitigating capabilities of afforestation, which could be further influenced by rising temperatures/modifications in rainfall. However, less attention has been directed at N_2O emissions from forest soils and there is little, if any, information on how climate change-associated modifications in temperature and/or water availability may affect soil N_2O fluxes, particularly in cool temperate forest

ecosystems. In this study, we investigated the responses of soil N_2O fluxes to artificially increased temperatures and variations in water availability, as well as a combination of the two factors, in an Irish Sitka spruce forest plantation. In these experiments, soil N_2O fluxes and associated microclimate parameters were measured in forest plots over a period of 24 months. The major objectives of this study were to assess the effect of atmospheric warming on soil N_2O fluxes and how this could be further impacted by alterations in water availability, together with the likely abiotic or biotic drivers.

2 Materials and methods

Experimental site

The field study area was located within Dooary Forest, Co. Laois, Central Ireland (52°57′N, 7°15′W; altitude of 260 m). The establishment of the forest and its management is under the control of Coillte, a semi-state company involved in forest-based land management. The forest was planted in 1988 on a previously unmanaged grassland, with a planting density of 2300 stems ha $^{-1}$, and currently consists of even aged stands of one main species, Sitka spruce. The size of the forest covers a total area of 42 ha and is made up of two management compartments, which are 25.8 ha and 16.2 ha in size, respectively. The measurements for this investigation were conducted in the 25.8 ha stand near an eddy covariance tower used for long-term (2002 -) measurements of C fluxes and climatic factors, as part of a long-term forest C sequestration and greenhouse gas emissions study. The climate for this region is cool temperate, with a mean annual temperature and precipitation of 9.9°C and 857 mm (1978–2007), respectively. The soil type is a wet mineral gley. The forest stand has been thinned four times, in 2006, 2008, 2012 and 2015, resulting in a more open canopy. Little understory vegetation was present except for some moss and fungi. However, some herbaceous vegetation was present in the thinning lines where trees were removed and the forest floor was open to receive radiation and rainfall directly. More information on the site and the measurements can be found in (Saunders et al. (2012), Saunders et al. (2014)) and Zou et al. (2018).

Experimental design

The experiment used a paired nested design with warming as the main factor and watering as a secondary factor. The main experimental treatments for this study were an ambient control (C), warming (W), water addition (P), and warming plus water addition (WP). Table S1 provides detailed information of the experimental design. In order to simulate future climate warming, the plots were warmed continuously using IR heaters (165 × 15 cm, MSR-2420, Kalglo Electronics Inc., Bethlehem, PA, USA), suspended 1.2 m above the soil surface. "Dummy" heaters made of aluminium with the same shape and size as the IR heaters were suspended at the same height. There were three replicate plots (each with an area of 2 × 2 m) for the warming and control treatments. Each of the warming and control plots were divided into two 2 × 1 m subplots. One subplot of each replicate was watered (~15 mm of water) approximately twice a month from May to October. The overall objective was to elevate soil temperature by ~2°C, consistent with many climate change projections and the amounts of water added each year (170 mm in

total each year) represented approximately 20% of the annual precipitation for this region. Please refer to Zou et al. (2018) for more information on the experimental treatments.

Field measurements

From March 2014 to February 2016, soil N_2O fluxes were measured approximately bi-weekly using a 1412 Photoacoustic Field Gas Monitor (PAS, INNOVA Air Tech Instruments, Ballerup, Denmark), and soil temperature and moisture at 5 cm depth were also determined in conjunction with each gas measurement using a portable sensor (WET sensor Delta-T Devices Ltd, Burwell, Cambridge, UK). For the measurements, the PAS was connected to a static chamber (16 cm inside diameter, 16 cm in height) placed on top of a permanently installed collar in each plot via 6 m plastic tubing of 3 mm diameter, allowing access to all plots. Before the actual flux measurements, ambient air was sucked into the PAS for about 30–40 minutes, until the readings for N_2O concentrations were stabilized. For each flux measurement, the first 3–4 readings were excluded after the chamber was closed and the next 5–6 PAS readings were subsequently used to calculate the flux rate, which took approximately 8–10 minutes Two additional readings of ambient air were also taken after the chamber was opened in between the flux measurements for about 2 minutes. Therefore, the total time required per plot was around 10 minutes, excluding the time required to move from one plot to the next. Air temperature and relative humidity (RH) were continuously monitored using a HC2S3-L probe (Campbell scientific Inc., UK) at 25 cm above ground level in each warming and control plot. Other data, such as precipitation, were obtained from a nearby meteorological station attached to an eddy covariance (EC) tower deployed on the same site (see Saunders et al. 2012, 2014).

Calculation of flux rates

Flux rates were calculated from the change in N_2O concentration with time (slope in ppm s^{-1}). Linear regressions of N_2O concentration against time were used to generate the slope and the flux rate calculated from the linear increase or decrease in gas concentration over time for each chamber. Quality checks were applied for all flux measurements: the R^2 value of the linear regression analysis had to be larger than 0.7, otherwise, the data were discarded. Flux rates in ppm s^{-1} were converted to μmol m^{-2} s^{-1} or kg ha yr^{-1} using the following equation.

$$\mu mol\ GHG\ m^{-2}s^{-1} = \frac{ppm\ GHG\ s^{-1} \times P \times V}{\frac{R}{1000} \times T \times A}$$

where

P = barometric pressure, e.g. 1 atm

T = air temperature within the enclosure during sampling in K (K = °C + 273.15)

R = the universal gas constant (0.0820575 L atm./K mol)

V = the internal volume of the chamber (enclosure) (m^3)

A = the soil area enclosed by the chamber (m^2)

Soil analysis

Soil samples (n = 3) were collected at 0–10 cm depth, near each chamber and subsequently mixed well to generate one composite sample. Soil pH was measured using water extracts with a pH meter/probe (Thermo Fisher Scientific Inc., Waltham, Michigan, USA). Soil ammonium (NH_4^+) and nitrate (NO_3^-) concentration, and dissolved C and N were determined on 5 g samples of fresh soil based on a 2 M KCl solution extraction procedure. Soil ammonium (NH_4^+) and nitrate (NO_3^-) concentrations were measured using a nutrient analyser (Lachat Quickchem®, 5600 Lindbergh Drive, Loveland, Colorado, USA). A sub-sample of the extracts was used to determine dissolved total N concentrations (DTN) with a TOC/TN analyser (TOC-V Shimadzu Corp. Tokyo, Japan). Soil microbial N (MBN) concentrations were determined using the chloroform fumigation-extraction method (Robertson et al. 1999). The soil MBN concentrations were calculated from the differences between fumigated and non-fumigated samples divided by 0.45 (Wu et al. 1990).

Statistical analysis

Cumulative N_2O fluxes for each treatment were calculated by summing the product of N_2O flux and the number of days between measurements. A repeated-measures split-plot ANOVA was used to test the main and interactive effects of warming and water addition on soil N_2O fluxes, soil temperature, and soil moisture. The effects were considered to be significantly different if $P < 0.05$. Comparisons of means were further carried out using Tukeys post-hoc test if the effects of treatments are statistically significant. Regression analysis was used to investigate relationships among soil N_2O fluxes, soil temperature and soil moisture. All these statistical analyses were performed using SAS software v9.3 (SAS Institute Inc., Cary, NC, USA).

3 Results

Impact of soil warming and water addition on soil N_2O fluxes and soil properties

Warming increased the mean air temperature by ~0.8°C (Figure 1) and the mean soil temperature by ~1.7°C (Table 1) in the forest plantation during the study period 2014–2016. In contrast, warming significantly ($P < 0.05$) reduced the mean soil moisture content by ~5% over the whole warming period (Table 1). Watering only significantly affected the soil moisture content (Table 1). The measured soil variables were generally not significantly influenced by warming or watering (Table S3).

Overall, the soils were a net N_2O sink during 2014–2016, although there were periods when there were both positive and negative values (Figure 2). There were more dates in 2014 with net soil N_2O emissions compared to 2015, when the soils generally became a sink for N_2O (Figure 2). Measurements of N_2O fluxes conducted at the start of the warming period indicated small variations (before June 2014, variations less than 15%)

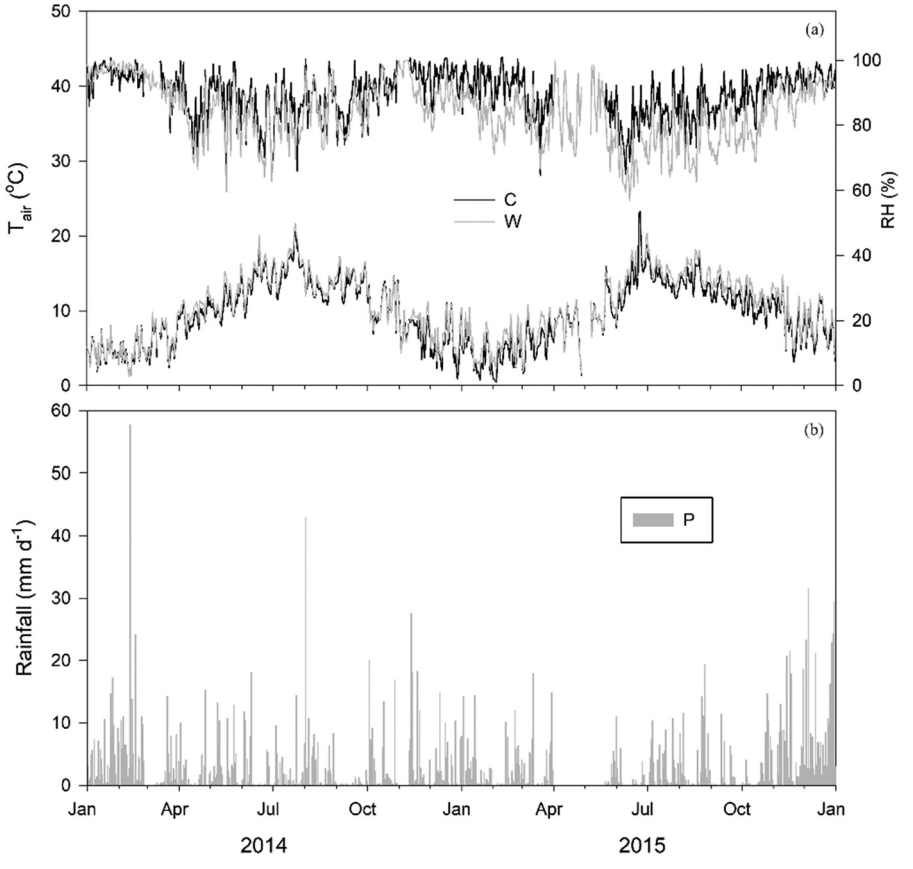

Figure 1. Seasonal variations in (a) daily air temperature (T_{air}, lower line and relative humidity (RH, upper line) for control (C) and warmed (W) plots in the forest and (b) seasonal variations in daily rainfall.

Table 1. Statistical significance (*P*-values) of the split-plot ANOVA for the effects of warming (W) and watering (P), and their interactions on the different variables. T, soil temperature, M, soil moisture; Df = degrees of freedom.

Factor	Df	T	M	N_2O
Warming (W)	1	<0.001	<0.001	0.596
Watering (P)	1	0.592	<0.001	0.410
W × P	1	0.727	0.645	0.474

among different experimental plots. Experimental warming did not significantly affect the mean N_2O fluxes across the study period ($P > 0.05$, Table 1).

Overall, seasonal variations in soil N_2O fluxes showed no significant relationships ($P < 0.05$) with soil temperature or soil moisture (Figure 3).

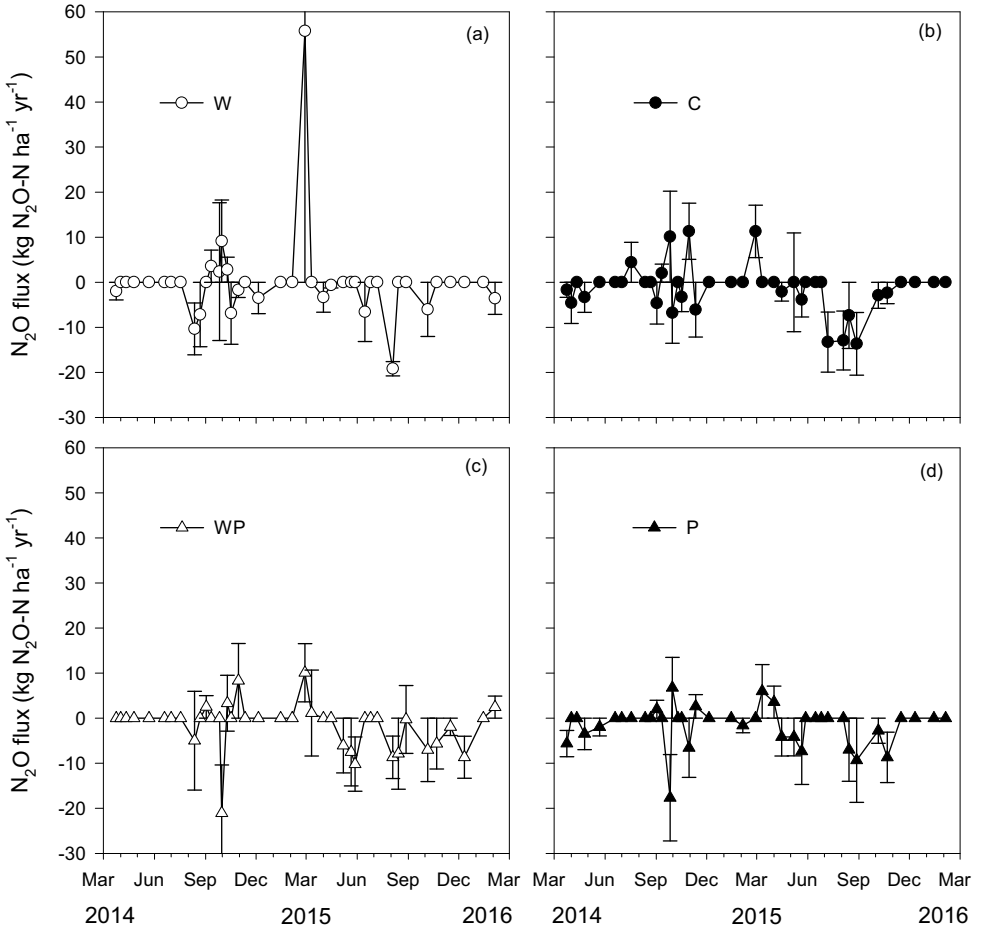

Figure 2. Seasonal variations in soil N_2O fluxes for (a) warmed, (b) control, (c) warming + watered and (d) watered plots.

Cumulative soil N_2O fluxes

The cumulative soil N_2O fluxes were −1.14 ± 0.41, −0.57 ± 0.49, −0.97 ± 0.33, −1.43 ± 0.29 kg N_2O-N ha^{-2} for the C, W, P and WP plot, respectively, over the course of the experiment. Although there was some evidence for a reduced sink for N_2O in response to warming, this was not significant. Also, no significant impact of watering on cumulative soil N_2O fluxes ($P > 0.05$) was found (Figure S3).

4 Discussion

In this study, IR heaters increased the mean air temperature by ~0.8°C (Figure 1) and the mean soil temperature by ~1.7°C (Table 1) during the two years, 2014 and 2015. Conversely, warming reduced soil moisture by ~5%. Rather surprisingly, neither warming nor watering had a significant impact on soil N_2O fluxes or soil chemical properties in this forest plantation. This is consistent with another climate simulation study conducted in

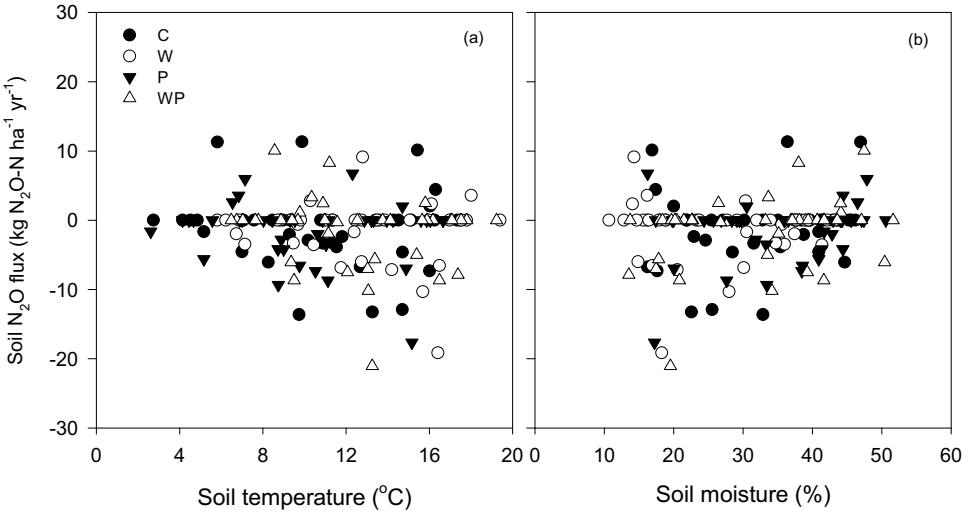

Figure 3. Relationships between soil N_2O fluxes and (a) soil temperature and (b) soil moisture for the different treatments. C, control; W, warming; P, watering; WP, warming + watering.

a montane grassland, where N_2O emissions (~0.3 kg N_2O-N m^{-2} yr^{-1}) remained small and unaffected by warming (Unteregelsbacher et al. 2013). Mean soil fluxes of N_2O over the course of this experiment ranged from −0.63 to 0.03 kg N_2O-N ha^{-1} yr^{-1} across the study period (Figure 1), falling well within the reported range (mostly from −1.1 to 3.4 kg N_2O-N ha^{-1} yr^{-1}) of mean annual N_2O fluxes from 13 temperature forest sites measured during the NitroEurope project (Gundersen et al. 2012). No relationships were found between the N_2O fluxes and any soil properties, including C and N concentration. Given that many studies have linked variations in N_2O emissions to differences in soil N availability these results may suggest that the absence of an effect of warming and/or watering might be due to the low and unchanged soil C and N properties.

The results of this study showed major intra-annual variations in soil N_2O fluxes, from net uptake to net emissions (Figure 2). This is in line with other studies that found that soils can intermittently switch between a sink and an N_2O source (Brummell et al. 2012; Benanti et al. 2014), depending on the conditions favourable for nitrification or denitrification (Chapuis-lardy et al. 2007; Muñoz et al. 2010). The cumulative N_2O fluxes indicated that the soils in this Sitka spruce forest plantation generally appeared to be net sinks for this gas. Whether this represents a true sink for N_2O or is due to the increased conversion of N_2O to N_2 gas (apparent sink) as indicated in some studies (Yu et al. 2000; Wrage et al. 2004) is yet to be determined. An uptake of N_2O has also been recorded from other forest soils, with similar links observed between N_2O fluxes and available nitrogen (Rosenkranz et al. 2006; Goldberg and Gebauer 2009); however, the influence of these factors seems to vary between experiments and no clear set of conditions that would favour negative fluxes from different soil types has been established. It remains plausible that various microbial processes in soils are able to remove N_2O from the atmosphere; however, the mechanisms and triggers for N_2O uptake need to be studied further in order to understand these processes in more detail (Chapuis-lardy et al. 2007). Furthermore, the spatial variability of N_2O emissions associated with the static chamber method was reported to

be very high, ranging from ~44 to ~175 kg N_2O-N ha^{-1} yr^{-1} for the same sampling day in a previous investigation (Benanti et al. 2014) conducted in a different area of the same forest plantation. Clearly, a better assessment of the variability in N_2O fluxes and the underlying drivers is required at this and similar forest sites in order to provide improved GHG budgets and their response to climate warming.

Interestingly, none of the individual warming/watering treatments nor their interactions had significant effects on soil N_2O fluxes (Table 1, Figure S3). These results seem to contradict the findings of Griffis et al. (2017) where information from a 6-year continuous monitoring data set, combined with inverse modelling, suggested that warmer and wetter conditions enhanced N_2O emissions. Bai et al. (2013) synthesized results from eight warming manipulation studies, which suggested that warming could increase soil N_2O emissions, but the results were not statistically significant. Six of the eight studies included in their analysis used a higher temperature increase compared to our study, while the other two studies with a similar temperature increase to our study showed consistent non-significant warming effects on soil N_2O fluxes. These results, together with our study might indicate that higher temperatures are required to induce a significant impact. Another important factor is the warming duration. In this study, warming was conducted over a two-year period and the results may not reflect the effects of longer periods of warming. Interestingly, a recent study of the effect of long-term warming on CO_2 fluxes has indicated that this can vary during different stages of the warming treatment, with periods where there are significant emissions and other periods where there is little or no effect (Melillo et al. 2017).

Warming can also enhance evapotranspiration rates, reducing soil water availability (De Boeck and Nijs 2011), which is also the case in this study (Table 1). It has been reported in many studies, where soils were subjected to experimental warming, that there is a strong relationship between N_2O fluxes and soil water content (Dobbie and Smith 2001; Bateman and Baggs 2005; Werner et al. 2011). However, in our study little of the variation was explained by soil water content or temperature, suggesting that these are not the major drivers. One of the key factors regulating the magnitude of denitrification is O_2 availability (Werner et al. 2011; Hall et al. 2012; Jarecke et al. 2016). Variations in water addition/availability can reduce O_2-availability/diffusion into the soil and bacteria may use nitrate as an alternative electron acceptor under these conditions (Knowles 1981; Firestone and Davidson 1989), leading to N_2 rather than N_2O being the major end product of denitrification, thus resulting in an increased N_2: N_2O ratio (Firestone and Davidson 1989; Davidson 1991; Scholefield et al. 1997). Typically, the soil moisture at this experimental site remains at a high level (>20% even during the summer months) thus the changes in water availability due to our watering treatments may not have been high enough to result in any modifications in soil N_2O emissions.

Many authors have also reported links between observed net negative N_2O fluxes at the soil surface and very low concentrations of inorganic N, even in soils with an appreciable total N content (Ryden 1983; Clayton et al. 1997; Wagner-Riddle et al. 1997; Khalil et al. 2002), because insufficient inorganic N is available for conversion to N_2 O. Consequently, N_2O may be the only electron acceptor left for denitrification due to the low concentrations of soil NO_3^- found in our site (Butterbach-Bahl et al. 1998; Goossens et al. 2001; Rosenkranz et al. 2006). A recent study has also reported that there may be little effect of nitrate on N_2O emissions until these are >1 mg g^{-1} (Parn

et al. 2018). As the soils in our study site had relatively small concentrations of NO_3^- (<2.5 mg kg^{-1}) regardless of the treatments, the emissions are likely to be limited by N availability, so that alterations in moisture or temperature would have had little impact. Given the low soil N availability in our study site, this may also have favoured N_2O "consumption" (Ryden 1983; Khalil et al. 2002; Rosenkranz et al. 2006). Whilst the reason(s) for actual or "apparent" net N_2O consumption has not been systematically studied, the results available so far (Mengis et al. 1997; Mühlherr and Hiscock 1998; Cavigelli and Robertson 2001; Butterbach-Bahl et al. 2002; LaMontagne et al. 2003; Punshon and Moore 2004) suggest that it might be important in several ecosystems and this also needs further consideration.

5 Conclusions

Our study provides evidence that, overall, soils in this forest site showed little emissions of N_2O, whether they were warmed or not, although peaks in emissions were seen on several dates. The reason(s) for these transitory emissions are not known and this warrants more attention. Changes in temperature and moisture availability had no significant impact on soil N_2O fluxes, most likely due to the overriding effect of low soil N availability in this ecosystem, given that N availability is often the major driver. Importantly, these results suggest that N_2O emissions will, at least in the short term, be unaffected by climate warming, or variations in water availability, over the range of values found in this study. However, a greater focus may need to be directed at an assessment of spatiotemporal variability in these and other forest ecosystems before coming to any general conclusions. As future atmospheric N_2O concentrations may also depend on the extent of N inputs, including atmospheric deposition, as well as ecosystem/soil properties, more studies across a broad geographical range are needed to explore interactions among global change factors and gaseous emissions.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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