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Fluxes and ^{13}C isotopic composition of dissolved carbon and pathways of methanogenesis in a fen soil exposed to experimental drought

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Abstract

The impact of drought and rewetting on carbon cycling in peatland ecosystems is currently debated. We studied the impact of experimental drought and rewetting on intact monoliths from a temperate fen over a period of ~300 days, using a permanently wet treatment and two treatments undergoing drought for 50 days. In one of the mesocosms vegetation had been removed. Net production of CH_4 was calculated from mass balances in the peat and emission using static chamber measurements and results compared to ^{13}C isotope budgets of CO_2 and CH_4 and energy yields of acetoclastic and hydrogenotrophic methanogenesis. Drought retarded methane production after rewetting for days to weeks and promoted methanotrophic activity. Based on isotope and flux budgets, aerobic soil respiration contributed 32–96% in the wet and 86–99% in the other treatments. Drying and rewetting did not shift methanogenic pathways according to $\delta^{13}C$ ratios of CH_4 and CO_2. Although $\delta^{13}C$ ratios indicated a prevalence of hydrogenotrophic methanogenesis, free energies of this process were small and often positive on the horizon scale, suggesting that methane was produced very locally. Fresh plant-derived carbon input apparently supported respiration in the rhizosphere and sustained methanogenesis in the unsaturated zone according to a ^{13}C-CO_2 labelling experiment. The study documents that drying and rewetting in a rich fen soil may have little effect on methanogenic pathways but result in rapid shifts between methanogenesis and methanotrophy. Such shifts may be promoted by roots and soil heterogeneity, as hydrogenotrophic methanogenesis occurred locally even when conditions were not conducive for this process in the bulk peat.

1 Introduction

Peatlands sequester carbon (C) sinks at estimated rates of 0.074–0.094 GtC yr^{-1} but also contribute approx. 2–10 % to the global release of methane into the atmosphere (Bousquet et al., 2006; Mikaloff Fletcher et al., 2004). These important processes

are both important in the global carbon cycle and sensitive to climate change, i.e. increases in temperature (Lafleur et al., 2005) or changes of water tables (Laiho, 2006). Increases in winter precipitation and drier summers with heavy convective rainfalls have been predicted for mid and higher latitudes (IPCC, 2001). Most peatlands are therefore subjected to rising temperature and changes in the hydrologic regime (Moore, 2002). This may increase decomposition and overall release of carbon from these ecosystems (Belyea and Malmer, 2004; Chimner and Cooper, 2003; Laiho, 2006), but probably lower the production of methane (Blodau and Moore, 2003a; Freeman et al., 2002). Methane emissions are, however, not always related to production in the subsurface (Smemo and Yavitt, 2006) and may be dominated by vegetation effects (Shannon and White, 1994). Understanding methane cycling and respiration pathways under changing environmental conditions is also important because effects are not straightforward to predict (Laiho, 2006).

Climate change induced disturbance, such as drying and rewetting events, may cause increased carbon mineralization but reduced CH_4 production by driving internal cycles of electron acceptors such as sulphate and iron (Roden and Wetzel, 1996). The time scale involved in depletion of electron acceptors and restart of methanogenesis is not yet well studied. Under fluctuating hydrological conditions an apparent coexistence of different redox processes was observed (Paul et al., 2006). Furthermore, the addition of alternative electron acceptors did not always inhibit CH_4 production (Dettling et al., 2006; Blodau and Moore, 2003b), and some methanogens were found to be able to shift to iron reduction (van Bodegom et al., 2004). The respiration dynamics is further complicated because methanogenesis is typically driven by input of fresh organic material and may occur in microenvironments (Wachinger et al., 2000)

The application of stable isotopes is a tool to identify the pathway by which methane is formed (Conrad, 2005; Whiticar, 1999). CH_4 produced by acetate cleavage is usually not as depleted in ^{13}C as CH_4 produced from CO_2 reduction with H_2. Fractionation factors for acetoclastic methanogenesis ranging from 1.000–1.032 compare to fractionation factors of hydrogenotrophic methanogenesis of 1.045–1.082 (Conrad, 2005;

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Whiticar, 1999 and references therein). Based on profiles of CH_4 stable isotope ratios in peat it was thus postulated that the upper profile was dominated by acetoclastic, the lower profile by hydrogenotrophic methanogenesis (Hornibrook et al., 2000a; Popp et al., 1999). A smaller depletion in ^{13}C of CH_4 in the upper profile is also caused by methanotrophic activity (Whiticar, 1999). Transport mediated by plants also preferentially removes ^{12}C-CH_4 from the soil and fractionation depends on transport mechanism, water table level, daytime, and season (Chanton, 2005; Popp et al., 1999). The isotopic composition of emitted methane resembled CH_4 of deeper soil layers (Popp et al., 1999), and the fractionation is thus likely smaller than for other relevant processes. Another tool to explain pathways of respiration is given by the calculation of Gibbs free energies (ΔG), which is also approximated using hydrogen concentrations, which control ΔG most strongly (Lovley and Goodwin 1988). This approach has recently been applied to study hydrogenotrophic versus acetoclastic methanogenesis in a ombrotrophic peatland (Beer and Blodau, 2007).

Controls on in situ CO_2 and CH_4 production, such as temperature and water table position, have been identified (e.g. Granberg et al., 1997; Roulet et al., 1992;Updegraff et al., 2001) but the impact of short term disturbances is still uncertain. This study addresses this gap by analyzing CO_2 and CH_4 dynamics and the ^{13}C isotopic composition of these pools and the peat. The specific objectives were to elucidate the impact of experimental drought and rewetting on (i) C-fluxes and their isotopic composition, (ii) below ground methane production and oxidation and on (iii) methanogenic pathways. Furthermore we identified in which part of the peat profile effects occur. To this end we used of mesocosms which allowed us to manipulate soil moisture but to hold other controls constant.

We incubated three peat mesocosms from a weakly acidic, northern temperate fen as individual treatments for ∼300 days and manipulated irrigation levels while keeping all other environmental conditions constant. To study the effect of plant cover on below ground C turnover, we also incubated a defoliated mesocosm. We expected that a simulated drought would result in prolonged periods of low or absent methane produc-

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tion after rewetting. Effects of drought and subsequent rewetting were traced using (i) turnover and (ii) flux calculations, (iii) changes in carbon isotopic composition of CO_2 and CH_4, (iv) isotope budgets, (v) changes in apparent isotope fractionation as well as (vi) thermodynamic calculations.

2 Material and methods

2.1 Treatments and sampling

Three intact peat cores with a diameter of 60 cm and a depth of 60 cm each ("meso-cosms") were collected in September 2005 at the Schlöppnerbrunnen fen site in north-eastern Bavaria (Fichtelgebirge, Germany, mean water table 19 ± 22 cm, for more site details see Paul et al., 2006). They were incubated in the laboratory for $\sim$300 days in a 15°C climate chamber ($\sim$60% rH, 12 h light/dark cycles, 660 μmol s^{-1} photosyn-thetic photon flux). The vegetation was left intact in two mesocosms. One of these was kept wet at high water table throughout the incubation treatment ("wet-vegetation" or "W-V"), while the other was subjected to a drying and wetting cycle as described below ("drying/wetting-vegetation" or "DW-V"). The third mesocosm – also subjected to drying and rewetting – was defoliated prior to sampling by covering the vegetation since spring 2005 and kept devoid of vegetation ("drying/wetting-defoliated" or "DW-D") to study vegetation effects.

The vegetation on DW-V mainly comprised of *Agrostis sp., Nardus stricta, Molinia coerulea, Sphagnum fallax, Brachythecium rivulare, Atrichum undulatum* and *Galium hercynicum*. In the W-V mesocosm, there was less *Agrostis*, but some more *Sphagnum* and exclusively here there was *Carex rostrata*. As *Carex* in W-V gained more domi-nance with increasing incubation time, increasing effects of *Carex* on soil processes had thus to be considered.

After 40 days at a water table of about 30 cm below surface (phase I), we adjusted the water table of all mesocosms to 10 cm below surface. Therefore, 30 (DW-V, DW-D) or

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40 mm (W-V) of irrigation were applied within two days. The water table was then kept at ~11.9 +/−1.3 cm (DW-V) or 9.9 +/−0.9 cm (DW-D) for the following 70 days (phase II), irrigating daily. Subsequently, two mesocosms, DW-V and DW-D, were dried by reducing irrigation (phase III), while the third, W-V, was kept at high water table. Within

5 50 days, the water table dropped to about 55 cm below surface. The treatment DW-D received no irrigation in this phase, while we applied ~1 mm d^{-1} on DW-V to induce a similar water table drop as in DW-D. Thereafter, we rapidly raised the water table back up to 10 cm (begin of phase IV). This required 54 (DW-V) and 53 mm (DW-D), applied within 2 (DW-V) or 5 (DW-D) days. During phase IV, the water table was held at 12.7

10 +/−1.8 (DW-V) or 9.8 +/−1.8 cm (DW-D) below surface till the end of the experiment.

Volumetric water contents (VWCs) were measured using calibrated TDR probes at 10, 20, 30 and 40 cm depth (IMKO, Germany), and water tables were monitored in piezometers at two depths (25 and 50 cm). Total porosity was determined by oven drying of 100 cm^3 samples.

15 The irrigation water was prepared according to field measurements (Lischeid, pers. comm.). It contained Na$^+$ (5 μmol L^{-1}), Ca^{2+} (6 μmol L^{-1}), SO$_4^{2-}$ (10 μmol L^{-1}), Cl$^-$ (12 μmol L^{-1}), NH$_4^+$ and NO$_3^-$ (40 μmol L^{-1}). The pH was adjusted to ~4.8 using H$_2$SO$_4$ and the solution had a DIC concentration of ~15 μmol L^{-1}.

Methane emission from the mesocosms was measured weekly, using shrouded

20 chambers on previously inserted collars of 20 cm in diameter. A total of 5–8 gas samples were taken every 5 min and concentration change over time was recalculated into a flux using linear regression over time (min. r^2>0.9). We sampled soil gases, at least weekly, from horizontally inserted silicon tubes at 5, 10, 15, 20, 30, 40 and 50 cm depth. With this technique, the gas phase in equilibrium with the solution is measured, thus it

25 can be applied in saturated and unsaturated soil (Kammann et al., 2001). Soil solution was sampled from Rhizon® samplers (microporous polymer, <0.2 μm pore size, fibre glass support).

At the end of the incubation, a ^{13}C-CO$_2$ pulse label was applied on each mesocosm to identify the zone of main root activity in the soil. We prepared a ~900 ppm,

~63% ^{13}C-CO_2 atmosphere by dissolving 250 mg of 95% ^{13}C Na_2CO_3 with 6N HCl in a transparent chamber and manual mixing of the gas phase. The chamber was placed on each mesocosm for 60 min and the label was traced in the upper soil gas for the following 90 h. Stable isotopic composition was analyzed as outlined below.

5   Finally, the solid phase of all mesocosms was sampled at 10–15 cm depth intervals..

2.2   Analytical techniques

CO_2 and CH_4 concentrations in gas samples were measured on a SRI 8610C gas chromatograph, equipped with FID and a CO_2 methanizer. H_2 was analyzed on a TA 3000 H_2-analyzer (Trace Analytical). Stable C isotope measurements of CO_2 and
10 CH_4 were performed using a GC-Combustion-Isotope ratio mass spectrometry (GC-C-IRMS) combination (delta^{plus}, Thermo Finnigan, MAT), equipped with a Carboxen 1010 PLOT column (0.32 mm×30 m, Supelco). The detection limit for CO_2 and CH_4 was ~350 ppm. Isotope signatures were expressed in the common δ notation in ‰ versus the VPDB-standard (Eq. 1).

15 $$\delta = \left[\frac{R_{sample}}{R_{standard}} - 1 \right] \times 1000 \ ^0/_{00} \qquad (1)$$

We calibrated the δ^{13}C measurements twice a day, using $NaCO_3$ with a known isotope signature of −8.84‰ (VPDB) and four working standards of CO_2 (5000 and 50 000 ppm, −33.53‰) and CH_4 (1000 and 10 000 ppm, −56.37 and −52.84‰). The standard deviation of multiple measurements was mostly below 0.5 for CO_2 and CH_4
20 except of CH_4 samples with a very low isotope signature of −80 to −110‰ (~2.5‰).
    Carbon and nitrogen content and isotope signature of the solid phase were determined on a Carlo Erba CN2500 elemental analyzer, connected via Conflo III interface to a delta^{plus} IR-MS (Thermo Finnigan, MAT). In liquid samples, pH was determined using a glass electrode (WTW), and acetate using a GC equipped with FID (Varian).

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2.3 Calculations

Volumetric gas content of the soil (VGC) was calculated from total porosity as determined by oven drying of $100\,cm^3$ samples and measured volumetric water content (VWC) from the TDR probes.

Dissolved inorganic carbon (DIC), CH_4 and H_2 concentrations in the soil gas were calculated using Henry's law constants for 15°C (Sander, 1999) ($K_{CO2}=0.0463\,mol\,L^{-1}\,atm^{-1}$, $K_{CH4}=0.0017\,mol\,L^{-1}\,atm^{-1}$). DIC speciation was calculated using pH values obtained from Rhizon® samples and equilibrium constants taken from Stumm and Morgan (1996).

Net turnover of CH_4 in the depth layers of the peat core could be calculated from mass balances of diffusive fluxes and changes in storage over time according to Eq. (2).

$$R_N = \frac{\Delta S_A}{\Delta t} + \left[D_A \frac{\Delta C_{A,\text{upper}}}{\Delta x} \right]_{\text{upper}} \cdot z^{-1} - \left[D_A \frac{\Delta C_{A,\text{lower}}}{\Delta x} \right]_{\text{lower}} \cdot z^{-1} \qquad (2)$$

in which R_N is the net turnover rate of a species A ($nmol\,cm^{-3}\,d^{-1}$), $\Delta S_A/\Delta t$ the change in storage of species A in a layer. The left-hand expression in parenthesis represents the diffusive flux of A at the upper boundary, the second expression is the flux at the lower boundary of a layer (D_A: diffusion coefficient in peat, $\Delta C_A/\Delta x$: concentration gradient at upper or lower end of segment, z: thickness of the layer).

The change in storage in an individual layer was calculated from concentration changes between two measurements. Concentration gradients over depth for these time points between samplings were obtained by calculating means of two consecutive profiles. The diffusion coefficients were corrected for porosity using $D=D_0\phi^2$ (Lerman, 1988) and in case of unsaturated conditions using gaseous diffusion coefficients (Lerman, 1988) and an correction function $\alpha(a)=a^2\phi^{-2/3}$ (α: correction factor at air content a, ϕ: soil porosity) (Jin and Jury, 1996).

To obtain information about the dominating CH_4 production pathway, we calculated

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an apparent isotope fractionation factor α_C between CO_2 and CH_4, using Eq. (3)(Conrad, 2005; Whiticar, 1999) and made a cross plot of $\delta^{13}C(CO_2)$ and $\delta^{13}C(CH_4)$.

$$\alpha_C = \frac{\delta^{13}C_{CO2} + 1000}{\delta^{13}C_{CH4} + 1000} \qquad (3)$$

Assuming no significant fractionation during breakdown of organic matter (Boehme et al., 1996) and no carbon losses from the system except CO_2 and CH_4, an isotope mass balance for different soil layers was calculated (Eq. 4). Using methane fluxes from chamber measurements we calculated an anaerobic CO_2 flux (Eqs. 5, 6) (Lansdown et al., 1992).

$$C_{tot} \cdot R_{OM} = C_{CO2} \cdot R_{CO2} + C_{CH4} \cdot R_{CH4} \qquad (4)$$

$$F_{tot} = F_{CO2} + F_{CH4} \qquad (5)$$

$$F_{tot} \cdot R_{OM} = F_{CO2} \cdot R_{CO2} + F_{CH4} \cdot R_{CH4} \qquad (6)$$

In which C_{CO2} and C_{CH4} are the concentrations of CO_2 and CH_4, respectively, and R_{CO2}, R_{CH4} and R_{OM} the isotope ratios of CO_2, CH_4, and the soil organic matter, respectively. C_{tot} should then equal the measured sum of the assumed mineralization end products CO_2 and CH_4. F_{CO2}, and F_{CH4} are the diffusive fluxes of CO_2 and CH_4, respectively, resulting in F_{tot}, the total diffusive C flux.

For the ^{13}C pulse label we calculated an isotope mass balance, tracing the label uptake into the soil DIC and CH_4 pool. This allowed to identify zones of high root associated respiration and to calculate a rate, at which the label was taken up (Eq. 7).

$$U_{CO2} = \frac{\Delta\left[^{13}C\right]_{soil}}{\Delta t \cdot f(^{13}C)_{label} \cdot A_{mesocosm}} \qquad (7)$$

In which $\Delta[^{13}C]_{soil}$ is the change in ^{13}C content in the total soil CO_2, Δt the time interval of labelling (1 h), $f(^{13}C)_{label}$ the fraction of ^{13}C in the total labelling gas phase (62.9%)

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and $A_{mesocosm}$ the area of the mesocosm in m^2, resulting in an uptake rate of CO_2 U_{CO2} in $mmol\,m^{-2}\,h^{-1}$.

The thermodynamic energy yield from hydrogenotrophic and acetoclastic methanogenesis and from homoacetogenesis was calculated using the reactions given in Table 1 (Eq. 9–11). Thermodynamic data was taken from Nordstrom and Munoz (1994) and concentrations of CH_4, CO_2, acetate and H_2 as measured.

As hydrogen measurements in environmental samples may be biased by clustered distribution of hydrogen producers and consumers (Hoehler et al., 2001), we applied another approach to estimate ΔG_{hm} for hydrogenotrophic methanogenesis from the fractionation factor α_C which had also been tested in peatland samples (Penning et al., 2005) (Eq. 8).

$$\Delta G_{hm} = 11.8376 - \sqrt{|\ln(\alpha_C - 1) - \ln(0.0919)| \cdot 12170} \qquad (8)$$

For visualization of concentrations over time and depth, we created contour plots of the data sets using natural neighbour interpolation as implemented in Surfer Version 8 (Golden Software).

3 Results

3.1 Solid phase data

Soil carbon content (w/w) was variable in the treatments over depth, ranging from ~29–34% in the top layers, through ~22–32% in the middle profile to 25–48% in 40–60 cm depth (Table 2). While the carbon content in the upper profile was comparable among treatments, treatment DW-V contained less carbon below 25 cm depth than W-V and DW-D.

The measured $\delta^{13}C$ in the total soil organic matter of the top soil was −27.1 (DW-D) to −27.7 (DW-V) (Table 2). In DW-V and DW-D, $\delta^{13}C$ values decreased to −27.9

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and −28.3‰, respectively. Highest values of −26.8 to −27.3‰ occurred in ∼10–15 cm depth.

3.2 Hydrological conditions

In the drought phase (III), maximum VGCs in the treatment DW-V reached 12, 6 and 2% in 10, 20 and 30 cm depth, just before rewetting. Only 3 days after readjusting the high water table, VGCs decreased to 2–3% again. In the treatment DW-D, VGCs of 12, 13 and 9% in 10, 20 and 30 cm depth, respectively, were measured. Here, it took about 30 days after rewetting until VGCs decreased to below 4%. When saturated at 10 cm depth, during phases II and IV, VGCs adjusted typically to 1% or below in this layer. At high water table, a mean volumetric gas content of 2% in the upper 5 cm of all treatments was assumed, as this was a value typically observed 5 cm above the water table when the water table was below. It has to be noted that a VGC of 1% would halve and of 3% double calculated fluxes at the surface, leaving general trends of changes in turnover unaffected, however.

3.3 Methane emission

During the first 60 days, no methane efflux was detected from any of the treatments using the closed chamber method. Thereafter, the permanently wet treatment W-V emitted CH_4 with increasing rates, reaching 18 ± 9.8 mmol m^{-2} d^{-1} by the second half of the experiment (Fig. 1). These fluxes remained, despite decreasing concentrations in the profile toward the end of the experiment. In DW-V and DW-D sporadic methane fluxes were generally close to the detection limit of this method (0.8–1.5 mmol m^{-2} d^{-1}).

3.4 Concentration and isotope signature of dissolved CO_2 (DIC)

At constantly high water table in the wet treatment W-V, concentrations of DIC increased for about 140 days to levels of 1–2 mmol L^{-1} in the unsaturated zone and up to 7.6 mmol L^{-1} in 30 cm depth. In the treatments DW-V and DW-D highest DIC

concentrations occurred just below the water table and right before the begin of the drought phase, reaching $4.5\,\mathrm{mmol\,L^{-1}}$ around day 100 in 15 cm depth in DW-V and $3.5\,\mathrm{mmol\,L^{-1}}$ on day 111 in 30 cm depth in DW-D. After rewetting, DIC concentrations recovered quickly to pre-drought levels within ~20 days and continued increasing there-

5   after (DIC data not shown).

Values of $\delta^{13}C$ of dissolved CO_2 ($\delta^{13}C_{CO2}$) showed a similar pattern in all meso-cosms (Fig. 2). Values of −26 to −27.5‰ occurred in the upper profile or shortly after rewetting, and highest values of −18 to −14‰ below 30 cm depth, particularly in the permanently wet treatment. A smaller maximum of $\delta^{13}C_{CO2}$ occurred around

10 5 cm depth in DW-V during wet conditions. Only after rewetting $\delta^{13}C_{CO2}$ approximately matched $\delta^{13}C$ measured in the soil solid phase ($\delta^{13}C_{OM}$). Drying and rewetting thus lowered $\delta^{13}C_{CO2}$ in the soil DIC pool.

Under vegetation, the ^{13}C pulse label was rapidly transferred into the soil DIC-pool in the upper 10 (DW-V) to 20 (W-V) cm changing $\delta^{13}C_{CO2}$ up to 3 in DW-V and 8‰ in

15 W-V, compared to before labelling (Fig. 3). Considering also the shifts in $\delta^{13}C_{CH4}$, this was equivalent to an uptake of 0.00, 0.21 and 0.57% of the total tracer amount in DW-D, DW-V, and W-V, respectively. Given a mean storage of ~150 mmol DIC in the upper 20 cm of all treatments and an application time of 1 h, this resulted in C-incorporation rates U_{CO2} of 0.00, 0.67 and $1.80\,\mathrm{mmol\,C\,m^{-2}\,d^{-1}}$ for DWD, DW-V and W-V.

20 3.5   Concentration and isotopic signature of methane

Concentrations of CH_4 peaked at $460\,\mu\mathrm{mol\,L^{-1}}$ and 50 cm depth in W-V, $150\,\mu\mathrm{mol\,L^{-1}}$ and 30 cm depth in DW-V and $100\,\mu\mathrm{mol\,L^{-1}}$ and 50 cm depth in DW-D (Fig. 4). In both mesoscocms with vegetation a secondary concentration maximum of 50–$150\,\mu\mathrm{mol\,L^{-1}}$ in W-V and 40–$100\,\mu\mathrm{mol\,L^{-1}}$ in DW-V (phases II and IV) occurred at (W-V) or above

25 (DW-V) the water table. This depth segment was densely rooted and showed the strongest changes in $\delta^{13}C_{CO2}$ and δ^{13}_{CH4} following the ^{13}C-CO_2 labelling pulse (Fig. 3). $\delta^{13}C_{CH4}$ reached up to 3‰ in W-V at 10 cm depth and in DW-V at 5 cm depth after 85

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and 45 h, respectively. A fraction of 1.3% and 1.7% of the incorporated label had been transformed into methane. During water table drawdown, CH_4 concentrations strongly diminished in the newly unsaturated peat. CH_4 pools were restored following rewetting within about 40 (DW-V) and 50 (DW-D) days (Fig. 1E, F). In the densely rooted up-
5 per 10 cm of the DW-V treatment, methanogenesis re-established more rapidly within 10 days.

The $\delta^{13}C_{CH4}$ was comparable in the DW-V and DW-D treatments and adjusted to −75 to −110‰ below a depth of 15–20 cm, with lowest values in 50 cm depth (Fig. 5). In DW-V, values of −65 to −75‰ were higher the upper 15 cm. The carbon isotopic com-
10 position of methane in W-V differed substantially, as $\delta^{13}C_{CH4}$ in this mesocosm was about −45 to −55‰ in the upper 15 cm and around −65‰ below. Drying and rewetting led to concomitant shifts in $\delta^{13}C_{CH4}$ in DW-V and DW-D (Fig. 5). A methanotrophic zone migrated downwards with the declining water table level because $\delta^{13}C_{CH4}$ increased by approx. 10–20‰ in DW-V and ∼5–10 in DW-D when the water table passed.
15 Methane in DW-D had a persistently higher $\delta^{13}C_{CH4}$ than in DW-V in the upper 30 cm after rewetting but in each treatment values were similar as before drying. The predominating CH_4 production pathway was thus not affected by drying and rewetting, in terms of $\delta^{13}C_{CH4}$.

3.6 Methane turnover

20 Calculated methane net turnover (Fig. 4) at constantly high water table in W-V reached 2 to 8 nmol cm^{-3} d^{-1} around the mean water table. After 120 days of incubation net CH_4 production ceased and CH_4 was net consumed. Methane production in DW-V peaked at 5 cm depth, reaching 10–15 nmol cm^{-3} d^{-1} at high water table. This coincided with a local maximum in $\delta^{13}C_{CO2}$, suggesting CO_2 to be the precursor. A second
25 but lower maximum of 0–3 nmol cm^{-3} d^{-1} occurred at a depth of 20–30 cm. In DW-D, methane production peaked near the water table. Methane production followed the water table downward in DW-V and DW-D. After rewetting, methane production re-

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bounded to >3 nmol cm^{-3} d^{-1} in 5 cm depth of DW-V within 10 days and increased to >11 nmol cm^{-3} d^{-1} and thus highest absolute net production rates. In DW-D, rates of 3 nmol cm^{-3} d^{-1} in 10 cm depth were exceeded only after 20 days and did not increase further.

3.7   Concentrations of acetate and hydrogen

Acetate concentrations generally ranged from 50 to 100 μmol L^{-1} (Fig. 6) but increased in the unsaturated peat of DW-V and DW-D to about 300–350 μmol L^{-1} before rewetting. Subsequently, acetate concentrations decreased to below 50 μmol L^{-1} and finally readjusted to pre-drought levels in about 30 days. Acetate consumption thus contributed to a post-rewetting respiration pulse. Concentrations were higher in W-V, especially in 5–10 cm and 50 cm depth, and often exceeded 350 μmol L^{-1}.

Hydrogen concentrations were mostly below 1 nmol L^{-1} (Fig. 6). In W-V and DW-V, higher concentrations occurred at 5–10 cm depth during wet periods and reached up to 2.5–5 nmol L^{-1}. The concentration maximum was thus related to the activity of roots and CH$_4$ production. In DW-D, H$_2$ concentration reached a maximum of 0.7–1.7 nmol L^{-1} in 50 cm depth, where also the maximum in CH$_4$ concentrations was measured. This depth was, however, not affected by the drying/rewetting cycle.

3.8   Diffusive C fluxes and their isotopic composition, CO$_2$/CH$_4$ ratios and isotope balance

Based on the concentration gradients at the water table, CO$_2$ fluxes from the saturated zone in the treatments W-V, DW-V and DW-D were 3.6, 1.1, and 7.6 mmol m^{-2} d^{-1} respectively and had an isotope signature of −21.8±9.3‰ (W-V), −22.7±7.7‰ (DW-V), and −19.9±6.3‰ (DW-D). Drying and rewetting shifted δ^{13}C of diffusive CO$_2$ fluxes temporarily from around 20 to −25‰ to values below −25‰ thus supporting the suppression of methanogenic activity, leading to less residual ^{13}C enrichment in the released CO$_2$.

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Methane fluxes at the water table were 0.08, 0.01 and 0.12 mmol m^{-2} d^{-1} in W-V, DW-V and DW-D, respectively, and had an isotope signature of −59.2±9.9‰ in W-V, −75.0±22.7‰ in DW-V, and −82.9±14.1‰ in DW-D. The methanogenic surface layer in DW-V emitted methane with a δ^{13}C of −60.9±13.9‰ and thus comparable to values observed in W-V. During the dry phase, treatment DW-V emitted CH$_4$ with lower δ^{13}C values, probably due to the release of previously stored highly ^{13}C depleted CH$_4$. After rewetting, the treatments W-V and DW-V emitted again CH$_4$ of comparable isotopic composition around −60‰ while in treatment DW-D without vegetation δ^{13}C of CH$_4$ fluxes were mostly below −70‰.

The diffusive CO$_2$ to CH$_4$ flux ratios were quite high in all treatments, reaching 45 (W-V), 106 (DW-V), and 61 (DW-D). Considering the isotope balance, however, these ratios were much smaller, i.e. 5.4, 9.7 and 7.2 in W-V, DW-V, and DW-D respectively. This would mean that either diffusive CO$_2$ fluxes were over- or diffusive CH$_4$ fluxes underestimated. Nevertheless, both drying and rewetting treatments had higher CO$_2$/CH$_4$ ratios.

Based on applying Eqs. (4–6), the contribution of anaerobic respiration to CO$_2$ fluxes was 64.0, 12.8 and 9.8 mmol m^{-2} d^{-1} in W-V, DW-V, and DW-D, respectively. These fluxes compare to a measured soil CO$_2$ flux in DW-D of 94 mmol m^{-2} d^{-1}. We then took the above mentioned fluxes from concentration gradients and isotope mass balance as an lower and upper estimate of anaerobic CO$_2$ fluxes and the 94 mmol m^{-2} d^{-1} CO$_2$ flux of DW-D as the total soil CO$_2$ flux reference for all treatments. This allowed to calculate the aerobic CO$_2$ fluxes from the soil to account for 32–96% (W-V), 86–99% (DW-V), and 89–92% (DW-D) of the total CO$_2$ flux.

3.9   Isotope ratio cross plot and apparent fractionation factors

As depicted in the isotope ratio cross-plot (Fig. 7) for DW-V and DW-D, most δ^{13}C$_{CH4}$ and δ^{13}C$_{CO2}$ pairs from below the water table showed apparent fractionation factors α_C of >1.065 (solid triangles and rectangles). Above the water table, values of 1.07–

1.04 were calculated, with only few exceptions <1.04 (open triangles and rectangles). Overall, fractionation factors in DW-V and DW-D increased with depth. This pattern was essentially not affected by drying/rewetting. Fractionation factors in the wet treatment W-V differed from the values observed in DW-V and DW-D. Values of α_C observed in W-V below the water table (solid circles) plotted between the lines for $\alpha_C = 1.055$ and $\alpha_C = 1.04$. Above the water table also $\alpha_C < 1.04$ was calculated (open circles). An increasing importance of acetoclastic methanogenesis or methanotrophy seemed thus likely (Fig. 7).

3.10 Thermodynamic calculations

The Gibbs free energy yield from hydrogenotrophic methanogenesis ΔG_{hm} was mostly positive (Fig. 8), i.e. this process was thermodynamically unfavourable. This finding was mostly caused by low hydrogen concentrations (see Eq. 9). Concentrations of >4 nmol L^{-1} would have been needed for methanogens to gain energy. This result is in apparent contradiction with the predominance of hydrogenotrophic methanogene-sis as derived from δ^{13}C analyses. The process became only temporarily exergonic in the upper 5–15 cm of the soil in DW-V, which coincided with high production rates in this depth. A similar pattern was found in the DW-D treatment. In W-V treatment hydrogenotrophic methanogenesis was only exergonic near the water table, again co-inciding with a production maximum of CH$_4$. Acetoclastic methanogenesis (Eq. 10) was a thermodynamically feasible process in all treatments with a ΔG_{am} of −30 to −60 kJ mol^{-1} (Fig. 8 ΔG_{am}), especially at shallow depths. Homoacetogenesis (ha) from CO$_2$ and H$_2$ (Eq. 11) required 9- >70 kJ mol^{-1} in all treatments, which makes an occurrence of this process unlikely. To make the process exergonic H$_2$ concentrations of >50 nmol L^{-1} would have been needed.

Using the relationship of ΔG_{hm} for hydrogenotrophic metanogenesis and the appar-ent fractionation factor α_C (Eq. 3) given in Penning et al. (2005) (Eq. 8), this process was always viable in all layers where we could quantify the isotopic composition of

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CH$_4$. Values of ΔG_{hm} shifted from positive values as calculated using the measured H$_2$ concentrations to values ranging from -2 to $-80\,\mathrm{kJ\,mol}^{-1}$ H$_2$ following Penning et al. (2005). According to these calculations, measured hydrogen concentrations may therefore be underestimated by about two orders of magnitude.

4 Discussion

The drying/rewetting cycle had substantial effects on methane production and dynamics in our mesocosms, as could be expected from previous work (Aerts and Ludwig, 1997; Blodau and Moore, 2003a; Shannon and White, 1994; Updegraff et al., 2001). Key finding of our study were (i) an effective suppression of methanogenesis and promotion of methanotrophy during drought and after rewetting, (ii) highest methanogenic activity under vegetation in the uppermost soil layers, (iii) apparent insensitivity of methanogenic pathways to drying and rewetting, and (iv) and a prevalence of hydrogenotrophic methanogenesis, despite this processes being mostly endergonic on the scale of observation.

4.1 Solid phase inventory

The carbon content of this minerotrophic temperate fen soil was in some parts of the profile low compared to other organic soils (Hornibrook et al., 2000c). The isotope signature was around $-27‰$ although a trend towards more negative δ^{13}C values existed in W-V and DW-D. This may be due to former changes in plant communities, as some wetland plants may have a δ^{13}C of less than $-30‰$ (Hornibrook et al., 2000c). Total nitrogen content of 1–2% was within the range reported for minerotrophic, acidic habitats (Bridgham et al., 1998). Nevertheless, the small differences in δ^{13}C in this peat suggested that the isotope signature of CO$_2$ formed by respiration should not vary much with depth and the major effects on δ^{13}C in CO$_2$ should thus be due to methanogenic activity (Whiticar, 1999).

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4.2   Impact of drying and rewetting on hydrological conditions

During experimental drought the water table dropped by 30–40 cm, which is also common at the field site (Paul et al., 2006). VGCs of up to >12% were high, compared to the study of Mainiero and Kazda (2005), who documented that a change in water content of ~2% may introduce oxygen into unsaturated peat. The rewetting event of 54 (DW-V) and 53 mm (DW-D) irrigation, was also akin to heavy rain naturally occurring at the site (Lischeid, personal communication). The experiment was thus successful in creating a realistic "extreme" drying/rewetting event. As the timescale of this experiment was ~300 days, it is reasonable to assume that the results should be relevant on the field scale. Inherent limitations remain, however, as the incubation temperature was higher than at the field site and in our mesocosm approach no advective transport or flow occurred.

4.3   Impact of drying and rewetting on methane dynamics

Generally, methane concentrations measured in this study were lower than observed in bog mesocosms (Blodau and Moore, 2003a) but comparable to other fen soils (Chasar et al., 2000; Smemo and Yavitt, 2006). During dry phases in DW-V and DW-D, methane concentrations rapidly decreased with the peat becoming unsaturated. After re-elevation of the water table, methane production was retarded, likely because electron acceptors were used for respiration preferentially (Peters and Conrad, 1996; Roden and Wetzel, 1996). Methane concentrations in the lower profile steadily and slowly increased after rewetting and more rapidly, within days, in the shallow and rooted peat of DW-V. Methanogenesis thus more quickly recovered than in mesocosm experiments with peat from a dry ombotrophic bog (Blodau and Moore, 2003a). In our study we could even observe methanogenic conditions above the water table (Knorr et al., 2008). More rapid production of methane at shallow depths of DW-V supported the idea of previous studies that methanogenesis was found to depend on input of fresh and labile carbon compounds provided by vegetation (Whiting and Chanton, 1993;

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Popp et al., 1999). Methane production above the water table was so far only documented with respect to potential methane production in laboratory incubations (Coles and Yavitt, 2004), but in this study it was found in intact soils.

In the wet treatment W-V, concentrations of methane and total dissolved carbon dioxide reached a steady state and were high enough to sustain measurable emission. By the second half of the experiment, CH_4 concentrations in W-V declined, but methane efflux measured in the static chamber continued to increase. Methane efflux was thus to some extent disconnected from the methane pool size. Carex roots can access deeper soil layers and which may lead to CH_4 bypassing of the soil (Popp et al., 1999). Furthermore, high productivity of plants and well developed root systems were shown to support methane production and emission (Joabsson and Christensen, 2001). To allow for such high CH_4 emission rates we thus speculate that Carex rostrata in the wet treatment promoted gross turnover also in the deeper soil. Slowly declining CH_4 concentrations during the growing season at *Carex* dominated sites were already reported by Joabsson and Christensen (2001) and the authors hypothesized that increased rooting increased methane oxidation in and emission from the rhizosphere.

4.4 Insights gained from the ^{13}C-labelling experiment

The applied ^{13}C-CO_2 label was quickly transferred into $\delta^{13}C_{CO2}$ of the soil, within 12 h. Changes in $\delta^{13}C$ of the soil CH_4 pool were detected after about 24 h. The transfer was in the same range as reported for arctic wet sedge tundra (King and Reeburgh, 2002). Although less than one percent of the tracer amount had actually been taken up, calculated CO_2 incorporation rates were 0.7–1.8 mmol C m^{-2} d^{-1} under vegetation, and thus in the same order of magnitude as depth integrated CH_4 production in the upper 20 cm. One may hypothesize that plants with aerenchyms could transport oxygen into the soil at comparable rates and thus provide effective oxidation potential for CH_4 or other electron acceptors.

About 1.3 to 1.7% of the label that had been taken up had been transformed into methane within 90 h. King and Reeburgh (2002) found less than 1 % of their label in

the emitted methane after two weeks. Thus, our findings suggest that in our meso-cosms recent photosynthetates and root associated CO_2 may contribute significantly more to CH_4 production. This confirmed the role of plant activity for below ground methanogenesis, especially at shallow depths (Strom et al., 2003).

Furthermore, the labelling experiment demonstrated the rhizosphere associated respiration in the fen soil, which was mainly limited to the upper 10-20 cm. This confirmed the findings of Coles and Yavitt (2004) that fresh organic matter input through plants fuelled anaerobic microbial activity to a great extent. Chimner and Cooper (2003) also found that manipulating the water table had most impact on soil respiration when manipulated within the range of the most active surficial zone.

4.5 Impact of drying and rewetting on isotopic composition of CO_2 and CH_4

A residual enrichment of ^{13}C in CO_2 as observed in this study was also observed in other studies (Hornibrook et al., 2000a; Lansdown et al., 1992; Waldron et al., 1999) and is typical for methanogenic environments due to strong fractionation during methanogenesis (Conrad, 2005; Whiticar, 1999). It was also frequently found that $\delta^{13}C_{CO2}$ does not match $\delta^{13}C$ of the solid phase probably due to methanogenic activity (Hornibrook et al., 2000a; Waldron et al., 1999).

Concerning the temporal dynamics of $\delta^{13}C_{CO2}$, increased respiration activity after rewetting was often observed (Fierer and Schimel, 2003; Blodau and Moore, 2003b). Our study demonstrated that almost the complete soil CO_2 pool must have been renewed, as the isotopic composition after rewetting matched the $\delta^{13}C$ of the solid phase. These results support that there is no isotope fractionation during breakdown of organic matter (Boehme et al., 1996), as the effect should be largest at the re-build-up of the soil CO_2 pool.

The isotopic composition of methane in this study was comparable (W-V) or lighter (DW-V, DW-D) than previously reported (Chasar et al., 2000; Lansdown et al., 1992; Popp et al., 1999; Waldron et al., 1999). As the zone of higher $\delta^{13}C_{CH4}$ values followed

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closely the water table drawdown and re-elevation, we suggest this ^{13}C enrichment in the CH_4 pool to be to a great extent attributed to CH_4 oxidation and residual ^{13}C enrichment (Popp et al., 1999; Whiticar, 1999). Another methanogenic pathway was probably effective in the wet treatment W-V and may have occurred in DW-V, as the
5   isotopic composition of methane in 5–10 cm depth was heavier than in DW-D. If the shift in isotopic composition observed in the upper profile was solely related to a different production pathway in the rhizosphere, one would, however, not expect this pattern to follow the water table.

  In the W-V mesocosm, the observed isotopic composition of methane was different
10 compared to DW-V and DW-D but also did not change during the course of the experiment. The measured values here were in accordance with $\delta^{13}C_{CH4}$ reported in other studies, though, particularly if sedges were present (Chasar et al., 2000; Popp et al., 1999).

4.6   Anaerobic and aerobic respiration as derived from δ^{13}C in diffusive fluxes of CO_2
15   and CH_4

The isotopic composition of the calculated diffusive CO_2 fluxes across the water table was in a narrow range of −20 to −23‰ in all treatments. In contrast to the study of Lansdown et al. (1992) CO_2 fluxes in our study were thus isotopically heavier than the soil organic matter. Only after rewetting, $\delta^{13}C_{CO2}$ temporarily approached the
20 δ^{13}C of solid phase. We interpret this to be caused by the temporal suppression of methanogens after rewetting due to consumption of alternative electron acceptors (Achtnich et al., 1995; Dettling et al., 2006). Therefore, the fractionating effect of methanogens on $\delta^{13}C_{CO2}$ was temporarily suppressed and $\delta^{13}C_{CO2}$ approached the isotopic signature of the solid phase.
25   The defoliated treatment DW-D had lowest observed δ^{13}C in the CH_4 diffusive flux. This number reflected the highly ^{13}C-depleted methane from bottom layers. Treatment DW-V and especially W-V emitted less ^{13}C-depleted methane, which was near the sur-

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face presumably produced from fresh plant material. Popp et al. (1999) also found at non-vegetated sites methane to be more depleted in ^{13}C than at vegetated sites and attributed to the presence of vegetation. Treatment DW-V showed a layered profile in terms of isotopic composition of methane, as during phases of low water table, the lower profile emitted highly ^{13}C depleted methane as observed in DW-D. At high water table level, the isotopic composition of the efflux was comparable to W-V. As in the near surface peat of DW-V the re-onset of methanogenesis was exceptionally fast we hypothesize that this was due to the input of fresh plant derived carbon near the surface. Roots did not penetrate below 15 cm in DW-V, thus a lower contribution of fresh plant derived compounds may have caused methane to be produced at lower rates and to have a different signature in the lower profile.

Ratios of CO_2/CH_4 of diffusive fluxes were high compared to other studies in methanogenic environments (Yavitt and Seidmann-Zager, 2006). Drying and rewetting raised the ratio to as much as 61 for DW-D and 106 for DW-V, thus supporting the suppressive effect of drying and rewetting on methanogenic activity (Achtnich et al., 1995; Dettling et al., 2006). Calculated from the isotope mass balance (Eqs .4–6), these numbers were much smaller, ranging from 7 (DW-D) to 10 (DW-V), and 5 in W-V. This may be due to a significant proportion of aerobic CO_2 production near the water table. By calculating diffusive fluxes from the saturated zone one cannot differentiate between CO_2 produced under aerobic or anaerobic pathways. Although a lack of replicates does not allow for attributing this solely to drying and rewetting, these treatments showed higher CO_2/CH_4 ratios.

Using the isotope mass balance and measured CH_4 chamber fluxes for W-V, we calculated an anaerobic CO_2 flux of 64 mmol m^{-2} d^{-1} for this treatment. This flux was much higher than reported for a bog in the study of Lansdown et al. (1992). We speculate that fresh carbon and electron accepting capacity input at greater depths through Carex roots may have contributed to this high flux. Assuming CH_4 fluxes at the detection limit of our chamber technique, one may also calculate anaerobic CO_2 fluxes for DW-V and DW-D, which were in a range of the numbers calculated by Lansdown et

al. (1992), although still a factor of 2–4 higher. This may be due to the higher temperature used for the incubation compared to field site temperatures.

Minding the inherent uncertainty due to a lack of replicates one may assume the non-vegetated treatment to be a rough estimate for soil respiration also for the other treatments. This allowed to calculate aerobic CO_2 fluxes for all treatments to account for 32–96% in W-V and 86–99% in DW-V and DW-D of the total CO_2 soil flux. Although speculative, these numbers supported the importance of the few cm top layers above the water table of fen sites that were found to consist of most easily degradable fresh organic carbon (Chimner and Cooper, 2003; Coles and Yavitt, 2004).

4.7 Impact of drying and rewetting on methanogenic pathways

Below the water table in DW-V and DW-D, high fractionation factors of >1.065 were observed. These values fell in the uppermost range of α_C reported by Conrad (2005) and Whiticar (1999) and should therefore justify the conclusion that CH_4 was to a great extend formed by hydrogenotrophic methanogens. Penning et al. (2005) suggested that high fractionation factors reflect thermodynamically unfavourable conditions for hydrogenotrophic methanogens. In this study this was presumably caused by the drying/rewetting event resulting in low hydrogen concentrations due to the presence of other electron acceptors in the bulk peat. Most α_C values calculated for above the water table (1.04–1.065) were in an overlap range of α_C from hydrogenotrophic and acetoclastic methanogenesis according to (Whiticar, 1999;Chasar et al., 2000), though, while most values of α_C for the latter pathway summarized by (Conrad, 2005) were still lower. Following Whiticar (1999), the observed shift in α_C may also be explained by occurrence of methanotrophic activity. This was supported by the net turnover calculations and as we could not measure any methane efflux using static chambers.

A $\delta^{13}C_{CH4}$ of around −70‰ of the methane formed in the shallow depths of DW-V and α_C=1.05–1.07 may thus lead to the assumtion that it was formed to a great extend by hydrogenotrophic methanogens and not by acetotrophs (Whiticar, 1999), as often reported for shallow peats (Chasar et al., 2000; Hornibrook et al., 2000a).

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This was supported by higher H_2 concentrations at shallow depths in this treatment. Methanotrophic activity at the aerobic/anaerobic interface may have shifted $\delta^{13}C_{CH4}$ to less negative values as observed at greater depths (Whiticar, 1999).

After rewetting of DW-V and DW-D, as soon as methane concentrations were high
5   enough to measure the isotopic composition, similarly high α_C values occurred as before the drought period. Drying and rewetting did thus not shift methanogenesis away from CO_2-reduction, as this would have been indicated resulted by lower apparent fractionation factors α_C (Whiticar, 1999; Conrad, 2005). The inverse pattern of $\delta^{13}C_{CO2}$ and $\delta^{13}C_{CH4}$, meaning an enrichment of ^{13}C in CO_2 in zones of production of CH_4 poor
10 in ^{13}C, therefore suggests that in this peat hydrogenotrophic methanogens also under transient conditions dominated. Up to now it was supposed that the latter dominate in surficial peat (Hornibrook et al., 2000a; Popp et al., 1999) under temporary occurrence of aerated conditions. As in the study of (Lafleur et al., 2005), due to the high water content in the upper profile even at a water table of 50 cm below surface, one
15 may assume that anoxic microenvironments (Wachinger et al., 2000) provided a suitable habitat during drought. Furthermore, some hydrogenotrophs were demonstrated to have a capacity for iron reduction and had possibly shifted their metabolic pathway (van Bodegom et al., 2004).

The observed range of fractionation factors in the wet treatment W-V would lead
20 to the conclusion that a significant part of methane was produced via acetoclastic methanogenesis. On the basis of our comprehensive data set, however, we did not exclusively follow this interpretation of values of α_C. Due to the inverse pattern of $\delta^{13}C_{CO2}$ and $\delta^{13}C_{CH4}$ also in this case and isotope mass balance considerations, a dominant contribution of hydrogenotrophic methanogens must have occurred. Additionally, val-
25 ues of α_C were still in the overlap of fractionation factors from both processes (Whiticar, 1999; Conrad, 2005). The measured $\delta^{13}C_{CH4}$ values also coincide well with data of greater depths from other fens where Carex species were found (Chasar et al., 2000; Popp et al., 1999), as it was the case in W-V.

The observed apparently low fractionation in the W-V treatment was in our opinion

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due to methanotrophic activity throughout the profile, which was possible only in the W-V mesocosm with Carex species being present. It is well documented that Carex species can transport oxygen into the soil and thus support the activity of methanotrophs (Popp et al., 1999; Mainiero and Kazda, 2005). From solid phase sampling it had became obvious that Carex roots had grown throughout the mesocosm down to 60 cm. The effects of Carex roots may be identified in the isotope ratio cross-plot (Fig. 7). The arrow shifting $\delta^{13}C_{CH4}$ towards less negative values but concomitantly decreasing $\delta^{13}C_{CO2}$ denotes methanotrophic activity. This effect, however, only partly explained the position of the $\delta^{13}C$ pairs of the W-V mesocosm. Another process, shifting the $\delta^{13}C_{CH4} - \delta^{13}C_{CO2}$ pairs along the lines of constant α_C towards both less negative $\delta^{13}C_{CH4}$ and $\delta^{13}C_{CO2}$ was needed. We propose that this shift is due to a "removal" of CH_4 which is especially obvious in the presence of Carex roots. This "removal" may be both, methanotrophy at and emission through the aerenchyms, but in both cases the lighter isotope is preferentially released in form of CO_2 or CH_4 through the plant aerenchym. Such selective enrichment of heavier isotopes was already described for lake sediments, where the lighter isotope tends to escape from methanogenic sediments by ebullition (Gu et al., 2004). Roots being able to transport gases may in this case cause the same effect in this case

Thermodynamic calculations revealed that no energy could be gained from hydrogenotrophic methanogenesis in any treatment when geochemical conditions were averaged on the scale of the sampling devices. It cannot be ruled out that the latter process occurred, though. Minding the above mentioned results and the considerations of Penning et al. (2005) it is still reasonable to assume CO_2 as the precursor of methane in our peat. Only in the permanently wet treatment W-V acetoclastic methanogenesis may have been more important, strongly negative ΔG_{am} coincided with lower values of α_C. In the DW-V and DW-D treatment, ΔG_{hm} of hydrogenotrophic methanogenesis was mostly dominated by the observed low hydrogen concentrations. Clustering of hydrogen producing and consuming bacteria in spatially heterogeneous samples was shown to lead to a severe underestimation of hydrogen concentrations when sampled

with common techniques.

Hydrogen measurements thus only serve as an indicator on the scale of the measuring device (Hoehler et al., 2001). Larger sampling devices may reflect hydrogen concentrations which are not representative for processes occurring in microenvironments. In our case, hydrogen concentrations on the sampling scale of 20 cm were thus presumably dominated by iron or sulphate reducing bacteria while methanogenesis was still possible in microenvironments. Although without further analysis of e.g. hydrogen isotopes or isotope analysis of acetate this point cannot be clarified (Conrad, 2005), a dominance of acetoclastic methanogenesis from our point of view seems unlikely, as such high values of α_C as observed in DW-V and DW-D were never reported in any study to date. The validity of the thermodynamic calculations may therefore be questionable under such dynamic or heterogeneously structured redox conditions, in which thermodynamic equilibrium may not be reached on the scale under study and the existence of different microenvironments is exceptionally likely. This discrepancy of conclusions derived from thermodynamic arguments and isotope fractionation factors may eventually be used to study biogeochemical heterogeneity in wetland soils.

A pathway of methanogenesis, in which CO_2 is first converted to acetate (homoacetogenesis) followed by disproportionating acetate into CO_2 and methane (acetoclastic methanogenesis) to close the isotope mass balance (Hornibrook et al., 2000b) seemed unlikely, as ΔG_{ha} for homoacetogenesis was always positive. For this process to become viable even higher H_2 concentrations of $>50\,nmol\,L^{-1}$ would have been needed. The above mentioned problems of our thermodynamic calculations do also apply in this case, though.

5 Conclusions

A key finding of this study was that short term manipulation of the water tables in the peat mesocosms did not translate into altered isotopic composition of the soil methane pool. The only effect observed was a zone of isotopically heavier CH_4 following the wa-

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ter table level, which indicated that CH_4-oxidation followed the water table level. Isotope budgets were a valuable tool to validate surface and diffusive below ground flux measurements. Knowing CH_4 fluxes enabled to recalculate anaerobic CO_2 fluxes at various depths. Taking a defoliated treatment as a rough estimate for soil respiration also al-
5 lowed estimating aerobic CO_2 production via isotope balancing. Isotope budgets and apparent fractionation factors furthermore supported the importance of the CO_2 reduction pathway in all treatments at all depths, despite being an unfavourable process in the bulk of the peat matrix following the thermodynamic calculations. Applying a more recent approach which links α_C and thermodynamics for hydrogenotrophic methano-
10 genesis (ΔG_{hm}) supported that CH_4 may have been formed by hydrogenotrophs; the very low energy gain was presumably due to the drying and rewetting cycle. Comparison of Gibbs free energies of respiration pathways with observed fractionations factors α_C may thus eventually serve as an indicator whether heterogeneity plays an important role on the scale under study. Despite suffering from a lack of replicates, the vegeta-
15 tion may possibly have had a strong effect on $\delta^{13}C$ of CH_4 as we observed consistently higher values in the permanently wet treatment W-V and this was the only treatment containing Carex species. We are aware that this conclusion is speculative, albeit reasonable. Mass balance considerations and isotope budgets supported a selective CH_4 removal, especially under Carex. Recalculation of fluxes and turnover by a combina-
20 tion of mass balance and isotope budgets may thus serve as useful tools also on the field scale. Regarding the importance of drying/wetting events, the study demonstrated an obvious impact on respiration pathways in the short term, expressed in temporary suppression of methanogenesis. There were, however, no sustainably altered process patterns in the long term but probably the proportion of CH_4 produced was lowered.

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Table 1. Stoichiometry of hydrogenotrophic and acetoclastic methanogenesis and thermodynamic data (Nordstrom and Munoz, 1994) as used to calculate the thermodynamic energy yield from each process.

Process	Stoichiometry	ΔG_r (kJ mol^{-1} at 15°C)	Eq.
Hydrogenotrophic methanogenesis:	$CO_2(aq) + 4H_2(aq) \rightarrow CH_4(aq) + 2H_2O(l)$	$\Delta G_{hm} = -194.3$	(9)
Acetoclastic methanogenesis:	$CH_3COO^-(aq) + H^+(aq) \rightarrow CO_2(aq) + CH_4(aq)$	$\Delta G_{am} = -49.8$	(10)
Homoacetogenesis	$2\,CO_2(aq) + 4H_2(aq) \rightarrow CH_3COO^-(aq) + 2H_2O(l) + H^+(aq)$	$\Delta G_{ha} = -144.5$	(11)

Table 2. Soil C and N content and δ^{13}C isotopic composition (δ^{13}C in ‰ vs. V-PDB) of soil organic matter in each mesocosm. Soil δ^{13}C and N content were measured four times (± standard deviation), for soil C-content n=2.

Treatment and depth (cm)	C-content (%)	δ^{13}C bulk SOM (‰)	N-content (%)
Permanantly wet treatment W-V			
5	30.5	−27.20 (±0.40)	1.57 (±0.52)
17.5	29.1	−27.36 (±0.24)	1.79 (±0.23)
32.5	32.4	−27.53 (±0.36)	1.45 (±0.51)
45	38.5	−27.90 (±0.22)	1.29 (±0.29)
55	37.3	−28.14 (±0.37)	1.26 (±0.08)
Vegetated drying / wetting treatment DW-V			
5	34.2	−27.69 (±0.59)	2.16 (±0.45)
17.5	26.7	−27.32 (±0.32)	1.54 (±0.39)
32.5	22.4	−27.34 (±0.45)	1.26 (±0.38)
45	15.8	−27.51 (±0.43)	0.92 (±0.44)
55	24.6	−27.89 (±0.42)	1.01 (±0.26)
Defoliated drying / wetting treatment DW-D			
5	28.7	−27.10 (±0.94)	1.76 (±0.65)
17.5	23.8	−26.85 (±0.34)	1.26 (±0.50)
32.5	30.1	−27.79 (±1.53)	1.16 (±0.23)
45	39.9	−28.18 (±0.22)	1.31 (±0.19)
55	47.5	−28.35 (±0.42)	1.52 (±0.23)
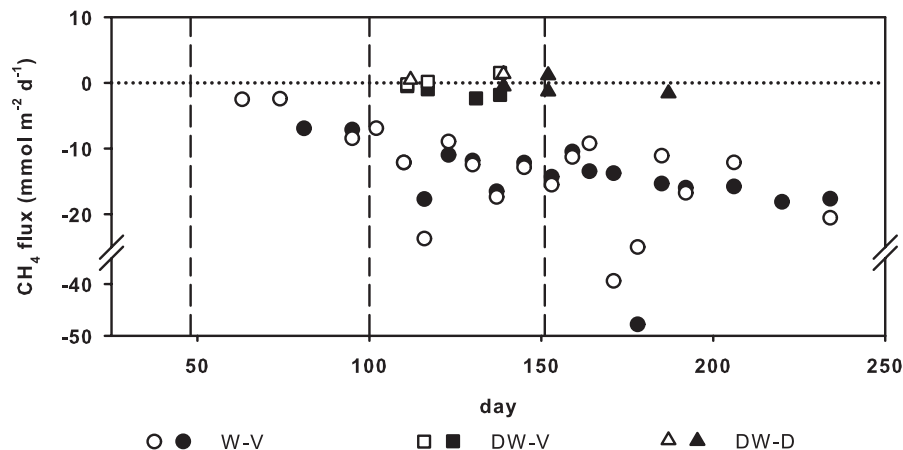
Fig. 1. Methane exchange of W-V, DW-V and DW-D measured with static chambers. Open and solid symbols denote two independent measurements per treatment. Fluxes were calculated from concentration over time through linear regression ($r^2 > 0.9$). Vertical dashed lines separate the different phases (I: initial dry, II: first wet, III: dry and IV: rewetted phase).
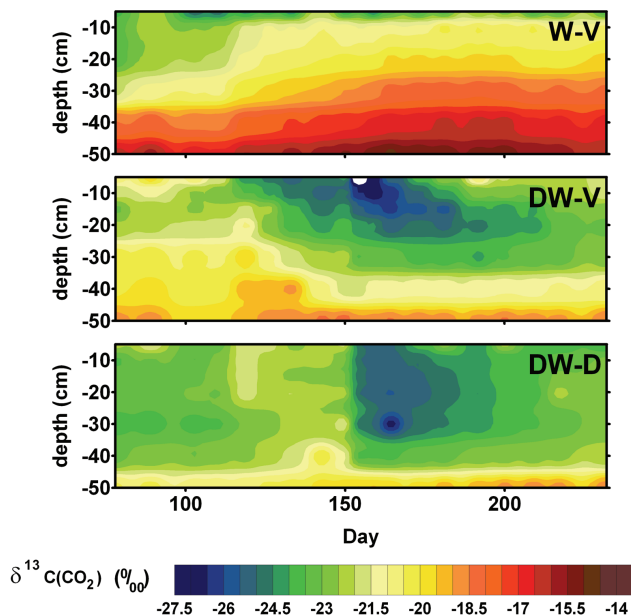
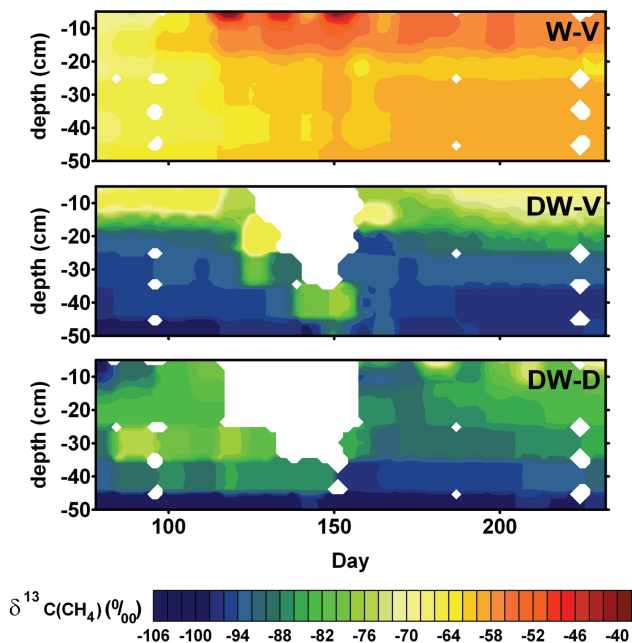**Fig. 2.** Values of $\delta^{13}C$ of CO_2 measured in the soil gas phase (saturated and unsaturated) of W-V (top), DW-V (middle) and DW-D (bottom). Color scales are similar for all treatments. For corresponding CO_2 concentrations, see text.
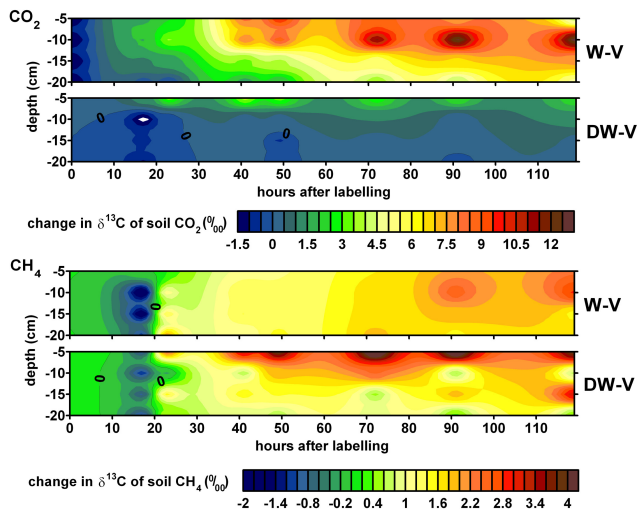
Fig. 3. Absolute changes in $\delta^{13}C$ (‰ vs. V-PDB) of soil CO_2 and CH_4 in the vegetated wet treatment W-V and drying/wetting treatment DW-V after application of the ^{13}C-CO_2 pulse label (time=0 h).
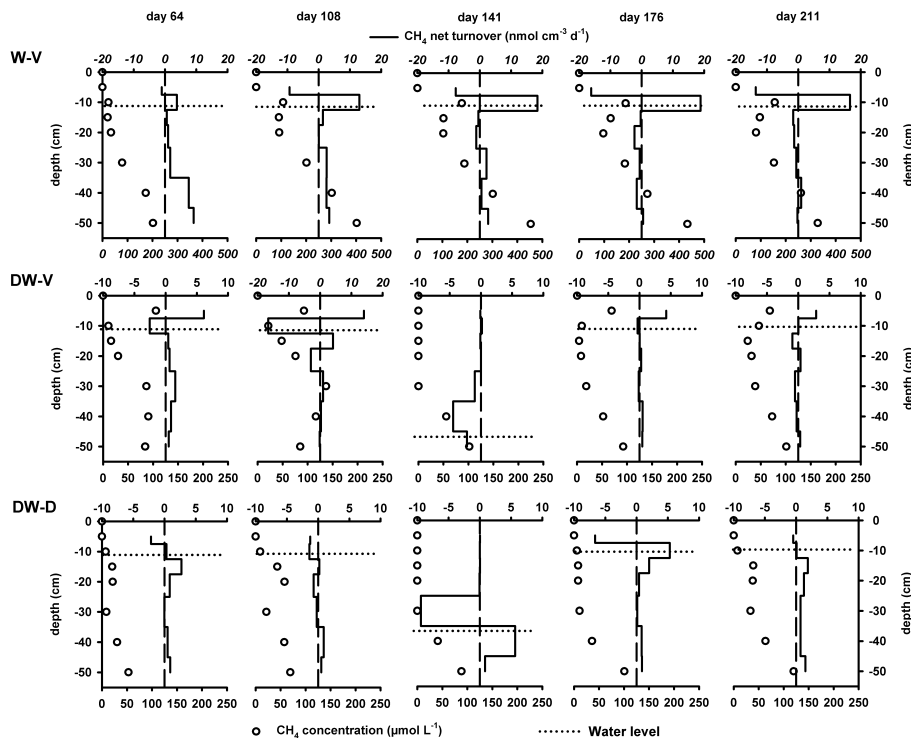
Fig. 4. Concentrations (lower x-axis), and calculated net turnover rates (upper x-axis) of CH$_4$ in the three treatments W-V, DW-V, and DW-D. Day 64 is after first wetting, day 108 begin of dry period, day 146 end of dry period, day 176 three weeks after rewetting and day 211 steady state rewetted. Different turnover and concentration scales on the x-axis are indicated by letters in italic. For calculation of turnover rates, see methods section.

Fig. 5. Values of δ^{13}C of CH_4 (vs. V-PDB) measured in the soil gas phase (saturated and unsaturated) of W-V (top), DW-V (middle) and DW-D (bottom). Colour scales are similar for all treatments. For corresponding CH_4 concentrations and turnover, see Fig. 3.
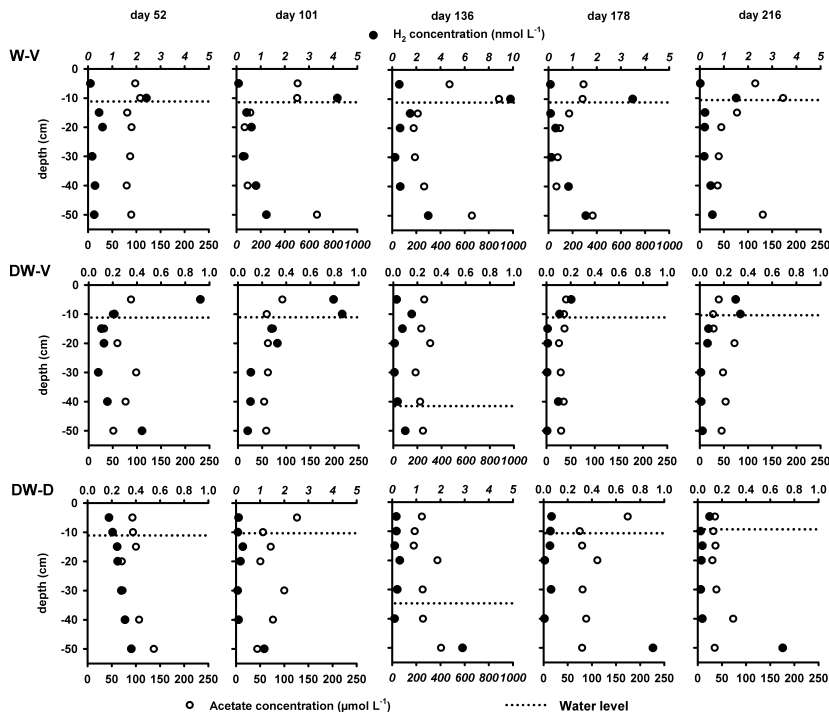
Fig. 6. Concentrations of hydrogen (upper x-axis) and acetate (lower x-axis) in the three treatments W-V, DW-V, and DW-D. Day 64 is after first wetting, day 108 begin of dry period, day 146 end of dry period, day 176 three weeks after rewetting and day 211 steady state rewetted. Different concentration scales on the x-axis are indicated by letters in italic.
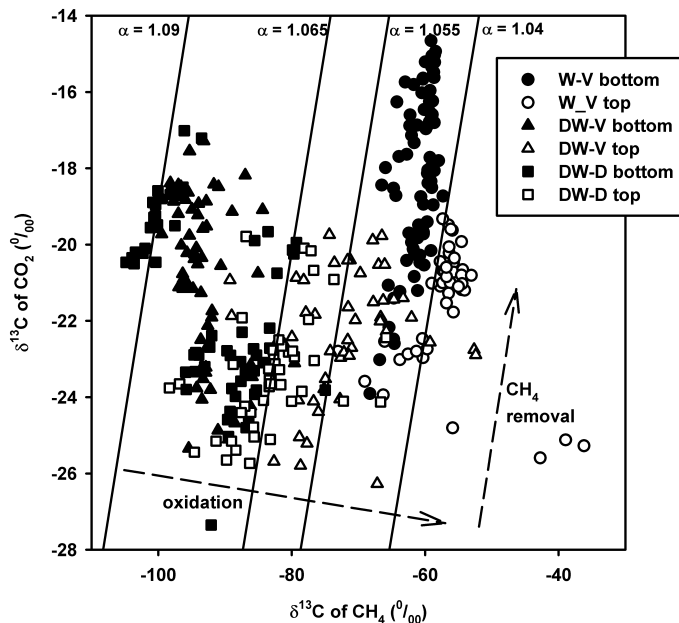
Fig. 7. Cross-plot of corresponding $\delta^{13}C_{CH4}$ and $\delta^{13}C_{CO2}$ values (‰) in the soil gas of the three treatments W-V, DW-V, and DW-D. Diagonal lines for different fractionation factors α_C (Whiticar, 1999; Conrad, 2005) are also given. The dashed arrows indicate directions in which pairs would be shifted by methane oxidation (oxidation) or removal from the system (CH$_4$ removal). For explanation see discussion section.
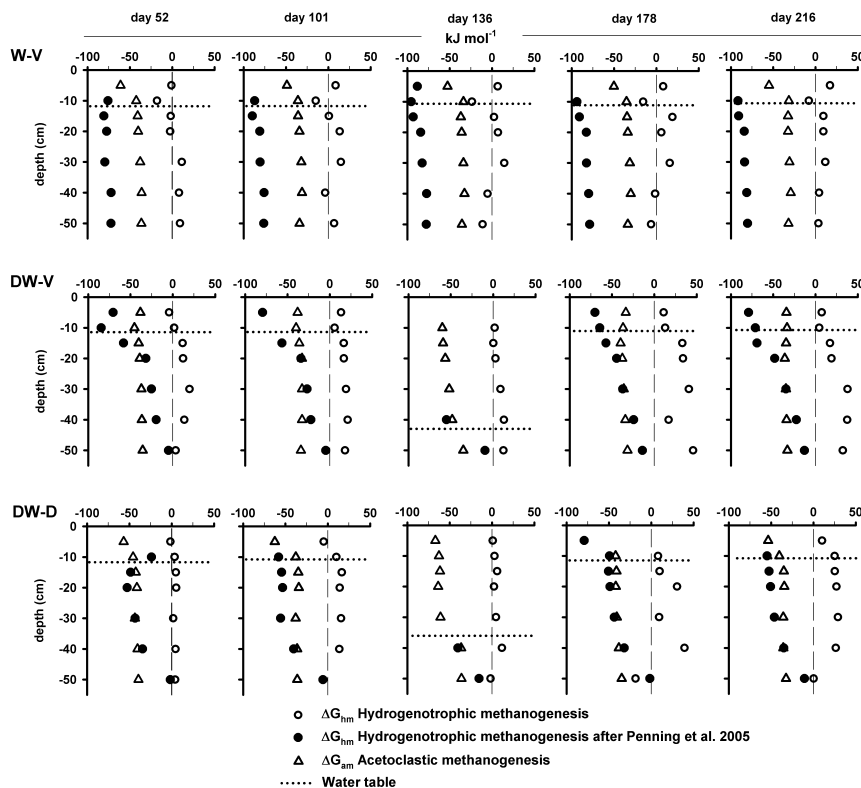
Fig. 8. Values of ΔG for hydrogenotrophic (ΔG$_{hm}$) and acetoclastic methanogenesis (ΔG$_{am}$) over depth and selected time points as calculated according to the stoichiometry given in Table 1. Note that ΔG$_{am}$ is mostly negative in all treatments, i.e. energy could be gained from this process according to the thermodynamic calculations. Contrarily, ΔG$_{hm}$ is mostly positive for hydrogenotrophic methanogenesis using measured hydrogen concentrations but again negative using the fractionation factor α_C (see also Fig. 6). Further explanations see text.