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Title: Effects of elevated atmospheric CO_2, prolonged summer drought and temperature increase on N_2O and CH_4 fluxes in a temperate heathland

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Abstract

In temperate regions, climate change is predicted to increase annual mean temperature and intensify the duration and frequency of summer droughts, which together with elevated atmospheric carbon dioxide (CO_2) concentrations, may affect the exchange of nitrous oxide (N_2O) and methane (CH_4) between terrestrial ecosystems and the atmosphere. We report results from the CLIMAITE experiment, where the effects of these three climate change parameters were investigated solely and in all combinations in a temperate heathland. Field measurements of N_2O and CH_4 fluxes took place 1-2 years after the climate change manipulations were initiated. The soil was generally a net sink for atmospheric CH_4. Elevated temperature (T) increased the CH_4 uptake by on average 10 µg C m^{-2} h^{-1}, corresponding to a rise in the uptake rate of about 20 %. However, during winter elevated CO_2 (CO_2) reduced the CH_4 uptake, which outweighed the positive effect of warming when analyzed across the study period. Emissions of N_2O were generally low (<10 µg N m^{-2} h^{-1}). As single experimental factors, elevated CO_2, temperature and summer drought (D) had no major effect on the N_2O fluxes, but the combination of CO_2 and warming (TCO_2) stimulated N_2O emission, whereas the N_2O emission ceased when CO_2 was combined with drought (DCO_2). We suggest that these N_2O responses are related to increased rhizodeposition under elevated CO_2 combined with increased and reduced nitrogen turnover rates caused by warming and drought, respectively. The N_2O flux in the multifactor treatment $TDCO_2$ was not different from the ambient control treatment. Overall, our study suggests that in the future, CH_4 uptake may increase slightly, while N_2O emission will remain unchanged in temperate ecosystems on well-aerated soils. However, we propose that continued exposure to altered climate could potentially change the greenhouse gas flux pattern in the investigated heathland.

1. Introduction

Nitrous oxide (N_2O) and methane (CH_4) are important greenhouse gases (IPCC, 2007), and N_2O is the dominant substance responsible for depletion of the stratospheric ozone layer (Ravishankara et al., 2009). On a global scale, N_2O emissions from unfertilized grassland/steppe, including heathlands, is estimated to be 0.4 Tg N_2O-N y^{-1} (Stehfest and Bouwman, 2006), which corresponds to about 2% of the total annual N_2O emissions (Fowler et al., 2009). Methane uptake by aerobic soils worldwide is estimated to be 30 Tg CH_4 y^{-1}, counteracting 6% of the total emission of CH_4 from natural and anthropogenic sources (Wuebbles and Hayhoe, 2002).

Nitrous oxide emitted from soil primarily originates from the two microbial processes nitrification and denitrification, which occur under aerobic and anaerobic soil conditions, respectively (Wrage et al., 2001). Thus, both processes are controlled by soil moisture that regulates oxygen (O_2) availability and by supply of substrates, ammonium (NH_4^+) or nitrate (NO_3^-). In addition, denitrifying bacteria need labile carbon (C) compounds as an energy source. Soils may act as a sink for atmospheric N_2O, which has been observed at low mineral nitrogen (N) availability and the responsible organisms could be denitrifying bacteria, but are probably also nitrifying organisms (Chapuis-Lardy et al., 2007).

The CH_4 flux between soil and atmosphere is the net result of CH_4 production by methanogenic archaea and CH_4 oxidation by methanotrophic bacteria (Le Mer and Roger, 2001). In aerobic soils, CH_4 oxidation typically proceeds at a greater rate than CH_4 production, resulting in net uptake of atmospheric CH_4. Maximum CH_4 oxidation usually occurs in deeper soil layers. Thus, CH_4 uptake is strongly controlled by the physical diffusion of atmospheric CH_4 through the soil profile, which is mainly regulated by the soil texture and water content (King, 1997), as molecular diffusion in water is four orders of magnitude slower than in air.

69 The effect of drought on fluxes of CH_4 and N_2O has only been investigated in a limited
70 number of studies in temperate ecosystems on aerobic soils. In a temperate spruce forest, Borken
71 et al. (2000) found that prolonged summer drought increased the annual CH_4 uptake by more than
72 40%. However, in a deciduous forest on well-drained soil, the CH_4 uptake was only increased by
73 7% because soil in the control plots already had a low water content due to effective drainage
74 (Borken et al., 2006). Goldberg and Gebauer (2009) reported that summer drought reduced the
75 N_2O emission from a spruce forest.
76 Elevated atmospheric carbon dioxide (CO_2) concentrations affect soil properties via plant-
77 mediated processes, and thereby potentially the fluxes of N_2O and CH_4. It is well known that
78 plants growing under elevated CO_2 may reduce their transpiration, leading to increased water use
79 efficiency and soil moisture contents (Morgan et al., 2004; Robredo et al., 2007). Furthermore, a
80 common plant response to elevated CO_2 is increased deposition of root-derived C into the
81 rhizosphere (Allard et al., 2006; Pendall et al., 2004).
82 The effect of elevated atmospheric CO_2 on N_2O fluxes has been investigated intensively
83 through the last decade in a variety of ecosystems. Long-term studies ($\geq$ 1 year) in natural or
84 semi-natural ecosystems on well-aerated soils with low N availability show either no response in
85 annual N_2O emissions rates to atmospheric CO_2 levels (Baggs et al., 2003; Mosier et al., 2002;
86 Phillips et al., 2001b) or increased N_2O emissions from soil under elevated CO_2 (Kammann et al.,
87 2008). The positive effect of elevated CO_2 on the N_2O emission was explained by increased
88 rhizodeposition of labile C substrates stimulating denitrification. In an N-limited pine forest,
89 Phillips et al. (2001b) observed seasonal variability in the N_2O response to elevated atmospheric
90 CO_2 compared to an ambient control. This included reduced N_2O emissions during the growing
91 season due to high plant-microbial competition for N, and enhanced N_2O emissions during winter
92 possibly because denitrification was stimulated by greater soil moisture and labile C sources

93 under elevated CO_2. In a short-term study during autumn, Arnone and Bohlen (1998) also found
94 that elevated atmospheric CO_2 increased N_2O emission, which they ascribed to improved soil
95 moisture conditions in a relatively dry grassland favouring the microbial transformation of N.
96 Reduced CH_4 consumption under elevated atmospheric CO_2 has been observed in several
97 ecosystems on undisturbed aerobic soils (Ambus and Robertson, 1999; Baggs and Blum, 2004;
98 Dubbs and Whalen, 2010; Ineson et al., 1998; Phillips et al., 2001a), but the exact mechanism is
99 not well known and may vary between ecosystems. Two possible mechanisms have been
100 suggested for the reducing effect of elevated CO_2 on net CH_4 consumption: i) decreased CH_4
101 oxidation due to higher soil water content and thereby reduced diffusion of CH_4 (Ambus and
102 Robertson, 1999; Baggs and Blum, 2004) and ii) increased CH_4 production due to anaerobic
103 microsites caused by reduced O_2 diffusion and increased respiration (McLain and Ahmann,
104 2008).
105 Effects of elevated temperature on N_2O and CH_4 fluxes have only been investigated in a
106 few field-scale warming experiments located in temperate ecosystems on well-aerated soils
107 (McHale et al., 1998; Peterjohn et al., 1994; Rustad and Fernandez, 1998). The artificial warming
108 had either no effect on CH_4 flux or it increased CH_4 uptake, which could be related to the
109 observed warming-induced declines in soil moisture in certain soil layers. Warming may reduce
110 soil moisture by increasing the evapotranspiration (Dermody et al., 2007). No effect of warming
111 on N_2O flux was observed (McHale et al., 1998; Peterjohn et al., 1994), although elevated
112 temperature is known to increase net N mineralization (Rustad et al., 2001).
113 In the current study, the effects of future climatic and atmospheric conditions on the
114 biosphere-atmosphere exchange of N_2O and CH_4 were investigated in a temperate heathland at
115 the CLIMAITE experimental site (www.climaite.dk). CLIMAITE was initiated in 2005 to
116 improve our understanding of how biological processes in natural terrestrial ecosystems may be

117 affected under future environmental conditions involving elevated temperature, elevated
118 concentration of atmospheric CO_2 and prolonged summer drought, simulating *in situ* the climatic
119 scenario as predicted for Denmark in year 2075 (Mikkelsen et al., 2008). Previous studies have
120 examined the effects on greenhouse gas fluxes of warming, elevated CO_2 and summer drought,
121 but to our knowledge field studies combining all three factors in a full-factorial design have not
122 been reported. We formulated four hypotheses for the responses in N_2O and CH_4 fluxes to the
123 climate change parameters investigated in the experiment.
124 1) Prolonged summer drought will stimulate CH_4 uptake and reduce N_2O emissions.
125 2) Elevated CO_2 will reduce CH_4 uptake due to higher soil moisture caused by plant water
126 saving mechanisms. Nitrous oxide emissions will remain unchanged under elevated CO_2
127 because the stimulating effects of increased soil moisture and availability of labile carbon
128 compounds will be offset by increased plant-microbial competition for N.
129 3) Warming will increase CH_4 uptake because of reduced soil moisture. On an annual basis N_2O
130 emission will be unaffected by warming, but this may include a reduction during spring and
131 summer due to reduced soil moisture and an increased emission in autumn due to higher N
132 turnover rates.
133 4) In the combinations of two or three treatments, the treatment effects will either counteract
134 each other, if in opposite directions, or intensify each other, if in the same direction.
135 To address these hypotheses, we conducted a full-factorial study including all treatments. In
136 addition, a more intense study involving a subset of five treatments was carried out to focus on
137 treatment effects during the experimental summer drought and the subsequent rewetting period.
138

2. Materials and methods

2.1 Field site

The study took place at the CLIMAITE experimental site (Mikkelsen et al., 2008) situated at Brandbjerg (55º53' N, 11º58' E) about 50 km NW of Copenhagen, Denmark. The site is a dry, temperate heathland on a hilly nutrient-poor sandy podzol (FAO classification). The mineral soil consists of 20.5% coarse sand, 71.6% fine sand, 5.8% silt and 2.2% clay, and has a pH_{H2O} of 4-5. Mean pore volume and field capacity of the upper 15 cm of the mineral soil is 42 and 17 vol%, respectively. The content of carbon and nitrogen decline sharply from 6.4% and 0.34% in the upper 2 cm of the mineral soil to 0.39% and 0.02% in the 10-30 cm layer, respectively. Above the mineral soil is an organic top layer of about 5 cm in depth containing approximately 23% carbon and 1.2% nitrogen. The vegetation is dominated by *Calluna vulgaris* (L.), *Deschampsia flexuosa* (L.) and various mosses. Annual mean temperature is 8.0 ºC, annual mean precipitation is 613 mm (www.DMI.dk) and the N deposition is 1.25 g N m^{-2} $year^{-1}$ (Ellermann et al., 2005). The study site was chosen to represent a semi-natural ecosystem having the required low vegetation to allow for experimental manipulations.

2.2 Experimental design

The climate change manipulations were initiated in October 2005 and consisted of eight treatments, *viz.* elevated temperature (T), prolonged summer drought (D), elevated atmospheric CO_2 concentrations (CO_2), all combinations of these treatments (TD, TCO_2, DCO_2 and $TDCO_2$) and untreated controls (A) (Mikkelsen et al., 2008). All treatments were applied to six replicate plots, *i.e.* 48 plots in total. The field site covered an area of about 2 ha and the experimental plots were distributed in twelve 6.8-m diameter octagons arranged pair-wise in six blocks, where one octagon in each block was exposed to elevated CO_2 concentrations (Fig. 1a). Each octagon

consisted of four plots in a split design with the treatments drought or elevated temperature solely or in combination, and a non-warmed, non-drought plot (Fig. 1b). Temperature was increased by passive night-time warming using automatic horizontal curtains that withdrew in case of precipitation during night. Periods of drought were achieved using automatic curtains, which extended above the plots during rain events. The atmospheric CO_2 concentration was increased from the ambient level of 380 ppm to 510 ppm by the Free Air Carbon Enrichment (FACE) technique that involved feedback control by monitored CO_2 concentration, wind speed and wind direction. The temperature increase produced by the passive night-time warming showed a seasonal pattern. More specifically, the mean and maximum temperature increase at 5 cm soil depth was higher in spring (0.6, 1.5 °C), summer (0.5, 1.3 °C) and autumn (0.3, 1.0 °C) than in the winter (0.2, 0.7 °C). The experimental drought periods took place in July 2006 and May-June 2007, and lasted one month after which the soil water content typically reached 5 vol% in the top 20 cm of the soil.

<Figure 1>

2.3 N_2O and CH_4 flux measurements

In October 2004, one stainless steel collar (60 cm × 60 cm × 10 cm deep) for gas flux measurements was installed in each plot to a maximum depth of 7 cm. In some plots, a section of the collar could not be inserted into the soil due to shallow *Calluna* roots, and instead a substantial border of soil was placed on the outside of the collar to ensure a complete seal against bulk air flow.

The field measurements of N_2O and CH_4 fluxes were conducted from June 2006 to October 2007 at weekly to monthly intervals. The aim was to examine treatment effects, and as

187 fluxes of N_2O are known to be low in dry soils, the measuring effort was targeted at rain events
188 when possible. Two studies were carried out: i) A full-factorial study including all eight
189 treatments and ii) a drought/rewetting study in relation to the summer drought in 2007 including
190 the treatments A, CO_2, D, DCO_2 and $TDCO_2$. The full-factorial study consisted of 11 measuring
191 campaigns evenly distributed between the warm and the cold season. Each measuring campaign
192 in the full-factorial study was conducted in four sub-campaigns over two consecutive days. Each
193 sub-campaign included at least one replicate of each treatment in order to minimize the influence
194 on possible treatment effects of any day-to-day fluctuation in emission rates, as well as
195 fluctuations between morning and midday. The drought/rewetting study began in April 2007 and
196 included only five of the treatments in order to enable the measurement of all plots on a single
197 day. This was performed at one to 20 days intervals over a five months period (n=11).
198 In a measuring campaign, fluxes of N_2O and CH_4 were determined by a static chamber
199 method using white PVC chambers placed on the metal collars installed in the plots. During
200 wintertime, 15-cm high chambers were used for 2-hour enclosure periods, whereas 45-cm high
201 chambers were used during summertime for 2.5-hour enclosure periods in order not to disturb the
202 vegetation. Each chamber had a sponge rubber edge trim, which fitted into a channel on the upper
203 edge of the soil collar and ensured a tight seal during measurements. The chambers were
204 equipped with a gas sampling port and a fan (50 × 50 mm) to mix the headspace air. Four times
205 during the enclosure period, two 30-ml samples of headspace air was taken with a syringe
206 through the sampling port and used to flush a 3.5-ml Venoject vial and a 2-ml crimp-seal vial,
207 respectively. To measure N_2O concentrations, the 3.5-ml vials were pressurized to 1.4 bar with
208 carrier gas immediately before analysis by gas chromatography (GC-14B, Shimadzu, Kyoto, JP).
209 The 2-ml samples for CH_4 determination were supplemented with 0.5 ml N_2 and the

concentrations were established by gas chromatography (HP 6890, Agilent, Santa Clara, US). Nitrous oxide fluxes and CH_4 emissions into the chamber were calculated using linear regression of headspace concentrations versus time (n=4), whereas CH_4 uptake was calculated by fitting a first-order function.

2.4 Soil moisture, temperature and nitrate

Each of the 48 plots in the climate change experiment had a soil temperature probe installed at 5 cm depth recording at 1 Hz and data was logged as hourly means. Time Domain Reflectometry (TDR) probes were installed at 0-20 cm and 0-60 cm depths to measure volumetric soil water content on a half-hourly basis. More specifically, the TDR probes measure the ability of the soil to transmit an electric field, which is converted into volumetric soil moisture using a soil type specific algorithm. The soil water content in the 20-60 cm layer was calculated from the measurements in 0-20 cm and 0-60 cm. In addition, manual measurements of soil moisture at 0-6 cm depth were conducted at each soil collar during some flux measuring campaigns (ThetaMeter HH1, Delta-T Devices, Cambridge, UK). During each measurement sub-campaign, air temperature was recorded outside two gas flux chambers to be used in the flux calculations. Precipitation was recorded at 2 m height by two independent weather stations at the field site (Fig. 1a).

Soil water was collected monthly from passive PVC soil water draining collectors below the organic soil layer (approximately 5 cm depth) in each experimental plot. Concentrations of NO_3^- were analyzed on an Autoanalyzer 3 (Bran+Luebbe Gmbh, Germany).

232 *2.5 Potential nitrification and denitrification*

233 In order to evaluate the long-term effect of the summer drought treatment, soil samples
234 were collected in late autumn, *viz.* on 5 November 2007. In each plot, three subsamples from the
235 0-8 cm soil layer under *Deschampsia flexuosa* were obtained using a 2.5-cm diameter soil core.
236 The subsamples were pooled, gently homogenized by hand and major roots were removed. Soil
237 samples were stored at 4 °C until analysis (less than 24 h). Potential nitrification was determined
238 by adding 3 g fresh soil to a 100-ml Erlenmeyer bottle together with 20 ml of Winogradsky
239 solution containing 7.6 mM ammonium sulphate. The bottle was placed on a shaker (100 rpm)
240 and incubated at 25 ^{o}C. After 0 and 168 h, a 10-ml sample was centrifuged (3500×*G*; 25 ^{o}C; 5
241 min) and the supernatant was analyzed for nitrite and nitrate (Fiastar 5000 Flow Analyzer, FOSS,
242 Hillerød, DK). Potential nitrification rates were estimated from the increase in nitrite+nitrate
243 concentrations between 0 and 168 h.

244 To measure potential denitrification, 10 g fresh soil was placed in a 100-ml incubation
245 bottle and 15 ml of 1 mM potassium nitrate, 0.5 mM glucose, 0.5 mM sodium acetate and 0.5
246 mM sodium succinate was added. The bottle was sealed with a butyl rubber stopper, flushed with
247 N_2 for 2 min, had 10% acetylene added, and was then placed horizontally on a shaker (200 rpm)
248 and incubated at 22 ^{o}C. After 30, 70, 120 and 180 min, 3 ml headspace was transferred to a pre-
249 evacuated 3-ml Venoject vial. The vials were pressurized with 1 ml N_2 before analysis for N_2O
250 on a gas chromatograph (HP 5890, Agilent, Santa Clara, US). Potential denitrification rates were
251 estimated from linear regression of total N_2O, *i.e.* headspace and aqueous phase N_2O (Weiss and
252 Price, 1980), versus time.

253

254 *2.6 Nitrous oxide reductase activity*

255 To determine N_2O reductase activity, NO_3^- was removed from soil samples by vortexing
256 10 g fresh soil with 30 ml of phosphate buffered saline (PBS) for 5 sec of followed by
257 centrifugation for 10 min at 3500×*G* and 5 ^oC. The supernatant was discarded and the pellet
258 resuspended in 30 ml PBS. This process was repeated three times; after the last, the pellet was
259 resuspended in 15 ml of 0.5 mM glucose, 0.5 mM sodium acetate, and 0.5 mM sodium succinate
260 and transferred to a 100-ml incubation bottle. The bottle was sealed with a butyl rubber stopper,
261 flushed with N_2 for 2 min, had 100 ppm N_2O added (final concentration), and was then placed
262 horizontally on a shaker (200 rpm) and incubated at 22 ^oC. After 0, 1, 3, 6 and 24 h, 3 ml of
263 headspace was transferred to a pre-evacuated 3-ml Venoject vial before analysis of N_2O as
264 described above. The decline in headspace and aqueous phase N_2O (Weiss and Price, 1980)
265 during the incubation was used to calculate N_2O reductase activity.
266
267 *2.7 Statistics*

268 Analyses of variance (ANOVA) were conducted by fitting mixed effects models using the
269 PROC MIXED procedure of SAS (SAS Institute, 2003). The random effects accounted for the
270 experimental design (*Random* Block Octagon). Data was transformed as required to obtain
271 normality and homogeneity of variance. In the full-factorial study, all CH_4 analyses were
272 performed on square root transformed fluxes, *viz.* $(-CH_4 \text{ flux}+110)^{0.5}$, where CH_4 uptake rates
273 were entered as negative values. For repeated measures, the optimal covariance structure was
274 selected using Akaike's An Information Criterion (AIC). In order to optimise the model, main
275 factor effects (CO_2, D, T) and interactions (CO_2×D, CO_2×T, D×T, CO_2×D×T) with $P \leq 0.25$ were

kept in the model during the step-wise reduction of the fixed effects in the model. Effects with $P \leq 0.05$ were considered to be significant.

Data from the full-factorial study and the drought/rewetting study were analyzed separately. The statistical analysis of the full-factorial study consisted of two parts. First, treatment effects on CH_4 and N_2O fluxes were examined with repeated measures models that also tested for treatment effects at specific measuring campaigns ($CO_2 \times D \times T \times Time$). Differences of least squares means was used to interpret significant treatment interactions and estimate absolute changes in response to the main treatments. Soil moisture and mean daytime soil temperature (sunrise to sunset) recorded on dates of the full-factorial study campaigns were analyzed in the same way. Secondly, other repeated measures models were used to evaluate how N_2O and CH_4 fluxes were influenced by the two covariates, soil moisture and daytime soil temperature. Dependency between soil moisture and soil temperature was determined by Pearson's Correlation Coefficient using the PROC CORR procedure of SAS.

Data from the drought/rewetting study was analyzed sequentially examining one measuring campaign at the time using a two-factor model including D, CO_2 and $CO_2 \times D$ (dataset excluding $TDCO_2$). In addition, single factor repeated measures models were used to analyze the N_2O and CH_4 fluxes across the drought and rewetting periods with time or soil moisture as covariates (dataset including $TDCO_2$).

3 Results

3.1 Soil moisture and temperature in the full-factorial study

Compared to the annual mean temperature and precipitation for the site, the years 2006 and 2007 where the study took place were generally warmer and wetter with a mean temperature of 10.2 ºC and an annual precipitation of 746 mm. Rain events were distributed throughout the

300 experimental period, but heavy rainfall was absent in the spring of 2007 (Fig. 2a). The soil water
301 content was generally higher in the 0-20 cm soil layer than in the 20-60 cm layer (Fig. 2b). Mean
302 daytime soil temperature at 5 cm depth measured during the full-factorial study campaigns
303 showed a steady decline from July 2006 to March 2007 (Fig. 2c). The passive night-time
304 warming increased the mean daytime soil temperature by 0.39 °C on average ($P<0.0001$) and the
305 temperature was slightly lower in drought treated plots compared to non-drought treated plots
306 ($P=0.05$; Table 1). Furthermore, the CO_2 treatment increased soil temperature but only in
307 unwarmed plots ($CO_2 \times T$; $P<0.0001$). This CO_2 effect could be due to reduced water consumption
308 by plants in response to elevated atmospheric CO_2 and thereby reduced loss of heat. Soil moisture
309 in the upper 20 cm was affected by the drought treatment at three measuring campaigns during
310 and following the experimental droughts, resulting in a 0.85 vol% lower soil water content in the
311 drought treated compared to the non-drought treated plots on average across the study period
312 ($P=0.0004$; Table 1). The drought effect on soil moisture was most pronounced in plots at
313 ambient CO_2 ($CO_2 \times D$; $P=0.0017$). Warming also had an effect on the soil water content in the top
314 soil during seven measuring campaigns, reducing it by 0.99 vol% on average ($P<0.0001$). In the
315 lower soil layer from 20 to 60 cm, drought and warming reduced the soil water content by 0.79
316 and 0.84 vol%, respectively ($P \leq 0.0036$). In March 2007, the water content in 0-6 cm was higher
317 in the plots exposed to elevated CO_2 compared to the ambient CO_2 plots ($P=0.0069$), however
318 soil moisture in 0-20 cm and 20-60 cm were not affected by the CO_2 treatment (data not shown).
319
320 <Figure 2>
321 <Table 1>
322

323 *3.2 CH_4 fluxes in the full-factorial study*

324 Fluxes of CH_4 were generally into the soil with 80% of the measured fluxes falling in the
325 range -1 to -130 µg CH_4-C m^{-2} h^{-1}. The CH_4 uptake was on average 10 µg CH_4-C m^{-2} h^{-1} higher
326 in the warmed plots compared to the unwarmed plots (P=0.044) (Fig. 3a, Fig. 4a). Furthermore,
327 an interaction appeared between the CO_2 treatment and time, which was the product of the lower
328 CH_4 uptake in elevated CO_2 plots compared to ambient CO_2 plots during one measuring
329 campaign in January 2007 (CO_2×Time; P<0.0001).

330 We tested the effect on the CH_4 flux of soil moisture recorded at the start of flux
331 measurement as well as mean daytime soil temperature. Daytime soil temperature at 5 cm depth
332 explained the variability in CH_4 fluxes to a greater extent (P<0.0001) than soil moisture in the
333 upper 20 cm (P=0.0049), which again explained the flux better than moisture in the 20-60 cm soil
334 layer (P=0.021). The less significant effect of soil moisture in the top layer compared to soil
335 temperature could arise from the linear modelling of the relationship between soil moisture and
336 the square root transformed CH_4 uptake. However, linear modelling might be misleading since
337 several studies have indicated that the relationship is hump-shaped, *i.e.* at high soil moisture the
338 relationship is negative because an increase in soil moisture reduces gas diffusivity, while at low
339 soil moisture the relationship may be positive because soil moisture limits the methanotrophic
340 activity (*e.g.* Del Grosso et al., 2000). As a test of the potential linearity, we excluded two
341 measuring campaigns where the lowest soil moisture values were recorded, *viz.* below 6 vol%.
342 However, excluding these data from the analysis removed the negative relationship between CH_4
343 uptake and soil moisture (P=0.61), whereas the positive relationship with soil temperature
344 remained (P=0.0083). Thus, linear modelling of soil moisture and CH_4 uptake seems reasonable
345 in the full-factorial study. Effects of soil temperature could be indirect via the effect of

346 temperature on soil moisture as the two parameters were 54% negatively correlated. However, an
347 analysis including both soil moisture in the top layer and soil temperature supported the stronger
348 positive effect of soil temperature (P=0.0004) compared to the negative effect of soil moisture
349 (P=0.074) on the CH_4 uptake (Table 2).

350 As stated above warming increased the CH_4 uptake by 10 µg CH_4-C m^{-2} h^{-1} on average.
351 To evaluate how much of this could be explained by changes in soil temperature and soil
352 moisture, respectively (Table 1), the model in Table 2 was used to calculate the increase in CH_4
353 uptake resulting from an 0.39 °C-increase in mean soil temperature across the study period and a
354 0.99 vol%-decline in mean soil water content. Based on the model, changes in soil temperature
355 and soil moisture due to the experimental warming caused an increase in the CH_4 uptake of 0.7
356 and 0.9 µg CH_4-C m^{-2} h^{-1}, respectively, all together explaining 16% of the measured increase in
357 the CH_4 uptake.

358

359 <Figure 3>

360 <Figure 4>

361 <Table 2>

362

363 *3.3 N_2O fluxes in the full-factorial study*

364 Generally, emission rates of N_2O were small (<10 µg N m^{-2} h^{-1}; Fig. 3b). The repeated
365 measures model fitted to the N_2O fluxes revealed an interaction between the CO_2 and drought
366 treatments (CO_2×D; P=0.047). Inspection of differences of least squares means showed that this
367 interaction arose because the N_2O emission decreased in response to drought, but only in plots
368 that were also exposed to elevated CO_2, *viz.* $CO_2 + TCO_2 > DCO_2 + TDCO_2$ (Fig. 4b). As single
369 experimental factors elevated CO_2, drought and warming had no significant effect on the N_2O

flux, but the combination of CO_2 and warming caused increased N_2O emission in the TCO_2 treatment, whereas the N_2O production ceased in the DCO_2 treatment, where CO_2 was combined with drought. The statistical analysis also revealed an interaction between the drought treatment and time (D×Time; $P<0.0001$), which was caused by reduced N_2O emission from drought treated plots compared to non-drought treated plots during one measuring campaign following a rain event during the summer drought treatment in 2006. Daytime soil temperature at 5 cm depth, soil moisture in the upper 20 cm and in the 20-60 cm layer all explained the N_2O flux equally well ($P<0.0001$).

3.4 Nitrate, potential nitrification and denitrification

Mean annual NO_3^- concentration in soil water was reduced in plots exposed to either elevated CO_2 or drought as single factor (CO_2×D×T; $P=0.0064$), whereas warming as main factor increased the NO_3^- concentration ($P=0.003$; Fig. 5). Soil samples collected under *Deschampsia* vegetation in November 2007 were incubated at uniform temperature conditions to assess potential nitrification and denitrification, which indicated the amount of nitrifying and denitrifying enzymes present in the 0-8 cm soil layer at the site. Potential nitrification was higher in the warmed plots than in the unwarmed plots ($P=0.0003$), especially in the TD treatment, whereas the activity tended to be low in plots exposed to CO_2 as a single factor (Fig. 6a). Potential denitrification was also increased in the warmed plots ($P=0.014$; Fig. 6b). Negligible N_2O reductase activity was observed in the soil samples with N_2O uptake rates less than 1 ng N g^{-1} dw h^{-1} in all samples (data not shown).

<Figure 5>

393 <Figure 6>

394

395 *3.5 Soil moisture and precipitation in the drought/rewetting study*

396 During the drought period from 21 May to 22 June 2007, 94 mm of precipitation was
397 excluded from the drought treated plots (Fig. 7a). As a result, soil moisture in the 0-6 cm layer
398 was lower in the drought treated compared to the non-drought treated plots during the drought
399 period (Fig. 7b). The drought effect on soil water persisted through the subsequent rewetting
400 period ($P<0.009$), where all plots received 53 mm of rain. Soil moisture at 0-6 cm depth peaked
401 on the second day after rewetting, which occurred on 27 June where 25 mm of rainfall was
402 recorded.

403

404 <Figure 7>

405

406 *3.6 CH_4 and N_2O fluxes in the drought/rewetting study*

407 During the drought and rewetting periods, drought effects on the CH_4 and N_2O fluxes
408 occurred on 30 May, the first day of measurement during the drought, where both CH_4 uptake
409 and N_2O emission decreased in the drought treated compared to the non-drought treated plots
410 ($P\leq0.022$; Fig. 7c,d). However, drought effects were absent during the last part of the drought
411 period and during the rewetting (*i.e.* 14 June to 2 July). Repeated measures models were used to
412 evaluate which other factors controlled the fluxes during this time span and revealed that the N_2O
413 flux was highly controlled by the soil water content ($P=0.024$). Furthermore, the N_2O emission
414 on the second day after rewetting was higher than the other days ($P=0.005$), thus the response in
415 the N_2O flux to rewetting was delayed by one day. In contrast, the CH_4 flux responded
416 immediately to rewetting by a general increase in CH_4 uptake across all treatments the first day

after rewetting compared to the other days (P=0.0087). As the soil water content continued to rise, the CH_4 oxidation was hindered, resulting in reduced CH_4 uptake and even net CH_4 emission in some plots during the last two campaigns in the rewetting period. Across the five treatments investigated, six of the 30 plots were CH_4 hotspots with emission rates above 100 μg C m^{-2} h^{-1}. No delayed effect of the drought treatment appeared in N_2O and CH_4 fluxes during four measuring campaigns from *medio* August to *primo* October 2007 (data not shown). Following 13 mm of rainfall in the beginning of October, consistently net CH_4 emission was observed again with 76% of the measured flux rates being CH_4 efflux.

4 Discussion

4.1 Effects of warming and elevated CO_2 on CH_4 fluxes

Methane fluxes ranged from net uptake to net emission, demonstrating that both methanotrophs (CH_4-oxidizing bacteria) and methanogens (CH_4-producing archaea) were present in the soil microbial community at the site. Generally, CH_4 oxidation exceeded CH_4 production with 80% of the measured flux rates in the full-factorial study falling in the range -1 to -130 μg C m^{-2} h^{-1}. This is comparable to the only other study that examined CH_4 fluxes in a shrubland on sandy soils, which happened to be in a Mediterranean sclerophyllous shrubland, where the CH_4 fluxes mainly varied between -12 and -94 μg C m^{-2} h^{-1} (Castaldi and Fierro, 2005).

Passive night-time warming increased CH_4 uptake by 10 μg C m^{-2} h^{-1} on average, corresponding to a rise in the uptake rate of about 20% (Fig. 4a). Enhanced CH_4 uptake in response to warming was also observed in studies conducted in a mixed deciduous forest and a spruce-fir forest (Peterjohn et al., 1994; Rustad and Fernandez, 1998). The warming treatment raised mean daytime soil temperature by 0.39 °C (Table 1). In addition, the warming treatment reduced soil moisture in the 0-20 cm soil layer by 0.99 vol%, thus potentially warming could

441 have affected the CH_4 flux solely via changes in soil moisture. However, most likely the CH_4
442 uptake was stimulated by changes in both variables. Our analysis revealed that a 0.39 °C-increase
443 in soil temperature and a 0.99 vol%-reduction of soil moisture would increase the CH_4 uptake to
444 almost the same extent, *i.e.* by 0.7 and 0.9 μg CH_4-C m^{-2} h^{-1}, respectively. However, together the
445 changes in these two variables only explained 16% of the observed rise in CH_4 uptake in
446 response to warming. Thus, other parameters than soil temperature and soil moisture were
447 apparently also involved in stimulating the net CH_4 uptake in the warmed plots.
448 In the full-factorial study, soil temperature appeared more important than soil moisture in
449 controlling the CH_4 oxidation in the sandy soil at our heathland site. This is in contrast to other
450 studies in temperate ecosystems on aerated soils, where soil moisture had a stronger influence on
451 the CH_4 uptake compared to soil temperature (McHale et al., 1998; Peterjohn et al., 1994; Rustad
452 and Fernandez, 1998). In most ecosystems, CH_4 uptake is controlled by the physical diffusion of
453 CH_4 from the atmosphere to the soil layer, where CH_4 oxidation takes place, and soil moisture is
454 a variable that influences the diffusive transport of CH_4 (Borken et al., 2006; King, 1997). On the
455 contrary, CH_4 uptake seems to be less controlled by biotic factors regulated by for instance soil
456 temperature as uptake rates are relatively similar across many different ecosystems worldwide
457 (Billings et al., 2000; King, 1997). In our study, a likely reason for the weaker negative influence
458 of soil moisture on CH_4 fluxes compared to the positive influence of soil temperature could be
459 that the sandy soil at the site is very well-drained. At field capacity, about 60 % of the pore space
460 is air-filled in the 0-15 cm layer of the mineral soil, thus CH_4 diffusion is seldom limited by
461 water-filled soil pores.
462 Accordingly, the drought treatment had no effect on the CH_4 flux although this treatment
463 reduced the soil water content in the 0-20 cm layer during three out of nine measuring campaigns.
464 The period of experimental drought in May-June 2007 also showed that the drought treatment

had limited direct or indirect effects on the CH_4 flux as the uptake was only affected by drought during one out of three occasions, and since no delayed drought response in the CH_4 flux occurred from June to October 2007. In fact, the methanotrophic activity was reduced due to drought stress on the first measuring day in the drought period (30 May; Fig. 7c). During the last part of the experimental drought, the methanotrophs were also drought stressed in the non-drought treated plots, as the CH_4 uptake increased across all treatment on the first day after rewetting (26 June). A similar response was observed by Priemé and Christensen (1999) at onset of the rainy season in the African savanna.

In summary, the methanotrophs were more susceptible to dry conditions in May-June 2007 compared to the drought period in July-August 2006, where the highest CH_4 uptake rates were recorded on 17-18 July at soil moisture levels of about 5 vol% in the 0-20 cm layer (Fig. 3a). One possible reason for the difference could be the relatively dry spring of 2007, where the site only received 39 mm of rainfall during the last two months prior to the experimental drought (Fig. 2a), whereas the pre-drought precipitation in 2006 was 122 mm. Thus, presumably the methanotrophs were only able to cope with dry conditions for a limited period.

The complex control of soil moisture on CH_4 fluxes was illustrated by the reduction in CH_4 consumption rates as the soil water content continued to rise during rewetting in 2007, resulting in net CH_4 emissions in some plots on the second day after rewetting (27 June; Fig. 7c). The CH_4 flux therefore seemed to be controlled by soil moisture in a non-linear way in the drought/rewetting study. A similar relationship between CH_4 uptake and soil moisture was found in a Dutch heather grassland on sandy soil (van den Pol-van Dasselaar et al., 1998). Furthermore, Dijkstra et al. (2010) eliminated constraints on CH_4 uptake due to gas diffusion and showed that methanotrophic activity increased under elevated CO_2, partly due to an increase in soil moisture.

In January 2007, the CH_4 uptake was reduced in the plots exposed to elevated CO_2 compared to the ambient CO_2 plots. A root in-growth study conducted at the site showed that elevated atmospheric CO_2 stimulated root growth of both *Deschampsia* and *Calluna* (Marie F. Arndal, pers. comm.). Thus, a likely reason for the temporary CO_2 effect on CH_4 fluxes is increased methanogenesis in soils under elevated atmospheric CO_2, fuelled by increased winter root decay resulting from a larger root biomass. McLain and Ahmann (2008) also suggested that reduced net CH_4 uptake under elevated CO_2 was due to increased CH_4 production. Another possible reason is reduced CH_4 oxidation in the CO_2 plots caused by increased soil water content in the upper most soil horizon (0-6 cm), which was observed in March 2007. Similar mechanisms were also put forward in other studies (Ambus and Robertson, 1999; Baggs and Blum, 2004).

Summarizing treatment effects on CH_4 fluxes, prolonged summer drought did not increase the CH_4 uptake as hypothesized because soil moisture at the site was already low and therefore not an obstacle for CH_4 diffusion. The warming treatment did increase the CH_4 uptake as hypothesized, however apparently not only via the effect on soil moisture that we expected, but also through a direct stimulation of the methanotrophic activity by increased soil temperatures and due to other unknown factors. Finally, elevated atmospheric CO_2 did decrease the CH_4 uptake as hypothesized, but only in January 2007 and the exact mechanism is not well understood. In the three-factor combination $TDCO_2$, the positive effect of warming on the CH_4 uptake was partly outweighed by the reduced CH_4 uptake under elevated CO_2 during winter, resulting in a slight but insignificant increase in the CH_4 uptake compared to the ambient treatment. We therefore accept the fourth hypothesis that individual treatment effects counteract each other if in opposite directions.

511 *4.2 N_2O response to elevated CO_2 combined with drought or warming*

512 Heterotrophic nitrification may be a significant source of N_2O in soils with low pH and
513 high availability of oxygen and organic material (Wrage et al., 2001). This process possibly
514 contributed to the N_2O emission from the acidic and well-aerated sandy soil at our site, along
515 with autotrophic nitrification and denitrification.

516 Neither elevated CO_2, drought or warming affected the N_2O flux as main treatments when
517 analyzed across all measuring campaigns in the full-factorial study, but the N_2O flux responded
518 to combinations of these treatments (Fig. 4b). Thus, in this study the effect of treatment
519 combinations could not be predicted from the effects of the single treatments. The N_2O emission
520 ceased when elevated CO_2 was combined with drought, whereas increased N_2O emissions
521 occurred when elevated CO_2 and warming was combined. Of the three main treatments, warming
522 affected soil moisture most frequently; however the reduction of soil moisture in the warmed
523 plots could not explain the increase of N_2O emissions in the TCO_2 treatment. Furthermore, the
524 poor indications of CO_2 effects on soil moisture via improved plant water use efficiency as well
525 as the temporally limited drought effects on soil water suggested that the N_2O responses in the
526 DCO_2 and TCO_2 treatments were not related to differences in soil water content between
527 treatments, but to one or more unknown parameters that will be discussed below.

528 Potential nitrification and denitrification rates were increased in the warmed plots (Fig. 6),
529 which indicates an increased content in the soil of enzymes involved in the two processes. This is
530 in contrast to studies in California and Wales, where natural and semi-natural grasslands were
531 exposed to elevated temperatures for about four years with no effect on nitrifying or denitrifying
532 enzyme activities (Barnard et al., 2004, 2006). In line with our results on potential nitrification
533 and denitrification, warming increased the concentration of NO_3^- in soil water, counteracting
534 negative effects of drought and CO_2 (Fig. 5). Additionally, compiled data from our site showed

535 that microbial NH_4^+ consumption was increased by warming (Larsen et al., 2011). In contrast,
536 drought reduced gross N mineralization and the fauna-related part of N mineralization, which
537 included N excretion by soil fauna and turnover of the soil fauna biomass. Thus, in general N
538 turnover was enhanced in the warmed plots, whereas drought tended to reduce N turnover at the
539 site. Apparently, the effects of drought and warming on N turnover only affected the N_2O flux in
540 the full-factorial study when each of the two treatments was combined with elevated CO_2.
541 Elevated CO_2 therefore seemed to trigger the N_2O response to drought and warming.
542 Elevated CO_2 is known to stimulate rhizodeposition (Allard et al., 2006; Pendall et al.,
543 2004). Studies at our heathland site showed increased root growth (Marie F. Arndal, pers.
544 comm.) and increased belowground respiration in response to elevated CO_2 (Merete B. Selsted,
545 pers. comm.), indicating enhanced carbon allocation belowground and possibly also increased
546 rhizodeposition. In the TCO_2 treatment, a larger input of labile C compounds possibly stimulated
547 the denitrifying microorganisms in combination with the increased N availability caused by the
548 warming treatment, resulting in enhanced N_2O production by denitrification (Fig. 4b). Increased
549 denitrification in the TCO_2 treatment, however, was not supported by measurements of potential
550 denitrification (Fig. 6b). An alternative source of N_2O in the TCO_2 treatment could be
551 heterotrophic nitrification that would be stimulated by the same mechanism and may have a
552 higher N_2O product ratio than autotrophic nitrification (Papen et al., 1989). In contrast, increased
553 C input via rhizodeposition probably shifted the balance even further between supply of reductant
554 (*e.g.* organic carbon) and oxidant (*e.g.* NO_3^-) for denitrifiers in the DCO_2 treatment, where the N
555 availability was already low due to the negative effects of drought on N turnover. In
556 denitrification, nearly all nitrogen oxide is reduced to N_2 when the availability of reductant
557 exceeds the supply of oxidant to a great extent (Hutchinson and Davidson, 1993). This could be
558 the reason for the low N_2O emission in the DCO_2 treatment (Fig. 4b), despite the potential

559 activity of enzymes involved in nitrification and denitrification being unaffected (Fig. 6). In line
560 with our results, Baggs et al. (2003) found that elevated atmospheric CO_2 caused increased N_2O
561 emission in highly N fertilized swards, whereas elevated CO_2 tended to reduce the N_2O emission
562 in low N swards. Furthermore, in nitrogen poor meadow mesocosms, Kanerva et al. (2007)
563 studied the effect of elevated CO_2 via increased C input to the soil, while controlling soil
564 moisture. They concluded that, in low N soils, the greater C availability under elevated CO_2 does
565 not lead to greater N_2O emissions.
566 In our study, all three treatments were combined in order to simulate the climatic and
567 atmospheric conditions in Denmark in year 2075. The positive effect of TCO_2 and the negative
568 effect of DCO_2 on the N_2O flux counteracted each other in the $TDCO_2$ treatment, resulting in no
569 change in the N_2O flux compared to the current climatic and atmospheric conditions given by the
570 ambient treatment, A. The lack of major changes in the N_2O emission from the TDCO2 treatment
571 is in line with a study on extensively managed grassland monoliths, which were exposed to
572 elevated temperature, summer drought and elevated atmospheric CO_2 using an additive
573 experimental design (Cantarel et al., 2011).
574 The strong control of soil moisture on the temporal changes in N_2O fluxes appeared from
575 both the full-factorial study and the drought/rewetting study. The one-day delay in the response
576 of N_2O fluxes to rewetting observed in the drought/rewetting study was probably related to
577 threshold levels for N_2O production, meaning that the soil water content needed to reach a certain
578 level before the development of anaerobic zones enabled the denitrification to occur (Smith et al.,
579 1998) (Fig. 7b,d). In addition to slowing the gas diffusion, rewetting of soil may promote the
580 development of anaerobic zones by stimulating the respiratory activity, leading to enhanced O_2
581 consumption (Ruser et al., 2006). The source of the short-term N_2O peak was possibly NO_3^- that
582 accumulated in the soil during the preceding drought period.

583 Revisiting our hypotheses, we expected that prolonged summer drought as main treatment
584 would reduce the N_2O emission, which we did not find when analysing across all measuring
585 campaigns in the full-factorial study. However, temporary drought effects occurred during the
586 experimental drought periods in 2006 (full-factorial study) and 2007 (drought/rewetting study).
587 Furthermore, in combination with elevated CO_2, drought reduced the N_2O emission. In line with
588 our hypotheses, we found no effect on the overall N_2O flux of elevated CO_2 or warming, but in
589 combination the two treatments enhanced the N_2O emission. Although the outcome of the two-
590 factor combinations, DCO_2 and TCO_2, could not be predicted from the single treatments, the
591 response in the three-factor combination fitted well with our last hypothesis that two opposing
592 two-factor responses would counteract each other.
593
594 *4.3 Depth distribution of microbial activity*
595 In contrast to field measurements conducted in October 2007, where significant N_2O
596 uptake occurred in some plots, laboratory incubations of soil from the 0-8 cm soil layer sampled
597 in November 2007 showed negligible N_2O reductase activity. One explanation could be that the
598 N_2O reduction took place in soil layers below the 0-8 cm layer. In a spruce forest, Goldberg and
599 Gebauer (2009) also found microbial N_2O consumption in the mineral horizon.
600 In dry soils, methane oxidation may occur well below the soil surface (Castaldi and
601 Fierro, 2005 and references within). However, in the drought/rewetting study drought stress on
602 the methanotrophs was released on the first day after rewetting (26 June; Fig. 7c) following a
603 rather modest rainfall of 6 mm that caused a general rise in soil moisture in the 0-20 cm soil
604 layer, but not in the 20-60 cm layer in all plots. This suggests that the main zone of CH_4
605 oxidation was localised in the upper part of the soil profile, which was supported by a stronger
606 influence on the CH_4 flux of soil moisture in the 0-20 cm layer compared to moisture in the 20-60

607 cm layer. Studies in forest soils also showed that CH_4 oxidation primarily occurred within the
608 upper 10 cm of the soil profile (Bender and Conrad, 1994; King, 1997).
609 On 27 June and 2 July 2007, high rates of CH_4 emissions (above 100 μg C m^{-2} h^{-1})
610 occurred in six specific plots across treatments. These CH_4 hotspots were presumably triggered
611 by the high soil water content reducing CH_4 oxidation and producing anaerobic microsites
612 combined with high local availability of substrates for methanogenesis. On 2 July, the plot with
613 the highest CH_4 emission also had the highest N_2O emission and soil water content in 0-6 cm,
614 supporting anaerobic conditions as an important driver for the CH_4 hotspots. The abundance of
615 *Deschampsia* and *Calluna* varied between plots at our heathland site, but the vegetation
616 composition had no effect on the measured CH_4 and N_2O fluxes in any of the studies.
617
618 *4.4 Conclusions*
619 Our study showed that warming as main factor increased the CH_4 uptake by about 20 %,
620 presumably due to the enhancing effects of increased soil temperatures and reduced soil moisture
621 on the microbial CH_4 oxidation process as well as some unknown factors. Elevated
622 concentrations of atmospheric CO_2 had no overall effect on the CH_4 flux, but reduced the CH_4
623 uptake during one measuring campaign in the winter season. In combination, the stimulating
624 effect of warming and the episodic reducing effect of CO_2 on the CH_4 uptake resulted in a
625 modest, but insignificant, increase in the CH_4 uptake when comparing the multifactor treatment
626 including elevated CO_2, warming and summer drought with the ambient treatment. Depending on
627 the duration of winter decline in CH_4 uptake under elevated CO_2, CH_4 oxidation in temperate
628 ecosystems on well-aerated soil could potentially have a negative feedback on global climate
629 change in future.

The study indicated that the N_2O flux in nitrogen poor natural ecosystems on well-aerated soils will probably not change under future climatic and atmospheric conditions. This apparent lack of response is the product of the complex interaction between the climate change parameters affecting the conditions for N_2O production in opposite directions. Overall, this study highlights the importance of evaluating climate change parameters in multifactor treatments as the response of CH_4 and N_2O flux rates to different two- and three-factor combinations may not be predicted from the responses to the individual treatments, and furthermore the effects of individual treatments may negate each other if they act in opposite directions.

The greenhouse gas fluxes reported here cover short-term (1-2 year) responses to climate change. We anticipate that after longer experimental manipulation, the treatment effects could differ from those initially observed. For example, the increased carbon input via stimulation of photosynthesis under elevated CO_2 (Albert et al., 2011a, 2011b) may result in changes in the quantity or character of the ecosystem carbon pools. Furthermore, the increased N turnover observed in response to warming (Larsen et al., 2011), also a short-term response, may be influenced by the properties of the pre-experimental organic matter. Therefore, a new equilibrium N turnover rate may develop as the properties of the soil organic matter also equilibrate; a process controlled by the continuous supply and decomposition of organic matter. Both the change in carbon pools and the potential change in the N turnover rate would alter the predictions for the future fluxes of N_2O and possibly CH_4.

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657

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Table 1. Absolute changes in soil temperature and soil moisture in response to the three main factors, elevated temperature (T), drought (D) and elevated atmospheric CO_2 concentrations (CO_2), and significant treatment interactions during measuring campaigns in the full-factorial study

	Main factor			Interaction
	T	D	CO_2	
Mean daytime soil temperature at 5 cm depth (Δ°C)	+ 0.39 ****	- 0.07 *	ns	CO_2×T ****
Soil moisture in 0-20 cm (Δ vol%)	- 0.99 ****	- 0.85 ***	ns	CO_2×D **; CO_2×D×T *
Soil moisture in 20-60 cm (Δ vol%)	- 0.84 **	- 0.79 **	ns	

Significance levels are: **** for $P \leq 0.0001$; *** for $P \leq 0.001$; ** for $P \leq 0.01$; * for $P \leq 0.05$; ns for not significant.

Table 2. Parameter estimates for a repeated measures model used to investigate the influence on CH_4 fluxes (μg C m^{-2} h^{-1}) of soil temperature at 5 cm depth (°C) and soil moisture in the 0-20 cm layer (vol%)

Parameter	Estimate	SE	P-value
μ	12.25	0.48	<0.0001
α(T = 0)	-0.35	0.20	0.082
α(T = 1)	0	.	.
β(D = 0)	0.28	0.20	0.17
β(D = 1)	0	.	.
γ(CO_2 = 0)	0.34	0.26	0.25
γ(CO_2 = 1)	0	.	.
δ(Temp)	0.070	0.019	0.0004
ε(Moist)	-0.034	0.019	0.074

Model structure:

$(-CH_4+110)^{0.5} = \mu + \alpha(T) + \beta(D) + \gamma(CO2) + \delta \cdot Temp + \varepsilon \cdot Moist$

The main factors were elevated temperature (T), drought (D) and elevated atmospheric CO_2 concentrations (CO_2). All two- and three-factor interactions had P-values >0.25 and were removed from the model.

831

832

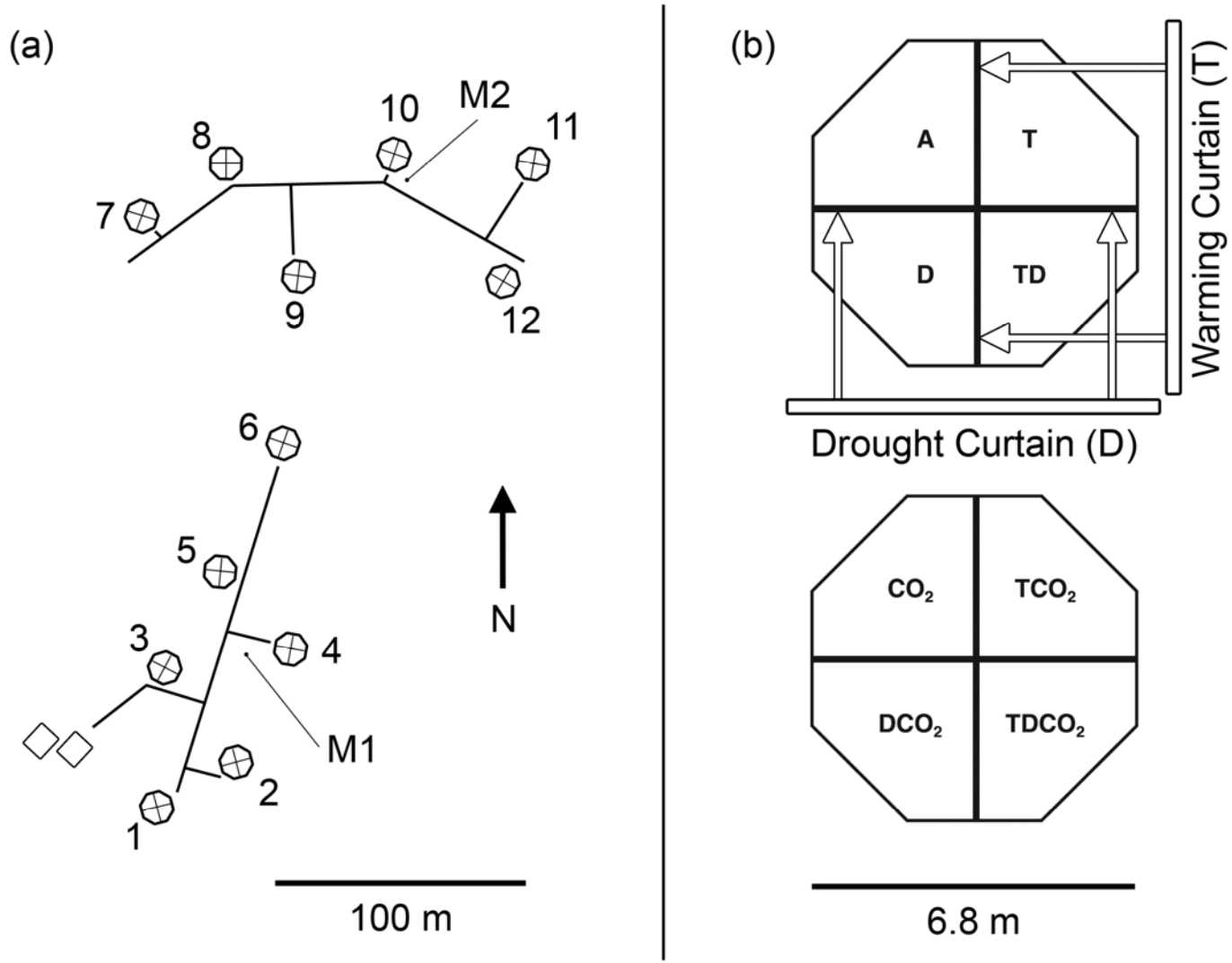
833 **Figure legends**

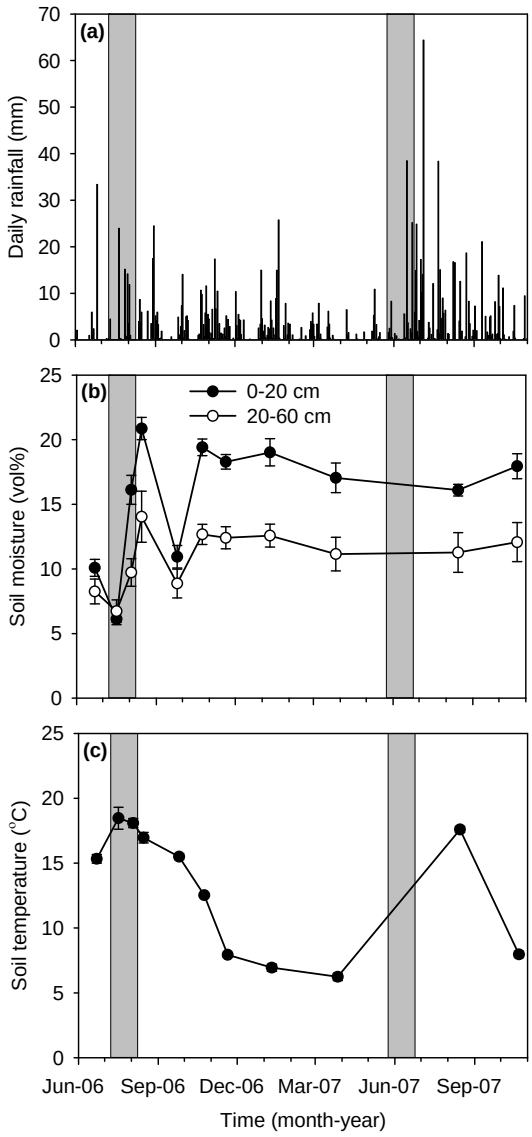
834 Fig. 1 The arrangement of the field experiment at Brandbjerg, Denmark (a) and the layout of a
835 single block of the experiment (b). In part (a), the octagons are labelled 1 through 12, with
836 consecutive pairs belonging to the same experimental block. Octagons 2, 4, 5, 8, 10 and 12
837 received elevated CO_2 concentrations. The boardwalks are shown by solid lines and the locations
838 of the two meteorological stations are shown as M1 and M2. The two rectangles represent
839 buildings that house computers, control systems and field laboratories. In part (b), the extension
840 direction of the curtains that provide the night-time warming (T) and drought (D) treatments is
841 shown on the upper octagon. One octagon is at ambient concentrations of CO_2 while the other
842 received elevated concentrations of CO_2 from the Free Air Carbon Enrichment (FACE) system.
843

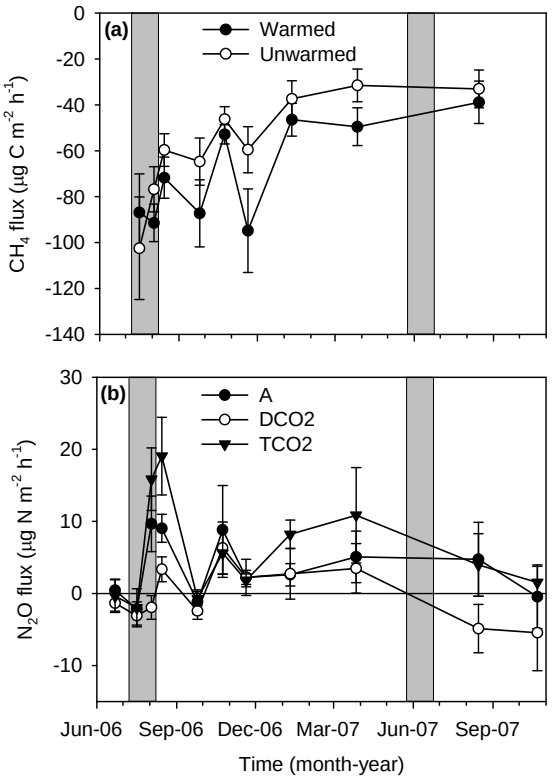
844 Fig. 2 Temporal variation in rainfall (a), soil moisture (b) and soil temperature (c) during
845 measuring campaigns in the full-factorial study. Soil moisture in the 0-20 cm and 20-60 cm soil
846 layers as well as mean daytime soil temperature at 5 cm depth were measured in the ambient
847 treatment (n=6; means ± SE). Periods of experimental drought are indicated by shaded areas.
848

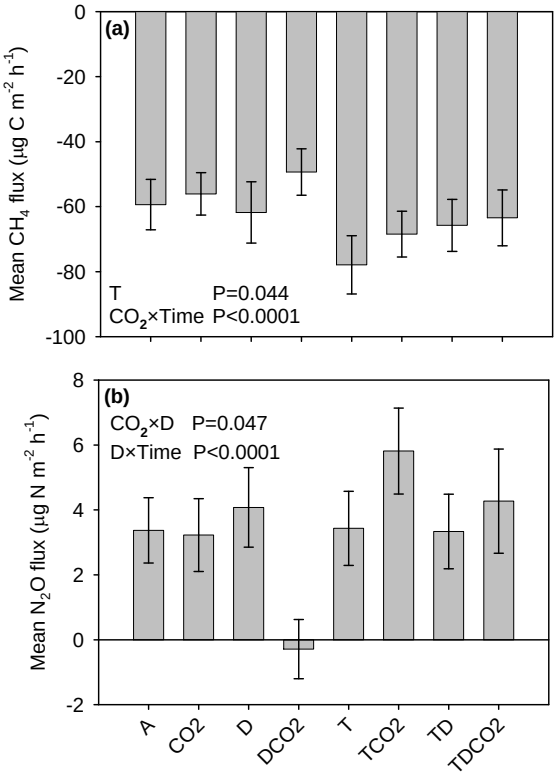
849 Fig. 3 Temporal variation in CH_4 (a) and N_2O fluxes (b) during measuring campaigns in the full-
850 factorial study. Fluxes of CH_4 are presented for warmed and unwarmed treatments separately
851 (n=24; means ± SE). Fluxes of N_2O in three treatments are presented, *viz.* the ambient treatment
852 (A), the combination of drought and elevated CO_2 (DCO_2) and the combination of warming and
853 elevated CO_2 (TCO_2) (n=6; means ± SE). The remaining five treatments were left out for clarity.
854 Periods of experimental drought are indicated by shaded areas.
855

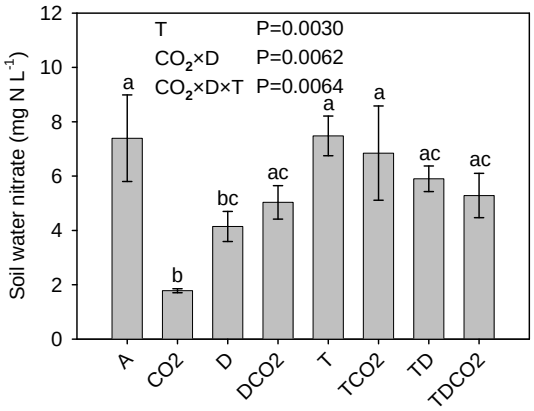
856 Fig. 4 Mean CH_4 fluxes (a) and mean N_2O fluxes (b) across measuring campaigns in the full-
857 factorial study for the ambient treatment (A) in addition to elevated CO_2 (CO_2), drought (D) and
858 elevated temperature (T) as single treatments and in all combinations (CH_4, n=54; N_2O, n=66;
859 means ± SE).
860
861 Fig. 5 Annual mean of NO_3^- concentrations in soil water collected at 5 cm depth in 2007 (n=6;
862 means ± SE)
863
864 Fig. 6 Potential nitrification (a) and potential denitrification (b) in soil collected in November
865 2007 in the ambient treatment (A) and in elevated CO_2 (CO_2), drought (D) and elevated
866 temperature (T) as single treatments as well as in all combinations (n=6; means ± SE). In part (a),
867 bars with same letter are not significantly different. The pair wise comparisons of the eight
868 treatments are based on differences of least squares means, and are enabled by the significant
869 CO_2×D×T interaction.
870
871 Fig. 7 Temporal variation in rainfall (a), soil moisture in 0-6 cm (b), CH_4 fluxes (c) and N_2O
872 fluxes (d) during measuring campaigns in the drought/rewetting study measured in the treatments
873 ambient (A), elevated CO_2 (CO_2), drought (D), combined drought and elevated CO_2 (DCO_2) and
874 the combination of elevated temperature, drought and CO_2 ($TDCO_2$) (n=6; means). Standard
875 error bars are not indicated for clarity. The mean coefficient of variation (CV) across treatments
876 and time for soil moisture, CH_4 and N_2O fluxes were 0.3, 3.0 and 3.6, respectively. The drought
877 period is indicated by shaded areas.

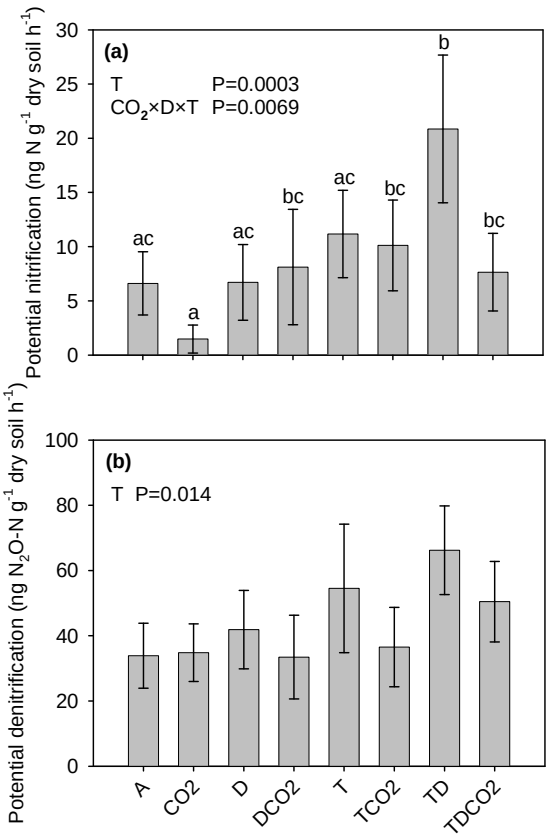

(a)
10
M2
11
8
7
9
12
6
5
N
3
4
M1
2
1
100 m
(b)
A
T
D
TD
Warming Curtain (T)
Drought Curtain (D)
CO2
TCO2
DCO2
TDCO2
6.8 m

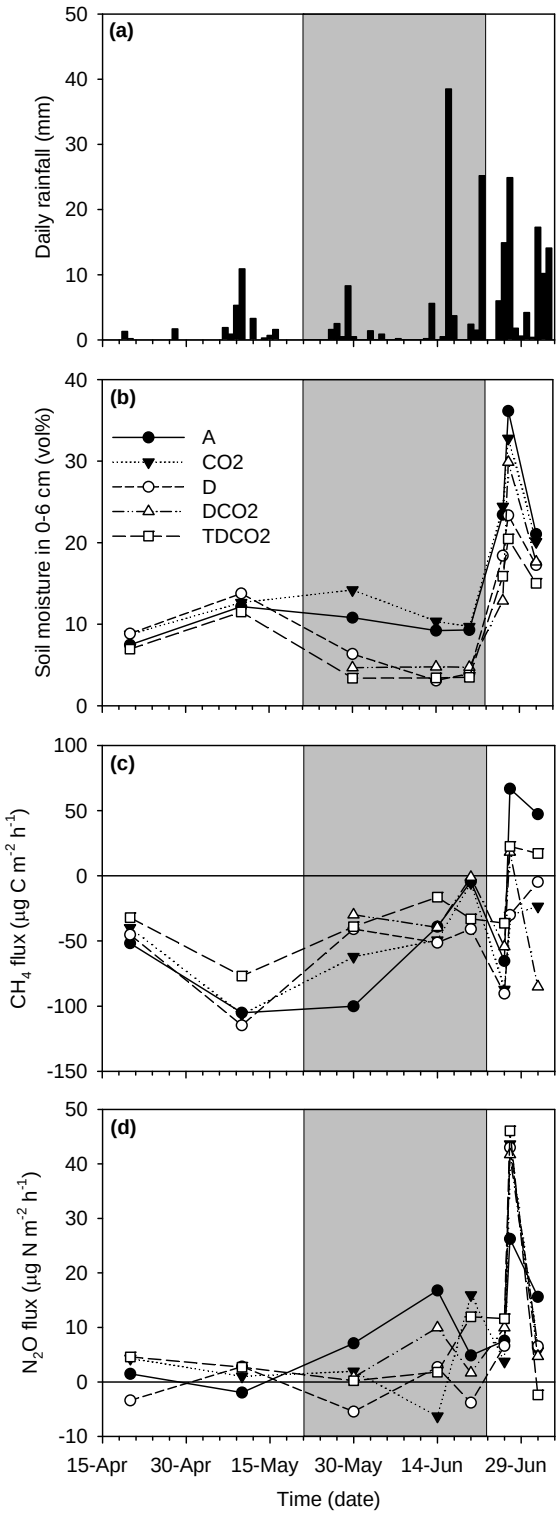

(a)
Daily rainfall (mm)
0
10
20
30
40
50
60
70
(b)
0-20 cm
20-60 cm
Soil moisture (vol%)
0
5
10
15
20
25
(c)
Soil temperature (°C)
0
5
10
15
20
25
Jun-06
Sep-06
Dec-06
Mar-07
Jun-07
Sep-07
Time (month-year)


(a)
Warmed
Unwarmed
CH4 flux (µg C m-2 h-1)
0
-20
-40
-60
-80
-100
-120
-140
(b)
A
DCO2
TCO2
N2O flux (µg N m-2 h-1)
30
20
10
0
-10
Jun-06
Sep-06
Dec-06
Mar-07
Jun-07
Sep-07
Time (month-year)


(a)
Mean CH4 flux (μg C m-2 h-1)
0
-20
-40
-60
-80
-100
T P=0.044
CO2×Time P<0.0001
(b)
Mean N2O flux (μg N m-2 h-1)
8
6
4
2
0
-2
CO2×D P=0.047
D×Time P<0.0001
A
CO2
D
DCO2
T
TCO2
TD
TDCO2


Soil water nitrate (mg N L-1)
12
10
8
6
4
2
0
T P=0.0030
CO2×D P=0.0062
CO2×D×T P=0.0064
a
b
bc
ac
a
a
ac
ac
A
CO2
D
DCO2
T
TCO2
TD
TDCO2


(a)
T P=0.0003
CO2×D×T P=0.0069
Potential nitrification (ng N g-1 dry soil h-1)
ac
a
ac
bc
ac
bc
b
bc
(b)
T P=0.014
Potential denitrification (ng N2O-N g-1 dry soil h-1)
A
CO2
D
DCO2
T
TCO2
TD
TDCO2


(a)
Daily rainfall (mm)
50
40
30
20
10
0
(b)
Soil moisture in 0-6 cm (vol%)
A
CO2
D
DCO2
TDCO2
40
30
20
10
0
(c)
CH4 flux (µg C m-2 h-1)
100
50
0
-50
-100
-150
(d)
N2O flux (µg N m-2 h-1)
50
40
30
20
10
0
-10
15-Apr
30-Apr
15-May
30-May
14-Jun
29-Jun
Time (date)