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How does elevated ozone reduce methane emissions from peatlands?

Sylvia Toet[a], Viktoria Oliver[a,1], Phil Ineson[b], Sophie McLoughlin[a], Thorunn Helgason[b], Simon Peacock[c,2], Andrew W. Stott [d], Jeremy Barnes[c] & Mike Ashmore[e]

[a] Environment Department, University of York, York, YO10 5NG, UK

[b] Department of Biology, University of York, York, YO10 5DD, UK

[c] School of Biology, Newcastle University, Newcastle upon Tyne, NE1 7RU, UK

[d] Natural Environment Research Council Life Sciences Mass Spectrometry Facility, Centre for Ecology and Hydrology, Lancaster, LA1 4AP, UK

[e] Stockholm Environment Institute, University of York, York, YO10 5NG, UK

Corresponding author: Sylvia Toet

tel. +44 1904 324018

fax +44 1904 432998

e-mail: sylvia.toet@york.ac.uk

[1] present address: The School of Biological Sciences, Aberdeen AB24 2TZ, UK

[2] present address: School of Agriculture, Food and Rural Development, Newcastle University, NE1 7RU, UK

Abstract

The effects of increased tropospheric ozone (O_3) pollution levels on methane (CH_4) emissions from peatlands, and their underlying mechanisms, remain unclear. In this study, we exposed peatland mesocosms from a temperate wet heath dominated by the sedge *Schoenus nigricans* and *Sphagnum papillosum* to four O_3 treatments in open-top chambers for 2.5 years, to investigate the O_3 impacts on CH_4 emissions and the processes that underpin these responses. Summer CH_4 emissions, were significantly reduced, by 27% over the experiment, due to summer daytime (8h day^{-1}) O_3 exposure to non-filtered air (NFA) plus 35 ppb O_3, but were not significantly affected by year-round, 24h day^{-1}, exposure to NFA plus 10 ppb or NFA plus 25 ppb O_3. There was no evidence that the reduced CH_4 emissions in response to elevated summer O_3 exposure were caused by reduced plant-derived carbon availability below-ground, because we found no significant effect of high summer O_3 exposure on root biomass, pore water dissolved organic carbon concentrations or the contribution of recent photosynthate to CH_4 emissions. Our CH_4 production potential and CH_4 oxidation potential measurements in the different O_3 treatments could also not explain the observed CH_4 emission responses to O_3. However, pore water ammonium concentrations at 20 cm depth were consistently reduced during the experiment by elevated summer O_3 exposure, and strong positive correlations were observed between CH_4 emission and pore water ammonium concentration at three peat depths over the 2.5-year study. Our results therefore imply that elevated regional O_3 exposures in summer, but not the small increases in northern hemisphere annual mean background O_3 concentrations predicted over this century, may lead to reduced CH_4 emissions from temperate peatlands as a consequence of reductions in soil inorganic nitrogen affecting methanogenic and/or methanotrophic activity.

Keywords: CH_4; mires; sedge; *Sphagnum papillosum*; nitrogen; ^{13}C.

1. Introduction

Tropospheric ozone (O_3) and methane (CH_4) are the second and third most important contributors to the human-induced greenhouse effect after carbon dioxide (IPCC, 2013). The concentrations of both gases in the background troposphere increased over the last century and, without strong emission control, are predicted to increase further during the 21st century (Dentener et al., 2006; Royal Society 2008; Wild et al., 2012). Recently, increased attention has been paid to the importance of measures to control atmospheric O_3 and CH_4 concentrations, because of their relatively short atmospheric lifetimes compared to CO_2 (Shindell et al., 2012). There are also important feedbacks between these two gases, since CH_4 emissions have contributed significantly to increases in global background O_3 concentrations (West & Fiore, 2005).

Ozone is also the most important gaseous air pollutant globally in terms of effects on ecosystem production and function (Ashmore, 2005) and northern hemisphere background levels of O_3 already exceed those at which significant effects on wild plant communities, crop yields and forest productivity can occur (Davison & Barnes, 1998; Averny et al., 2011; Ainsworth et al., 2012). Global modelling of O_3 effects on CO_2 uptake and sequestration suggest that these indirect effects may be as important as the direct effects of O_3 on radiative forcing (Sitch et al., 2007). However, these simulations do not consider the possibility that O_3 may affect CH_4 fluxes from managed and unmanaged wetlands. Methane emissions from natural wetlands may have contributed significantly to recent increases in global CH_4 levels (Kirschke et al., 2013), while predicted future releases of CH_4 from Arctic permafrost

thawing could be large enough to substantially increase tropospheric O_3 levels (Isaksen et al., 2014).

We have previously reported (Toet et al., 2011) that exposure to environmentally-relevant levels of elevated O_3 in the daytime decreased summer CH_4 emissions from temperate peatland mesocosms by *ca.* 25%. In contrast, Mörsky et al. (2008) reported that open-field exposure of boreal peatland microcosms to a similar increase in O_3 concentration in Central Finland only caused a decrease in CH_4 emission at the end of the first growing season, which was lost in the three subsequent growing seasons. Recently, Williamson et al. (2016) reported both increases and decreases in CH_4 emissions from temperate upland bog mesocosms in response to increasing background O_3 exposures in a short-term summer experiment. Studies on rice paddies, all also elevating O_3 concentrations for 7-8h in the daytime in summer, have demonstrated that CH_4 emissions were reduced in response to the pollutant (e.g. Bhatia et al., 2011; Zheng et al., 2011), but not in all cases (Kou et al., 2015). However, none of these previous peatland or paddy studies have explored the impacts of the small year-round increases in 24h mean background O_3 concentrations that are now affecting many areas of the northern hemisphere (Royal Society, 2008), which may be significant since substantial CH_4 emissions to the atmosphere have been reported in winter from boreal bogs and fens (e.g. Alm et al. 1999). Therefore, there is a need to assess year-round, long-term effects of elevated background annual mean O_3 concentrations on CH_4 emissions, as well as simply for summer peak exposures.

Furthermore, the mechanistic basis of any effects of O_3 on CH_4 emissions remains uncertain. It is unlikely to be related to direct effects of O_3 on microbial populations below-ground, because ^{18}O labelling studies have shown that O_3 penetration into the soil is limited to the top few mm, especially in wet soils (Toet et al., 2009). Consequently, O_3 effects on microbial activity are more likely to be indirectly controlled by processes mediated through

vascular plants. Although O_3 has been reported to have little effect on above-ground biomass of peatland vegetation (Mörsky et al., 2011; Toet et al., 2011; Williamson et al., 2016), allocation of vascular plant biomass into below-ground components may be reduced (Ashmore, 2005), leading to reductions in substrate availability for methanogens. The potential for such effects was shown by Jones et al. (2009), who found a rapid decrease in dissolved organic carbon (DOC) concentrations in fen mesocosms after O_3 exposure, with a change in molecular composition of DOC indicating a switch in the substrate for micro-organisms from root-derived carbon (C) to soil C; similar effects were not found in mesocosms dominated by *Sphagnum* moss. Such indirect effects of elevated O_3 in peatlands might be expected to affect CH_4 production, although both Rinnan et al. (2003) and Mörsky et al. (2008) reported that elevated O_3 had no significant effect on CH_4 production potential.

Elevated O_3 could also reduce CH_4 emissions indirectly by impacts on N cycling. This may be through reduced litter quantity or quality, although effects of O_3 on nitrification, denitrification, microbial biomass and plant uptake of N have also been reported (Wittig et al., 2009; Li et al. 2010; Bhatia et al., 2011; Pereira et al., 2011; Bassin et al., 2015). In nitrogen (N) poor systems such as peatlands, reduced below-ground allocation of N could cause reduced activity of heterotrophic soil microorganisms, such as methanogens (Kanerva et al., 2007). However, lower availability of ammonium (NH_4), the dominant form of inorganic N in peatlands, could also promote methanotrophic activity (Keller et al., 2006), and O_3 has been reported to reduce soil NH_4 concentrations in meadows (Kanerva et al. (2006) and soybean crops (Pereira et al., 2011). A direct adverse effect of O_3 on methanotrophs in the top layers of the moss cover of peatlands may also play a role, with Raghoebarsing et al. (2005) showing CH_4 consumption by *Sphagnum* plants through partly-endophytic methanotrophs in hyaline cells and on stem leaves.

We report here results from a peatland mesocosm study carried out over 2.5 years in open

top chambers (OTCs), with two major aims. The first was to test the hypothesis that increases in global background O_3 concentrations, as well as elevated O_3 exposure during summertime, may reduce CH_4 emissions from peatlands. Our second aim was to identify the mechanistic basis for any observed effects of elevated O_3 concentrations on CH_4 emissions, paying specific attention to the following hypotheses:-

1. Elevated O_3 reduces plant C allocation below-ground, whilst not affecting overall above-ground plant productivity
2. Elevated O_3 reduces the contribution of recent photosynthate to CH_4 emission
3. Elevated O_3 reduces CH_4 production potentials
4. Elevated O_3 decreases aerobic CH_4 oxidation potentials associated with the top peat layer (including living *Sphagnum* moss)
5. Elevated O_3 increases the aerobic CH_4 oxidation potentials deeper down the peat profile due to reduced pore water NH_4 concentrations.

2. Materials and methods

2.1. Experimental design

Mesocosms were collected from the wetter parts of a wet heath in the south western part of the Isle of Skye, Scotland (NGR: SV409227, latitude 57°13' N, longitude 6°18' W, 16 m a.s.l.) where annual average air temperature was 6.6°C and annual average precipitation 2825 mm over the period 1981-2010. The vegetation was dominated by the peat moss *Sphagnum papillosum* and the sedge *Schoenus nigricans*, with *Erica tetralix*, *Molinea caerulea* and *Narthecium ossifragum* regularly present at very low abundance. Other species found

intermittently and at very low abundance included *Scirpus cespitosus*, *Eriophorum vaginatum, Drosera rotundifolia* and other *Sphagnum* species such as *S. recurvum* and *S. palustre*.

Intact mesocosms (diameter 19 cm, length 35 cm) were cored in PVC tubes in early April 2008, sealed at the bottom and placed in deionised water in 22-l containers (diameter 30.5 cm). The water level was kept similar to the mean water table depth at the site by free drainage of the water through four 12-mm diameter holes 5 cm below the *Sphagnum* surface. The mesocosms were transported to the open-top chamber (OTC) facility, and left outside for a month to settle after coring; there was no evidence of significant damage to the vegetation as a result of cutting roots.

Ozone exposure was conducted in twelve rigid OTCs, situated at Heddon-on-the-Wall, Northumberland (NGR: NZ128659, latitude 54°59' N, longitude 1°48' W, 25 m a.s.l.). The octagonal OTCs (3.5 m (max) diameter x 3.3 m tall) and their O_3 delivery and control systems are described in detail in Gonzalez-Fernandez et al. (2008). All OTCs were ventilated with non-filtered air (NFA) at a rate sufficient to achieve 2 air changes min^{-1}. Twelve, of sixteen, OTCs were randomly assigned to one of four different O_3 treatments (three OTCs per treatment). In addition to the 'ambient air' treatment, which received only NFA, we included one treatment which, as in our previous study (Toet et al., 2011) and in the studies of rice paddies, raised O_3 levels for 8h during summer daytime to NFA plus 35 ppb (April - early October) and for 8h during winter daytime to NFA plus 10 ppb ('NFA+35/10'). The other two O_3 treatments raised background O_3 levels in the same range as the high O_3 treatment, but 24h throughout the year, to either NFA plus 10 ppb ('NFA+10'), corresponding to the upper end of IPCC predictions for 2050 under SRES scenarios (Wild et al., 2012), or to a more pessimistic NFA plus 25 ppb ('NFA+25'). The use of 24h exposures reflects the fact that, in rural areas throughout the UK, O_3 concentrations stay well above zero during the night and early morning (Royal Society 2008).

Three mesocosms were randomly placed in each OTC on 6 May 2008, resulting in nine mesocosms per O_3 treatment for the main experiment Additional mesocosms were placed in the OTCs for a $^{13}CO_2$ pulse-labelling experiment (see below). Methane emission, sedge green leaf density, soil temperature and pore water chemistry in all 36 mesocosms constituting the main experiment were determined prior to the start of O_3 exposure. Methane emission rates (which were very low) and the other measured variables were not significantly different between the four groups of mesocosms assigned to each O_3 treatment at the beginning of the experiment. The mesocosms were regularly rotated within the central part of each OTC to minimise any positional effects.

The effects of the OTCs on microclimatic conditions, determined from measurements made during the course of the experiment, were similar to those observed in other OTC studies (discussed in Toet et al. 2011). Precipitation was on average 14% lower than outside and the mean air temperature within the OTCs was on average 1.3°C higher than outside. The much lower annual precipitation at the OTC facility than at the source field site was compensated by maintaining a prescribed water table depth in the mesocosms at a level comparable to the field site, by regular additions of deionised water. The air temperature outside the OTCs was on average 2.5^{o}C higher than at the source field site. The higher temperature may have had some stimulating effect on plant and microbial activity of the mesocosms compared to the field situation, but the temperature increase was similar across all O_3 treatments, whilst the water in the containers also reduced impacts of lateral heat fluxes on soil temperature.

2.2. Main experiment

2.2.1. Methane emission

Daytime methane emissions from all 36 mesocosms were measured 3-5 times each summer and three times each winter over the 2.5-year experiment, using the methods described by Toet et al. (2011). Briefly, static, opaque chambers (25 cm high) covered with reflective insulation material to reduce temperature increases in the chamber during measurements were placed on the mesocosms, and gas samples (20 ml) were collected from the headspace at regular intervals for periods of 80-120 min. and stored in evacuated 12-ml Exetainers (Labco Limited, High Wycombe, UK). The gas in the Exetainers was analysed for CH_4 concentration on a PerkinElmer-Arnel gas chromatograph (GC, AutoSystem XL, PerkinElmer Instruments, Shelton, CT, USA) equipped with a flame ionization detector (FID) and a 3.7 m Porapak Q 60/80 mesh column within 7 days. Methane emission rates were calculated from the slope of regressions of CH_4 concentrations with time in each chamber; regressions with $r^2 < 0.90$ (0.8% of the total) being rejected.

2.2.2. Plant and soil variables

Sedge green leaf density was determined for each mesocosm at each sampling date. Soil temperature was measured in each mesocosm at 2.5, 10 and 20 cm below the *Sphagnum* surface immediately after CH_4 emission measurements using alcohol thermometers. Root biomass was determined at the end of the experiment in mesocosms exposed for 2.5 years to ambient O_3 or NFA+35/10 only. A peat sample was collected over the entire length of the peat profile of each mesocosm. The volume of each peat sample was determined by water displacement to enable determination of the root biomass in the entire mesocosm. Roots were collected from each sample, dried at 70˚C for two days, and weighed.

Peat water samples at 2.5, 10 and 20 cm below the *Sphagnum* surface were collected in each mesocosm on all sampling dates with Rhizon samplers (Eijkelkamp Agrisearch

Equipment, Giesbeek, The Netherlands). The pH of each sample was measured (Thermo Orion 420Aplus, Thermo Orion Europe, Witchford, UK), and each sample was analysed colorimetrically for NH_4 and nitrate + nitrite (NO_3+NO_2) using a Bran and Luebbe Autoanalyser 3 (Bran and Luebbe, Norderstedt, Germany), and for DOC using a TOC analyser (liquiTOC, Elementar Analysensysteme, Hanau, Germany).

Additional peat water samples were sampled via Rhizon samplers in 20-ml Exetainers from three mesocosms at each of the four O_3 treatments at 2.5, 10, 20 and 30 cm depth on 29 July 2010 to determine the apparent fractionation factor (α_C) indicative of the predominant methanogenic pathway, i.e. acetoclastic or hydrogenotrophic methanogenesis (Conrad, 2005). Peat water samples were immediately acidified with 20% sulphuric acid and N_2 was added to the headspace up to ambient pressure. The samples were left to equilibrate at 20˚C for at least a week. Gas from the headspace was analysed for δ^{13}C-CH_4 using a pre-concentrated cryofocussing isotope ratio mass spectrometer (GC-IRMS) setup described below (see $^{13}CO_2$ pulse-labelling experiment). The δ^{13}C of the CO_2 in gas samples was measured using the GC-IRMS system described in Subke et al. (2009) consisting of an IRMS (SIRAS Series 2, Micromass, UK) with non-ionising electromagnetic radiation (NIER) type ion impact source and triple Faraday collector system employing a rotary/turbo-molecular pumping vacuum system, interfaced to a Microsoft Windows^{TM} data system (model name 'PVS12', built by Pro-Vac Services, Crewe, UK). The apparent fractionation factor (α_C) was calculated using the equation $\alpha_C = (\delta^{13}CO_2 + 10^3)/(\delta^{13}CH_4 + 10^3)$ (Conrad, 2005).

2.2.3. Potential methane production and oxidation

At the end of the experiment, potential CH_4 production and oxidation were determined during a lab incubation in darkness at 19˚C using a method adapted from Saarnio et al. (1998). Pilot experiments at different peat depths (0-5, 10-15 and 20-25 cm depth), showed that whilst

potential aerobic CH_4 oxidation occurred throughout the peat profile, potential CH_4 production increased with depth (data not shown). Due to time constraints, the potential CH_4 production and oxidation measurements in the four O_3 treatments focussed on the top layer including green *Sphagnum* (0-5 cm) and the layer at 20-25 cm depth. The pilot tests were also used to identify over what incubation period the decrease or increase in CH_4 concentration remained linear. Potential CH_4 oxidation rates were also determined over a range of initial CH_4 concentrations (20-10,000 ppm), with potential aerobic CH_4 oxidation rates increasing with initial CH_4 concentration, but not any further once the initial CH_4 concentration exceeded 5,000 ppm. Therefore a starting CH_4 concentration of 10,000 ppm was used at which CH_4 oxidation was not limiting and which was similar to what was used for potential CH_4 oxidation measurements of *Sphagnum* from peat bogs by Raghoebarsing et al. (2005) and Larmola et al. (2010).

For potential CH_4 production measurements, a pooled subsample of the 20-25 cm layer from each mesocosm was immediately wrapped in aluminium foil and transferred to an anaerobic box flushed with N_2 to keep soil conditions as anaerobic as possible. To 125-ml Wheaton glass serum bottles (Wheaton UK, Rochdale, UK), 20 g of peat (after removal of large roots) and 10 ml of oxygen-poor deionised water (flushed with N_2 for 20 min) were added. The headspace was flushed with N_2 for 2 min before closing the bottle with a butyl rubber stopper and crimp cap. Three empty control bottles were also flushed with N_2. Two minutes after adding another 10 ml of N_2 to each Wheaton bottle, 2 ml of headspace was sampled from each bottle (t0) and stored in 3-ml evacuated Exetainers to which 5 ml of N_2 was added. The headspace was sampled daily over a 4-day incubation in the dark at 19°C.

For potential aerobic CH_4 oxidation measurements, pooled sub-samples of 20 g peat (and living moss, after removal of sedge shoots and large roots) from 0-5 cm or 20-25 cm depth were added to Wheaton bottles. The peat/moss was left to aerate in the bottles without

septa overnight in the dark at 19˚C. The next morning, bottles were left outside for 15 min to establish ambient air headspace conditions and they were then sealed with crimped rubber stoppers. Another 10 ml of air with concentrated CH_4 was added, to create a start concentration of 10,000 ppm CH_4 in the headspace. The headspace was sampled 2 min after adding the CH_4 (t0) and again after 4, 8, 24 and 48 hours. Each time, 1 ml of headspace was collected in 3-ml evacuated Exetainers and 6 ml N_2 was added.

The gas samples were analysed for CH_4 concentration on the gas chromatograph, as described earlier. The samples were also analysed for CO_2 concentration to determine soil respiration under aerobic and anaerobic conditions. On the GC, CO_2 was first converted to CH_4 before detection using FID by use of a Ni reduction catalyst. The rates of potential CH_4 production and oxidation, and the rates of potential CO_2 emission under anaerobic and aerobic conditions were determined from regressions, as described earlier in the text. The r^2 of all the regressions exceeded 0.90 and therefore none were rejected. The amounts of CH_4 and inorganic C dissolved in the water were also included in the flux calculations using Henry's law and the first dissociation constant of carbonic acid for dissolved inorganic C; for the latter the pH of the water was measured at the start and end of incubation and interpolated for the intermediate sampling times. No changes in CH_4 and CO_2 concentrations were observed in the control bottles during incubation.

2.3. $^{13}CO_2$ pulse-labelling experiment

Additional mesocosms that had been exposed to ambient O_3 or NFA+35/10 for 2.5 years were exposed to ^{13}C labelled CO_2 during the daytime on 10 August 2010 to assess the effect of the two O_3 treatments on the contribution of recent photosynthate to CH_4 emission during a pulse-chase experiment. For each O_3 treatment, nine mesocosms received a $^{13}CO_2$ pulse of 6.3 hours by enclosing the vegetation in each mesocosm using a transparent Perspex chamber

(height= 25 cm, internal diameter= 19.4 cm) which received 380 ppm $^{13}CO_2$ in synthetic air (99 atom%; Spectra Gases, Littleport, UK) at one air change every 2 min. A dark control mesocosm covered by dark and reflective material was also similarly exposed to $^{13}CO_2$ to take into account for any $^{13}CO_2$ diffusing into aerenchyma of the sedges or directly into the peat soil during the pulse, whilst a control mesocosm exposed to ambient air CO_2 (natural abundance) was also included for each O_3 treatment. A photoacoustic gas monitor (INNOVA 1412i, LumaSense Technologies, Ballerup, Denmark) was regularly connected to the outlet of chambers to check that the CO_2 concentration within the chambers remained at ambient levels.

After the $^{13}CO_2$ pulse, O_3 exposure of the mesocosms in the OTCs was continued for another 51 days. Methane emissions were determined the day before and 1, 3, 5, 7, 10, 14, 21, 30 and 51 days after the pulse as described earlier by sampling the headspace regularly over a 100 min period. At the end of the 100-min period, an additional 20 ml gas sample was collected from the headspace and stored in a 12-ml Exetainer for subsequent ^{13}C-CH_4 analysis. Ambient air from each OTC was sampled in five Exetainers on each sampling date, which was transferred to a 100-ml Young's gas flask in the laboratory. Water was removed from these gas samples via a perchlorate chemical trap, and the CH_4 was oxidised to CO_2, which was cryogenically pre-concentrated using a trace gas pre-concentrator (Isoprime, Stockport, UK), prior to GC column (Poraplot Q) separation and introduction to an IRMS via open split (Isoprime, Stockport, UK) to determine the $\delta^{13}C$ of the CH_4 in the gas samples relative to V-PDB at the NERC Life Sciences Mass Spectrometry Facility, Lancaster (precision better than 0.5‰; instrument calibrated with NIST 8562 certified reference gas).

Methane emissions were determined through regressions over time as described previously (all regressions: $r^2 > 0.90$). Cumulative CH_4 emission over the entire 51-day chase period and the first 7 days was calculated using linear interpolation between sampling dates.

The contribution of recently fixed C to the CH_4 emitted from the mesocosms was determined by summation of the daily $^{13}CH_4$ emitted by the pulse using linear interpolation for days between sampling dates, and multiplying by the ratio of average day length (measured as when PAR in the OTCs was above 50 μmol m^{-2} s^{-1}) and the length of the pulse (6.3 hours) on a weekly basis.

2.4. Statistical analyses

All data were analysed using IBM SPSS Statistics 20.0 (IBM Corp., Armonk, NY, USA). Data were tested for normality and log-transformed when variances were proportional to the means. Studentised residuals of statistical tests of the measured variables were screened for any OTC pattern within each O_3 treatment or pattern in their position at the field station. No consistent OTC or position patterns were observed; consequently, statistical test results with the nine replicates per O_3 treatment were used to interpret the data.

The effects of O_3 and time on CH_4 emission and ancillary variables were tested using linear mixed models. Data were analysed over the whole 2.5-year period and additionally for the summer (April - early October) and winter (early October - March) periods separately. Sedge green leaf density was included as a covariate in the model if $p < 0.100$. Multiple comparisons with Bonferroni correction were carried out amongst the means of the O_3 treatments. An independent samples t-test was carried out to compare the difference in sedge root biomass between the NFA+35/10 and ambient O_3 treatments. Linear relationships between CH_4 emission, sedge green leaf density and pore water variables were identified using Pearson's correlation tests during the summer and winter periods of the 2.5 year study, for all O_3 treatments together and for individual O_3 treatments.

A two-way ANOVA without replication with mesocosm as the second source was performed to determine the effect of peat depth on the apparent fractionation factor (α_C).

Responses of aerobic CH_4 oxidation potential to the four O_3 treatments at two peat depths was tested using a two-way ANOVA, with OTC as a random factor nested within O_3. For multiple comparisons among the means the Tukey test was used, and a modification of the standard error was calculated in the nested model (Zar, 1984). The same approach was used for CH_4 production potential, but just for one peat depth.

The effects of O_3 and time on the $\delta^{13}C$ of the emitted CH_4 during the chase was tested as described above, and independent samples t-tests were carried out to compare differences in sedge green leaf density and pore water variables between the NFA+35/10 and ambient O_3 treatments.

3. Results

3.1. O_3 exposure

The 24h mean O_3 concentration in the ambient O_3 treatment over the entire 2.5 year experiment (April 2008-August 2010) was 24 ppb, with little variation (23-26 ppb) between years and seasons (Table 1). The mean O_3 concentration over the course of the experiment showed only a weak diurnal pattern, varying between 26.1 ppb mid afternoon and 22.1 ppb early morning (See Supplementary Information Figure S1). In NFA+10, the overall increase in 24h mean concentration was 8 ppb, with a seasonal range of 7-10 ppb, except in the winter of 2008/9 when it was 2 ppb.

Table 1 Summary of O_3 concentration treatments (ppb) in the OTCs with non-filter air (NFA: 'Ambient O_3') , NFA plus 10 ppb O_3 ('NFA+10'), NFA plus 25 ppb O_3 ('NFA+25') and NFA plus 35/10 ppb O_3 '(NFA+35/10') by season. All O_3 treatments were supplied 24h day^{-1}, except for NFA+35/10, which was supplied for 8h in the daytime (9:00-17:00) at NFA plus

35 ppb during summer (April - early October) and at NFA plus 10 ppb during winter (early October - March). (mean ± SE, n= 3).

season	24h mean O_3 concentration				8h daytime mean O_3 conc.
	Ambient O_3	NFA+10	NFA+25	NFA+35/10	NFA+35/10
summer 2008	26 ± 1	33 ± 1	47 ± 1	39 ± 0	62 ± 1
winter 2008/9	24 ± 0	26 ± 1	48 ± 0	31 ± 0	36 ± 0
summer 2009	24 ± 0	34 ± 1	59 ± 0	42 ± 0	64 ± 0
winter 2009/10	23 ± 0	32 ± 0	48 ± 0	27 ± 0	33 ± 0
summer 2010	24 ± 0	34 ± 0	53 ± 0	38 ± 0	61 ± 0

In NFA+25, the overall increase of 27 ppb was also close to the target, with a seasonal range of 22-29 ppb, except in the summer of 2009 when it was 35 ppb. The daytime 8h mean O_3 concentrations and the 24h mean O_3 concentrations were very similar for each of these treatments. The mean 8h-concentrations of the NFA+35/10 treatment ranged between 34 and 39 ppb in summer and between 10 and 12 ppb in winter. The overall increase in 24h mean concentration in NFA+35/10 was 12 ppb overall (14-20 ppb in summers; 4-7 ppb in winters); hence in terms of 24h mean concentrations this treatment lay between the NFA+10 and the NFA+25 treatments (Supplementary Information Table S2 for AOT40 values).

3.2. Methane emission

Reductions in CH_4 emissions at elevated O_3 were apparent from the end of July in the first growing season (Fig. 1). Methane emissions were significantly affected by O_3 in summer

over the entire 2.5 years of the experiment (Table 2). Summer CH_4 emissions were reduced (P= 0.06) by 27% in the NFA+35/10 treatment (Table 3), but the effects of NFA+10 and NFA+25 treatments were not statistically significant. Methane emissions were low in winter and there was no evidence of a significant O_3 effect at this time (Table 2). In the third summer there appeared to be a consistent increase in CH_4 emission in the NFA+10 treatment, but this effect was not significant.

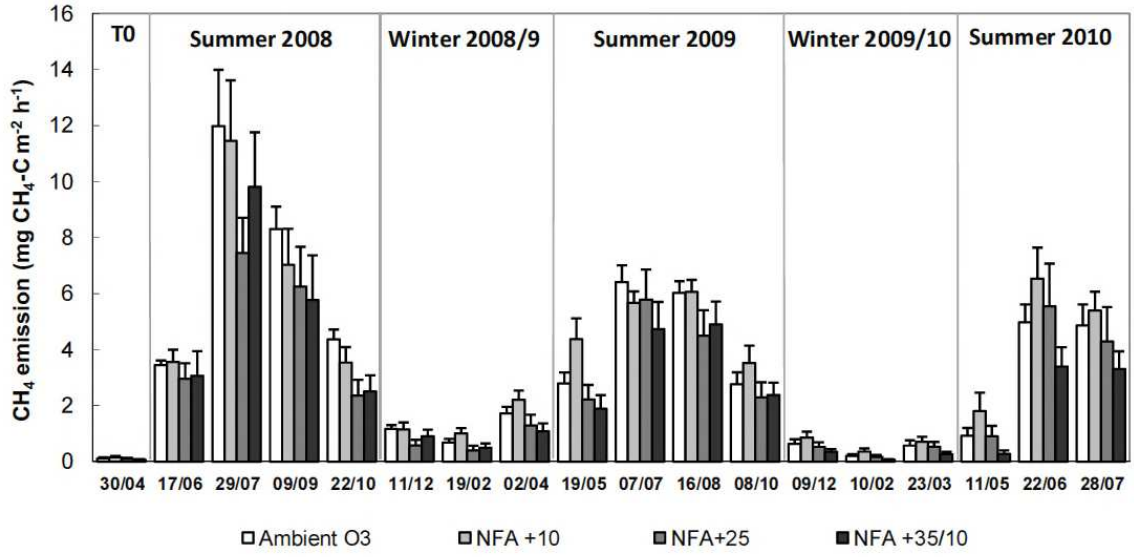


Fig. 1 Methane emission of wet heath mesocosms at ambient O_3 (non-filtered air (NFA), white bars), NFA plus 10 ppb O_3 (NFA+10, light grey bars), NFA plus 25 ppb O_3 (NFA+25, medium grey bars) and NFA plus 35/10 ppb O_3 (NFA+35/10, dark grey bars ppb) from April 2008 - July 2010. All O_3 treatments were supplied 24h day^{-1}, except for NFA+35/10, which was supplied for 8h in the daytime (9:00-17:00) at NFA plus 35 ppb during summer (April - early October) and at NFA plus 10 ppb during winter (early October - March). Values are expressed as m~~M~~ean ± SE (n= 9). See Table 2 for overview statistical analysis results.

Table 2 Summary of statistical analysis of O_3 effects on CH_4 emission for the whole period, and for the three summer periods only or the two winter periods only (ozone: n= 9; time: n= 17, 11, 6 for the whole period, summers and winters only, respectively). Green sedge leaf density was only included as a covariate when P< 0.100.

period	ozone		time		ozone*time		leaf density (covariate)	
	F	P	F	P	F	P	F	P
whole period	2.30	0.096	51.10	<0.001	3.32	<0.001	3.25	0.075
summer	3.22	0.036	72.78	<0.001	1.58	0.104		
winter	1.69	0.191	21.83	<0.001	1.56	0.143	6.74	0.011

3.3. Plant and soil variables

Sedge green leaf density increased on average by 142% (SE: ±25%) from the first to the third summer, but was not significantly influenced by O_3 (summers: F= 0.04 P= 0.33; winters: F= 0.35, P= 0.55; Table 3). Sedge green leaf density was a significant covariate for CH_4 emissions over the whole period and for the winter periods (Table 2). Methane emission was negatively correlated with sedge green leaf density during winter for all O_3 treatments (r= 0.53; P= 0.001), and NFA+10 (r= -0.68, P= 0.04). At the end of the experiment, exposure to NFA+35/10 had not significantly affected root biomass (mean ± SE: 2593 ± 307 g m^{-2} and 2378 ± 172 g m^{-2} for ambient O_3 and NFA+35/10, respectively; t= 0.62; P= 0.55).

Table 3 Methane emission, sedge green leaf density, dissolved organic carbon (DOC) concentration, pH and NH_4-N concentration of pore water at 20 cm depth at the four O_3 treatments (see legend Table 1 for details of O_3 treatments) by season averaged over the 2.5-year study. Values are expressed as mean ± SE (n= 9). Over the entire 2.5-year period, CH_4 emission ($P< 0.10$) and pore water NH_4 concentration at 20 cm depth ($P< 0.05$) in summer were significantly lower at NFA+35/10 compared to ambient O_3. Pore water pH at 20 cm depth was significantly lower at some of the sampling dates at elevated O_3 ($P< 0.05$), whilst green leaf sedge density and pore water DOC concentration at 20 cm depth were not significantly affected by elevated O_3 during the experiment.

period	Ambient O_3	NFA + 10	NFA + 25	NFA + 35/10
CH_4 emission (mg C m^{-2} h^{-1})				
summer	4.47 ± 0.30	4.98 ± 0.41	3.87 ± 0.75	3.27 ± 0.58
winter	0.65 ± 0.14	0.84 ± 0.16	0.49 ± 0.15	0.38 ± 0.09
Sedge green leaf density (no. leaves m^{-2})				
winter	4507 ± 527	3353 ± 588	4450 ± 547	3897 ± 365
winter	2804 ± 348	1994 ± 423	2596 ± 320	2927 ± 307
DOC concentration at 20 cm depth (mg l^{-1})				
summer	39.8 ± 4.3	52.1 ± 7.0	46.0 ± 4.3	48.7 ± 6.7
winter	20.4 ± 1.8	32.4 ± 6.8	24.5 ± 2.0	19.1 ± 2.0

NH_4-N concentration at 20 cm depth (mg l^{-1})				
summer	3.67 ± 0.60	4.19 ± 1.37	2.23 ± 0.70	1.67 ± 0.38
winter	3.22 ± 0.52	3.33 ± 1.15	2.24 ± 0.86	1.53 ± 0.40
pH at 20 cm depth				
summer	5.81 ± 0.04	5.58 ± 0.10	5.44 ± 0.10	5.55 ± 0.09
winter	5.85 ± 0.07	5.75 ± 0.10	5.56 ± 0.04	5.65 ± 0.09

The DOC concentration of the peat pore water at 20 cm below the *Sphagnum* surface did not respond significantly to the O_3 treatments (summer: F= 0.47, P= 0.70; winter: F= 1.43, P= 0.25; Table 3), although the concentrations tended to be higher in the three elevated O_3 treatments than for ambient O_3. There were also no significant effects of O_3 on DOC concentrations at 2.5 cm and 10 cm depth (data not shown).

In contrast, the effect of elevated O_3 on NH_4 concentrations in the pore water at 20 cm depth was significant in summer during the whole experiment (P= 0.04; Table 4), but there was also a significant O_3*time interaction (P= 0.007). The O_3 effect, like that on CH_4 emissions shown in Figure 1, became apparent from the first summer (Fig.2). During every summer, the NH_4 concentrations were reduced significantly in NFA+35/10 compared to ambient O_3 (by 50% in 2008 (P< 0.05), by 56% in 2009 (P< 0.05), and by 61% in 2010 (P< 0.10), with an overall reduction of 54% (Table 3; mean values ± SE of NH_4-N concentrations at 20 cm depth for each summer period are shown in the Supplementary Information: Table S3). The NH_4-N concentrations were also reduced in the two other elevated O_3 treatments during the first summer (P< 0.10), and for NFA+25 also in summer 2009 (P< 0.10). Overall in winter, the NH_4 concentrations at 20 cm were reduced by 52% in NFA+35/10 (P< 0.10; Table 3). Like CH_4 emissions, NH_4 concentrations tended to become higher in NFA+10 than

ambient O_3 treatments in the final year of the experiment (Fig. 2), but this effect was not significant. Elevated O_3 did not significantly affect pore water NH_4 concentrations at 2.5 cm and 10 cm depth, although similar trends in response to O_3 were observed (data not shown). The NO_3+NO_2 concentrations in pore water were in general consistently very low throughout the peat profile.

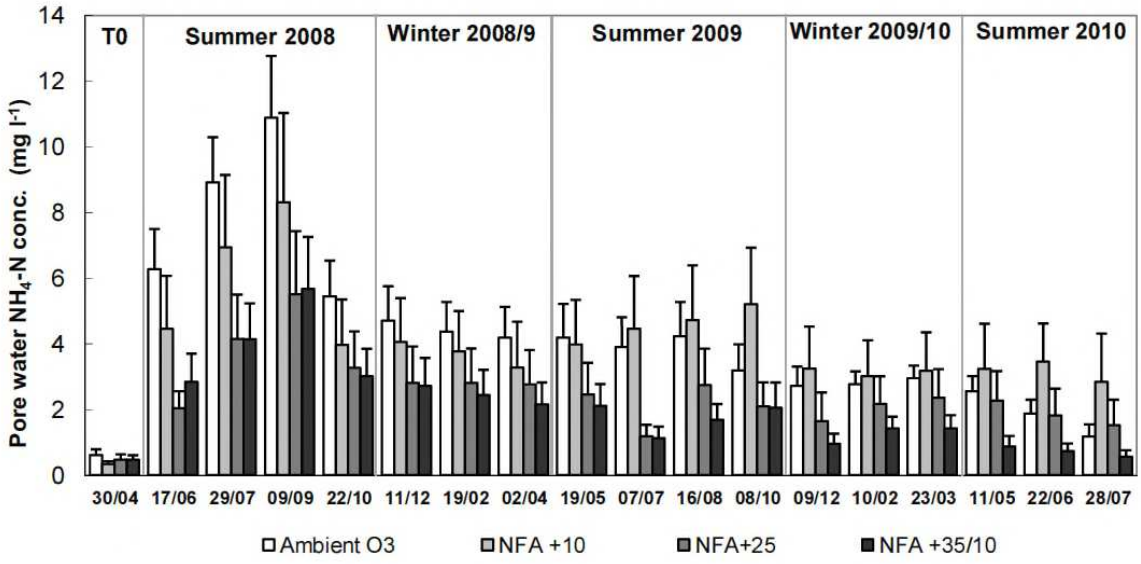


Fig. 2 Ammonium concentration of pore water at 20 cm below the moss surface of wet heath mesocosms at four O_3 treatments (see legend Fig. 1 for details of O_3 treatments) from April 2008 - July 2010. Values are expressed as mean ± SE (n= 9). See Table 4 for overview statistical analysis results.

Table 4 Summary of statistical analysis of O_3 effects on NH_4 concentration of pore water at 20 cm below the moss surface for the whole period, and for the three summer periods only or the two winter periods only (ozone: n= 9; time: n= 17, 11, 6 for the whole period, summer and winter only, respectively). Green sedge leaf density was only included as a covariate when $P < 0.100$.

period	ozone		time		ozone*time		leaf density (covariate)	
	F	P	F	P	F	P	F	P
whole period	2.44	0.063	48.54	<0.001	2.17	0.012	12.38	0.001
summer	3.04	0.043	29.43	<0.001	2.42	0.007	23.67	<0.001
winter	1.96	0.142	3.05	0.022	1.00	0.480	14.25	<0.001

The pH of pore water at 20 cm was significantly affected by O_3 during the summer periods (F= 3.58, P= 0.02; Table 3) throughout the experiment, but only during the second of the winter periods (F= 3.05, P= 0.043). There was also a highly significant interaction of O_3 with time in summer (F= 3.63, P< 0.001) which was also present for the first two individual summer periods; pH was significantly lower in NFA+10 than ambient O_3 on three summer measurement dates, and significantly lower in NFA+35/10 than ambient O_3 on five. During the second winter, pH was also significantly lower at NFA+25 (P< 0.05). Similar O_3 effects were observed for pH at 10 cm and 2.5 cm, though the effects were significant at fewer sampling dates (data not shown).

Pore water NH_4 concentrations at all three depths were positively correlated with CH_4 emissions in both summer and winter when data for all O_3 treatments were included (Table 5). At the two deeper depths, this was also the case for the NFA+35/10 treatment in summer and winter, and for most other O_3 treatments in winter (data not shown). Pore water pH also correlated positively with CH_4 emission when all O_3 treatments were included; this effect was significant in the winters throughout the peat profile, and in the summers at 10 cm and 20 cm depth (Table 5). At the two deeper depths, significant positive correlations were also

frequently found for individual O_3 treatments, particularly in winter (data not shown). The pH always showed strong significant positive correlations with NH_4 concentrations at each depth ($0.001 < P < 0.002$).

Table 5 Pearson correlation coefficients (r) and associated P-values of methane emission (mg C m^{-2} h^{-1}) with pore water NH_4-N concentration (mg l^{-1}) and pH at 2.5, 10 and 20 cm below the *Sphagnum* surface (n= 36), across all O_3 treatments split by summer and winter impacts during the 2.5-year study (n= 36). Significant correlations ($P < 0.05$) are indicated in bold. Untransformed data were used, except for NH_4-N concentrations at all three depths in summer, and at 2 cm and 10 cm depth in winter.

period	2.5 cm depth		10 cm depth		20 cm depth	
	r	P	r	P	r	P
NH_4-N						
summer	**0.496**	**0.002**	**0.649**	**<0.001**	**0.673**	**<0.001**
winter	**0.609**	**<0.001**	**0.653**	**<0.001**	**0.626**	**<0.001**
pH						
summer	0.188	0.271	**0.390**	**0.019**	**0.469**	**0.004**
winter	**0.352**	**0.035**	**0.648**	**<0.001**	**0.455**	**0.005**

Soil temperature at 2.5 cm in summer, and at 10 cm in both summer and winter, showed significant overall O_3 treatment differences. However, the soil temperature differences between elevated O_3 treatments and ambient O_3 were not consistent over time at

2.5 and 20 cm, ranging between -0.4 and 0.7˚C at 2.5 cm depth, and between -0.1 and 0.3˚C at 20 cm depth. There were more consistent soil temperature differences (P< 0.10) at 10 cm depth, with soil temperature in NFA+10 in summer and NFA+35/10 in winter being 0.5˚C higher than at ambient O_3. Overall, soil temperature differences between elevated O_3 treatments and ambient O_3 at 10 cm depth ranged between -0.1 and 0.5˚C in summer and between 0.1°C and 0.5°C in winter.

3.4. $^{13}CO_2$ pulse labelling experiment

The $\delta^{13}C$ of the emitted CH_4 was enhanced one day after the application of the $^{13}CO_2$ pulse, peaked after 10-14 days, and then gradually decreased again (time effect: F= 63.67, P< 0.001; Fig. 3). However, the $\delta^{13}C$-CH_4 value was not significantly affected by O_3 over the 51 days of the experiment (F= 0.29, P= 0.59), and there was no significant O_3*time interaction (F= 0.15, P= 1.00). Sedge green leaf density was included in the model (F= 103.0, P< 0.001).

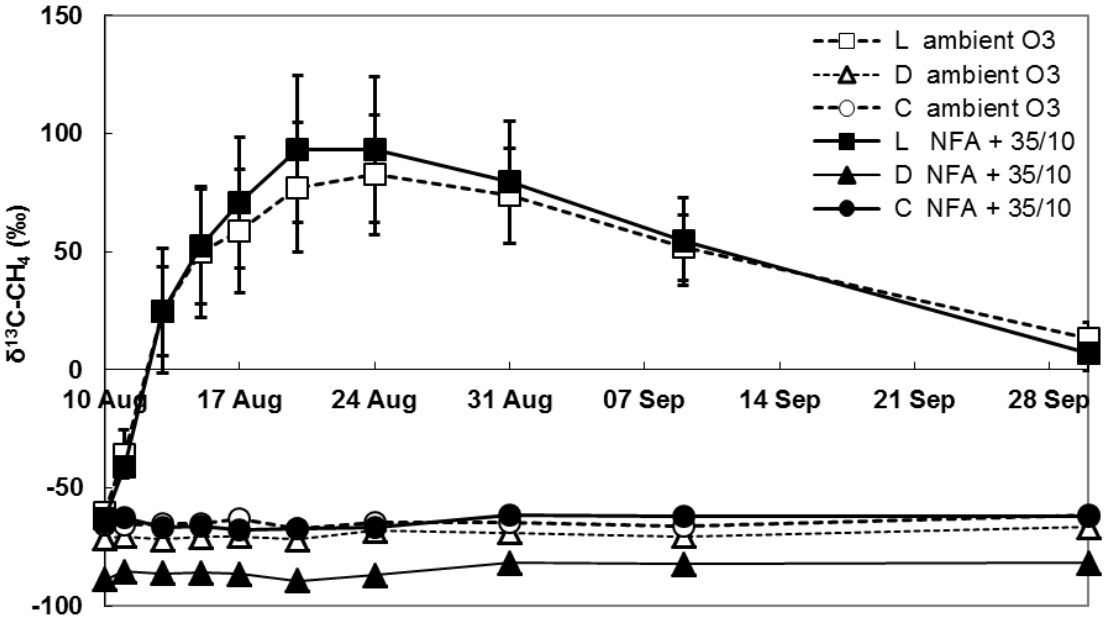


Fig. 3 The $\delta^{13}C$ of the emitted CH_4 after the $^{13}CO_2$ pulse labelling of 6.3h hours on 10 August 2010 at ambient O_3 and NFA+35/10 (see legend Fig. 1 for details of O_3 treatments), using transparent chambers during the $^{13}CO_2$ pulse in ambient light (L), identical transparent chambers with CO_2 at natural abundance in ambient light ('light controls', C), and identical but opaque chambers during the $^{13}CO_2$ pulse in dark conditions ('dark controls', D). For L: values are expressed as mean ± SE (n= 9), For C and D controls: n=1. No significant difference between ambient O_3 and the NFA+35/10 treatments was observed at $P< 0.05$.

The contribution of recent photosynthate to CH_4 emission over the 51-day period was not significantly affected by O_3 (t= -0.24, P= 0.81) and was relatively small (on average 12% for both O_3 treatments; Table 6). Little $^{13}CH_4$ was emitted from the dark control mesocosms after the $^{13}CO_2$ pulse (contribution of recent photosynthates to CH_4 emission: 0.1-0.4%), confirming that the $^{13}CH_4$ emitted in light conditions was predominantly derived from newly fixed C.

Table 6 Cumulative CH_4 emission, and plant and soil chemistry variables of the wet heath mesocosms of the $^{13}CO_2$ pulse labelling experiment at ambient O_3 and NFA+35/10 treatments (see legend Table 1 for details of O_3 treatments). '-5 cm' and '-20 cm' are measurements at 5 and 20 cm below the *Sphagnum* surface, respectively. Values are expressed as mean ± SE (n= 9). Significant correlations ($P< 0.10$) are indicated in bold.

variable	Ambient O_3	NFA + 35/10
CH_4 emission (mg CH_4-C m^{-2} h^{-1})		
0-7 days	**4.27 ± 0.48**	**3.09 ± 0.46**

0-51 days	4.00 ± 0.48	2.97 ± 0.45
Contribution of recent photosynthates to CH_4 emission (%)		
0-51 days	11.7 ± 1.8	12.4 ± 2.3
Sedge green leaf density (no. leaves m^{-2})		
day 0	4852 ± 1365	4562 ± 1016
Pore water chemistry at day 0 (concentrations in mg l^{-1})		
NH_4-N conc.: 2.5 cm depth	0.10 ± 0.04	0.16 ± 0.05
NH_4-N conc.: 20 cm depth	2.76 ± 0.99	2.08 ± 0.84
DOC conc.: 2.5 cm depth	**8.4 ± 0.5**	**12.1 ± 2.0**
DOC conc.: 20 cm depth	19.5 ± 3.4	25.9 ± 7.4
pH: 2.5 cm depth	5.37 ± 0.04	5.41 ± 0.06
pH: 20 cm depth	5.66 ± 0.04	5.60 ± 0.09

Cumulative CH_4 emission over 0-7 days was 28% lower in the NFA+35/10 treatment (t= 1.83, P= 0.09), but this effect was not significant over days 0-51 (F= 1.34, P= 0.20). The effects of the NFA+35/10 treatment on sedge green leaf density and pore water chemistry are also summarised in Table 6; there were no significant O_3 effects except for DOC at 2.5 cm depth which tended to be higher at NFA+35/10 (t= 1.81, P= 0.09).

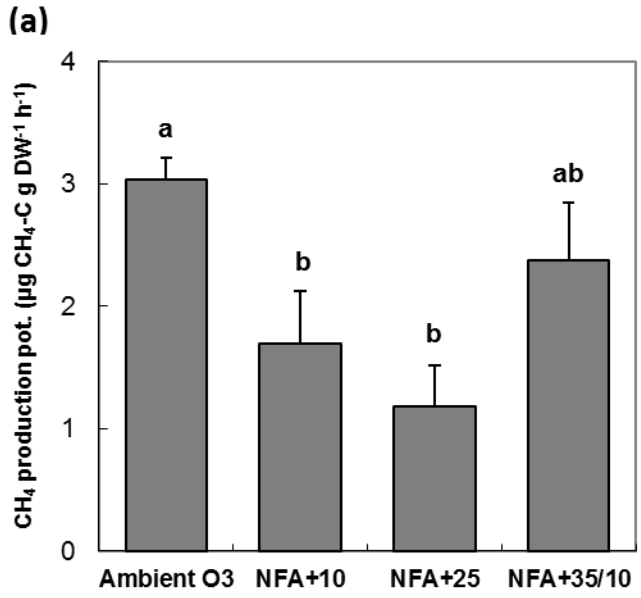
3.5. Predominant methanogenic pathway

The apparent fractionation factor (α_C) was significantly lower at 2.5 cm and 10 cm (mean ± SE: 1.048 ± 0.001 and 1.049 ± 0.001) than at 20 cm and 30 cm depth (1.055 ± 0.001 and

1.057 ± 0.001; depth effect: F= 17.61, P< 0.001). There was no significant effect of O_3.

3.6. Potential methane production and oxidation

The overall effect of O_3 on CH_4 production potential was highly significant (F= 8.29, P= 0.01; Fig. 4a). The CH_4 production potential in NFA+10 was reduced by 44% compared to ambient O_3, while that in NFA+25 was reduced by 61%; post-hoc tests showed that both these effects were significant (P< 0.05). However, the CH_4 production potential in NFA+35/10 was only reduced by 22%, a value that was not significantly lower than ambient O_3.



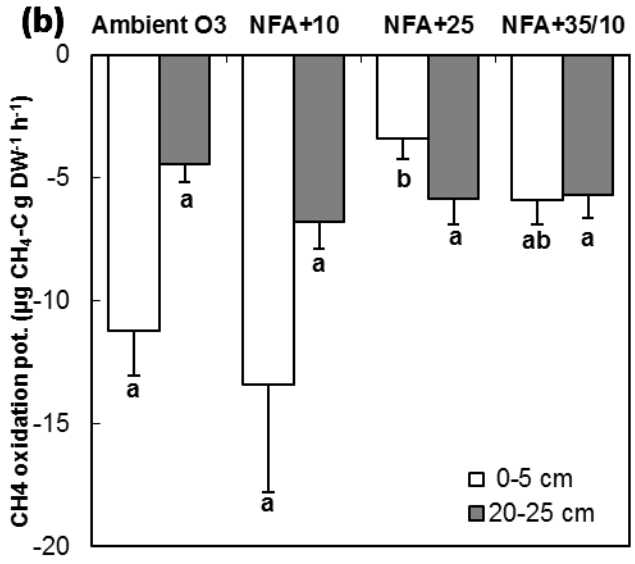


Fig. 4 Methane production potential at 20-25 cm below the *Sphagnum* surface (a) and aerobic CH_4 oxidation potential at 0-5 cm (white bars) and 20-25 cm (grey bars) depth below the *Sphagnum* surface (b) at the four O_3 treatments in August 2010 (see legend Fig. 1 for details of O_3 treatments). Values are expressed as mean ± SE (n= 9). Letters indicate significant differences among O_3 treatments within the same soil depth at $P< 0.05$.

The aerobic CH_4 oxidation potential was significantly reduced by elevated O_3 in the top 5 cm of the mesocosms ($F= 14.94$, $P= 0.001$; Fig. 4b); the values were reduced by 70% ($P< 0.05$) and 56% ($P< 0.10$) in NFA+25 and NFA+35/10 respectively, compared to ambient O_3. However, there was no significant O_3 effect at 20-25 cm ($F= 0.64$, $P= 0.61$).

Table 7 summarises O_3 effects on other variables in these mesocosms. Anaerobic respiration at 20-25 cm was not affected by O_3 ($F= 0.78$, $P= 0.53$), nor was aerobic respiration measured at 0-5 cm and 20-25 cm (overall: $F= 1.11$, $P= 0.40$). Sedge green leaf density was affected by O_3 ($F= 3.60$, $P= 0.07$), with values in the three elevated O_3 treatments tending to be lower than at ambient O_3 (only for NFA+10: $P< 0.10$). Extractable NH_4 concentrations at 20 cm had a tendency to decline with increasing O_3, and pore water DOC

concentration at both depths tended to be higher at elevated O_3, but neither of these effects was significant. Soil pH was not significantly affected by O_3 at 20 cm, but there was a significant effect at 2.5 cm depth (O_3: F= 3.04, P< 0.05), with significantly lower values in NFA+25 than at NFA+35/10.

Table 7 Soil respiration rates, and plant and soil chemistry variables of peat incubations used for CH_4 production and aerobic CH_4 oxidation potentials at the four different O_3 treatments (see legend Table 1 for details of O_3 treatments) in August 2010. Values are expressed as mean ± SE (n= 9). Anaerobic and aerobic respiration, extractable NH_4 concentration at 20-25 cm depth and most pore water variables were not significantly affected by elevated O_3, except for sedge green leaf density which was lower at the three elevated O_3 treatments (P< 0.10), and for pore water pH at 2.5 cm depth which was significantly lower at NFA+25 than at NFA+35/10 (P< 0.05).

variable	Ambient O_3	NFA + 10	NFA + 25	NFA + 35/10
Anaerobic respiration (µg CO_2-C g DW^{-1} h^{-1})				
20-25 cm depth	6.4 ± 0.4	6.5 ± 0.8	7.3 ± 0.8	6.3 ± 0.9
Aerobic respiration (µg CO_2-C g DW^{-1} h^{-1})				
0-5 cm depth	31.8 ± 2.3	36.7 ± 2.0	35.4 ± 4.0	31.6 ± 1.1
20-25 cm depth	12.0 ± 0.6	14.9 ± 0.9	13.8 ± 1.3	14.0 ± 1.2
Sedge green leaf density (no. leaves m^{-2})				

	7058 ± 959	4204 ± 1352	5829 ± 846	4999 ± 542
Pore water chemistry (concentrations in mg l^{-1})				
NH_4-N conc.: 2.5 cm depth	0.02 ± 0.01	0.24 ± 0.18	0.05 ± 0.04	0.02 ± 0.01
NH_4-N conc.: 20 cm depth	1.18 ± 0.37	2.85 ± 1.47	1.52 ± 0.78	0.56 ± 0.20
DOC conc.: 2.5 cm depth	9.5 ± 1.4	14.6 ± 2.4	15.2 ± 2.9	14.8 ± 3.4
DOC conc.: 20 cm depth	21.0 ± 3.4	34.9 ± 6.7	31.6 ± 3.7	35.2 ± 9.4
pH: 2.5 cm depth	5.35 ± 0.09	5.36 ± 0.10	5.08 ± 0.09	5.47 ± 0.10
pH: 20 cm depth	5.69 ± 0.04	5.53 ± 0.08	5.48 ± 0.11	5.49 ± 0.05
Extractable NH_4-N concentration (0.5 M NaCl; in mg kg^{-1} DW)				
20-25 cm depth	101 ± 10	86.0 ± 22.4	74.1 ± 21.7	64.5 ± 11.5

4. Discussion

4.1. Ozone impacts on CH_4 emission

Exposure to the NFA+35/10 O_3 treatment resulted in a 27% reduction in CH_4 emission during summer. This finding is consistent with results from our earlier study (Toet et al., 2011) using a different temperate peatland community in response to comparable elevated O_3 exposures, suggesting that the effect may be more widespread. However, the two treatments involving 24h year-round elevation of O_3 concentrations (NFA+10 and NFA+25), resulted in no significant decrease in seasonal CH_4 emissions, despite the 24h mean O_3 concentration

and AOT40 values (see Supplementary Information Table S2), being higher in NFA+25 than in NFA+35/10; indeed, in the final summer, CH_4 emissions tended to increase in NFA+10. This difference was not an artefact of CH_4 emissions being measured during the day, when NFA+35/10 exposures were higher, as in both ambient O_3 and NFA+35/10, CH_4 emissions showed relatively weak diurnal variation (data not shown). Part of the reason for the lack of response to 24h exposure may be stomatal closure at night-time, and hence reduced stomatal flux into sedge leaves; however, stomata may also have been (partly) open at night as found in several plant species including sedges (Caird et al., 2007, Gebauer et al., 1998). Ozone exposure has also been observed to make stomata sluggish, increasing nocturnal transpiration and O_3 uptake (Davison & Barnes, 2002; Hoshika et al., 2013). Although the air temperature was on average 3.8˚C higher in the OTCs than at the Scottish field site, the mean summer CH_4 emission rates of 2-12 mg C m^{-2} h^{-1} were within the range of field flux rates in other sedge-dominated peatlands within the UK (Greenup et al., 2000; Gauci et al., 2002; McNamara et al., 2008; Toet et al., 2011; Levy et al., 2012: 0.1-14 mg m^{-2} h^{-1}) and Northern Europe (Granberg et al. 2001, Rinnan et al. 2003, Mörsky et al. 2008: 0.7-16 mg m^{-2} h^{-1}).

However, the results from both this and our previous study in open-top chambers (OTCs) (Toet et al. 2011) were not consistent with the findings of the four-year mire open-air fumigation study of Mörsky et al. (2008), who reported no overall long-term responses of CH_4 emissions to elevated O_3, which were comparable to our NFA+35/10 treatment. The use of OTCs, rather than a field release system, may modify the size of the effect of a given ozone concentration, but these different findings may also be due to a range of other factors, including local climate, soil microbiota, and peat chemistry. Our results are also inconsistent with the summer-long closed-chamber experiment of Williamson et al. (2016), although the non-significant tendency for increased CH_4 emissions in the lower background O_3 treatment by the end of the experiment, was consistent with their report that relative small increases in

background O_3 exposure may increase CH_4 emissions, which suggests a hormetic dose response relationship (Calabrese 2005). Rinnan et al. (2013) suggested that CH_4 responses to elevated O_3 are rather small in these boreal peatlands, compared to other environmental factors (e.g. temperature, water table and light). However, consistent with our findings at NFA+35/10, decreased CH_4 emissions have been reported for three OTC studies on rice paddies in which O_3 concentrations were elevated for 7-8h during the day (Bhatia et al., 2011; Zheng et al., 2011; Tang et al., 2015). Tang et al. (2015) derived a dose-response relationship from these three experiments; CH_4 emissions declined by 2.3% for every 1 ppm.h increase in annual AOT40. This is a stronger effect than the 0.7% for every 1 ppm.h we calculated for impacts in the NFA+35/10 treatment in the experiment reported here.

4.2. Plant-mediated ozone responses

Elevated O_3 effects on CH_4 emissions are probably not caused by direct impacts on soil microbial populations since O_3 is very reactive, and O_3 and its reaction products have been shown not to penetrate deeper than the top few mm of soils (Toet et al., 2009). Ozone is also unlikely to penetrate through the aerenchyma, as it is expected to react rapidly on contact with moist plant surfaces. We cannot exclude the possibility of reduced transport of CH_4 through the aerenchyma of sedge plants due to elevated O_3, although Mörsky et al. (2008) reported no significant effect of O_3 on the proportion of aerenchymatous tissue in *Eriophorum vaginatum* leaves, and there was no significant effect of O_3 on sedge green leaf density of the sedges in our study; furthermore, sedge leaf density did not correlate positively with CH_4 emissions.

We concluded from this that the effects of elevated O_3 are likely to be linked to processes occurring below-ground that are mediated indirectly by plants, e.g. through reduced root biomass and turnover, altered root exudation, litter biomass or litter quality. There is

evidence from rice paddies that O_3-induced reductions in CH_4 emissions are due to reduced C allocation below-ground leading to reduced availability of soil organic C and hence decreased microbial activity. For example, Bhatia et al. (2011) reported reduced CH_4 emissions associated with lower root activity (as measured by reduced dehydrogenase activity), and lower DOC concentrations. Similarly, Tang et al. (2015) attributed reduced CH_4 emissions to reduced biomass allocation below-ground (root biomass declined by 35%) which they linked to an associated inhibition of CH_4 production potential. Feng et al. (2013) also reported that DOC concentrations were reduced by 20% in this study.

Sedge root biomass was not significantly affected by elevated O_3 during our study (S. Toet, unpublished data), contrary to expectation, and pore water DOC concentrations were also not significantly affected by O_3; indeed the trend was for increased, rather than decreased, DOC concentrations. Effects on root biomass were not reported by Mörsky et al. (2008), although they found increased pore water concentrations of organic acids (including acetate) under elevated O_3. Jones et al. (2009) reported that O_3 had no significant effect on DOC concentrations in bog microcosms dominated by *Sphagnum* species, but caused a large reduction in DOC concentrations in microcosms dominated by *Festuca* and *Juncus* species; this was attributed to a reduction in root exudates, causing a shift in microbial consumption from root exudates to soil C pools.

Results from our ^{13}C pulse labelling study clearly demonstrate that elevated O_3 did not alter the contribution of recent photosynthate to CH_4 emissions, even though CH_4 emissions from these labelled cores were reduced by 28%. Recent photosynthate was estimated to contribute only 12% of CH_4 emissions in August-September, a relatively low percentage compared to other peatlands with sedges (>75%: King et al., 2002; 32%: unpublished data, S. Toet) and anaerobic rice paddies (22-45% for the entire growth period, Minoda et al., 1996; Tokida et al., 2011). Impacts on recent photosynthate are therefore not

the main source for CH_4 production in this wet heath, at least not in early August, suggesting that the hydrogenotrophic pathway using less recent C sources rather than the acetoclastic pathway using more labile organic C may have been more prevalent in CH_4 production in the mesocosms (e.g. Hornibrook et al., 2000). This was not clearly supported by the apparent fractionation factors of 1.055 and 1.057 for pore water observed at 20 cm and 30 cm depth, as they are borderline in indicating the dominance of either pathway (Whiticar et al., 1986; Whiticar, 1999; Conrad, 2005; Holmes et al., 2015). These values were, however, probably lowered, indicating more prevalence towards acetoclastic methanogenesis, due to C isotopic fractionation of CH_4 during aerobic, anaerobic or facultative CH_4 oxidation when the CH_4 passed the peat profile to the moss surface (Semrau et al., 2011; Smemo & Yavitt, 2011; Whiticar, 1999). Dominance of the hydrogenotrophic pathway has been observed in several sedge-dominated peatlands (Whiticar et al., 1986; Mörsky et al., 2008; Holmes et al., 2015) and may also explain the lack of increase in CH_4 emission despite the increase in organic acid concentrations (including acetate) in the peat pore water at elevated O_3 in the peatland microcosm study of Mörsky et al. (2008). Transient shifts in pathway dominance are less likely as similar contributions of recent photosynthates to CH_4 emission and also no effect of elevated O_3 were observed in the previous summer (data not shown), but seasonal shifts cannot be excluded. Overall, and in contrast to our original hypotheses, and findings from rice paddies, there is no evidence that the observed decrease in CH_4 emission caused by elevated O_3 was due to reduced organic C substrate availability.

4.3. CH_4 production and oxidation potential

Methane production potential in the final summer of our study was significantly reduced in the two treatments providing 24h fumigation (NFA+10 and NFA+25), but not in NFA+35/10. The greatest reduction in production potential, of 61%, was found in the highest 24h mean O_3

exposure (NFA+25). Methane production potential was only measured at 20-25 cm, the depth with highest production potential in a pilot study. However, there was still considerable production potential at 10-15 cm, and we cannot rule out the possibility that O_3 had different effects at shallower depths.

Aerobic CH_4 oxidation potential was reduced, by an average of 63% in the top, partly green, *Sphagnum* layer in the NFA+25 and NFA+35/10 treatments. The high aerobic CH_4 oxidation potential in the *Sphagnum* layer is consistent with the findings of Raghoebarsing et al. (2005) and Larmola et al. (2010), who showed CH_4 consumption by *Sphagnum* plants through partly endophytic methanotrophs in hyaline cells and on stem leaves. However, reduced CH_4 oxidation rates should result in higher CH_4 emission rates, rather than the lower CH_4 emission rates that we found in NFA+35/10. There was no significant elevated O_3 effect on oxidation potential at 20-25 cm although, as for the CH_4 production potential, elevated O_3 may have had different effects at other depths. It is also possible that the higher O_2 availability in the potential measurements mean that actual aerobic CH_4 oxidation rates, especially below the water table, may have been lower *in situ*, or that anaerobic CH_4 oxidation may have been important (Smemo & Yavitt, 2011).

Mörsky et al. (2008) measured CH_4 production and aerobic consumption potential at a depth of 8-12 cm at the end of the third summer. There was no significant effect of elevated O_3 on either production or oxidation potential, a result that is consistent with the lack of O_3 effects on CH_4 emissions in their experiment. However, Feng et al. (2013) reported a strong reduction in methanogenic activity by elevated O_3 in paddy soils in an experiment in which elevated O_3 reduced both soil DOC and acetate concentrations, and Tang et al. (2015) reported a significant decrease in CH_4 emissions in elevated O_3. Importantly, Feng et al. (2013) were able to link this finding to changes in the diversity and richness of methanogenic archaea, and reductions in the proportion in certain dominant groups such as the acetoclastic

Methanosaeta. Earlier studies have also suggested that O_3 can affect the soil microbial community of rice paddies. Chen et al. (2010) used PFLA and C source utilisation to show that elevated O_3 significantly decreased total microbial biomass and changed soil microbial composition at the end of the growing season, while Feng et al. (2011) reported that elevated O_3 reduced the abundance and genetic diversity of anoxygenic purple phototrophic bacteria. Furthermore, Mörsky et al. (2008) also reported, using PFLA biomarkers, that elevated O_3 reduced total microbial biomass and altered microbial composition in their peatland microcosms. Hence, although we did not find effects of elevated O_3 on CH_4 production or oxidation potentials that were consistent with the observed long-term effects on CH_4 emissions, it remains likely that changes in the methanogenic and/or methanotrophic communities were the key underlying explanation.

4.4. Ozone impacts on N cycling

An alternative explanation for changes in the activity or composition of microbial communities involved in CH_4 emissions may relate to indirect effects of elevated O_3 on soil N cycling. In particular, elevated O_3 significantly reduced pore water NH_4 concentrations at 20 cm throughout the experiment. Similar trends were observed at shallower depths but they were not significant. The soil NH_4 response to elevated O_3 was apparent from the first summer of the experiment, when O_3 effects on CH_4 emission were also first observed. By the final year, NH_4 concentrations appeared to increase in the NFA+10 treatment, as did CH_4 emission. Overall, a strong positive correlation was found, both between and within O_3 treatments, between pore water NH_4 concentrations at all depths and CH_4 emission. Similar positive correlations were found with pH, which was therefore also closely correlated with NH_4 concentrations.

Previous long-term studies of elevated O_3 effects on peatland ecosystems (e.g. Mörsky et al., 2008) have not reported effects on NH_4 concentrations or on N cycling, but negative responses of soil NH_4 concentrations to elevated O_3 have been observed in rice paddies (Bhatia et al., 2011), meadows (Kanerva et al., 2006) and soybean crops (Pereira et al., 2011). A positive link between soil inorganic N availability and methanogenic activity is plausible, and CH_4 emission in paddy soils has been positively correlated to levels of soil mineralisable N (e.g. Zheng et al., 2006). However, there may be important differences between these heavily fertilised systems and natural wetlands. The lower NH_4 availability also might have promoted methanotrophic activity in the mesocosms (Wang & Ineson 2003, Keller et al., 2006) and hence reduced CH_4 emissions, although there was no evidence of enhanced soil aerobic CH_4 oxidation potential at NFA+35/10 in our study; in fact the opposite effect was observed in the top layer of the mesocosms.

Most studies attributed reduced soil NH_4 concentrations or changes in soil N concentration or microbial biomass N in response to elevated O_3 to reduced below-ground C inputs, reduced litter quality (Kanerva et al., 2006; Bhatia et al., 2011; Pereira et al., 2011) or higher nitrification and denitrification rates (Li et al., 2010; Pereira et al., 2011). We have no evidence of such changes in C inputs in elevated O_3 in our study. Aerobic and anaerobic soil respiration potentials were not affected by elevated O_3 in the final summer of our experiment. Low peat pore water NO_2+NO_3 concentrations and negligible N_2O emissions (S. Toet, unpublished data) suggest that enhanced nitrification and/or denitrification responses to elevated O_3 were also less likely. The mechanism leading to reduced soil NH_4^+ concentrations in our study is therefore uncertain.

Other possible explanations for decreased NH_4 concentrations may include increased plant uptake of N, increased microbial biomass (Kanerva et al., 2006; Bassin et al., 2015) or decreased N_2 fixation rate (Pausch et al., 1996; Li et al., 2013). Increased N concentrations of

leaves in response to elevated O_3 have been observed in trees and grasslands (Wittig et al., 2009; Bassin et al., 2015), but this has rather been attributed to increased retranslocation of N after early senescence of part of the leaves, reduced plant size (Wittig et al., 2009) and reduced N resorption from senescing leaves (Uddling et al., 2006). Moreover, leaf N concentrations of the green sedge leaves in the final summer of our experiment were, lower at NFA+35/10 than at ambient O_3 (S. Toet, unpublished data), and, together, with no significant O_3 effects on sedge green leaf density and root biomass imply that increased plant uptake of N at elevated O_3 is not very likely. Recent findings suggest that methanotrophy and N_2 fixation in peatlands may be linked (Ho & Bodelier, 2015); Larmola et al. (2014) observed that aerobic methanotrophs contributed to up to 40% of N_2 fixation. Lower rates of energy-demanding N_2 fixation at elevated O_3 may therefore have resulted in higher CH_4 oxidation rates and consequently lower CH_4 emissions, although this was not confirmed by our aerobic CH_4 oxidation potential measurements.

Finally, more than one mechanism may explain the observed effects of elevated O_3 on CH_4 fluxes: mechanisms may be transient or occur simultaneously. It may be significant that reductions in both CH_4 emissions and soil NH_4 concentrations were observed within a few weeks of initiation of the O_3 treatments. Similar large changes in pore water chemistry (in this case DOC) over a few weeks were reported by Jones et al. (2009), and rapid changes in root respiration in response to O_3 have been reported (Andersen, 2003). In contrast, the long-term responses to elevated O_3 that led to the changes in CH_4 production and oxidation potential that were found in the final summer of the experiment may be linked to different (contributions of) mechanisms.

In conclusion, our data provide evidence of reduced CH_4 emissions in temperate peatlands exposed to seasonal 8h mean O_3 concentrations during summer of about 60 ppb. A recent analysis of global ecosystem exposure to O_3 (Fuhrer et al., under review), using the

Community Earth System Model which has been applied globally, for example by Tai et al. (2014), identified large areas of northern hemisphere temperate ecosystems which experience spring and summer 12h mean O_3 exposures above 55 ppb. Hence, our results imply that temperate CH_4 emissions across the northern hemisphere are already significantly reduced by O_3, and this effect may become greater in future in regions (such as Asia) where precursor emissions are predicted to increase. However, our data also suggest that increases in global background annual mean O_3 concentrations within the range predicted for 2050 will have little, if any, effect on CH_4 emissions from peatland communities. We reject most of our original five hypotheses about the mechanisms underlying O_3 effects on CH_4 emissions from peatlands, but we have identified a close association between effects on NH_4 concentrations and on CH_4 emissions across O_3 treatments and time, which suggests that they are mechanistically linked, through effects on the methanogenic and/or methanotrophic communities. This also implies that the global increases in N deposition which may affect plant species composition, and hence ecosystem processes of temperate ecosystems, including peatlands (Bobbink et al., 2010), may also directly increase CH_4 emissions.

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