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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. Peatlands contain a carbon stock of global concern and significantly contribute to the global methane burden. The impact of drought and rewetting on carbon cycling in peatland ecosystems is thus 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. Results were 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 treatment 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. This suggests 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.

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1 Introduction

Peatlands sequester carbon (C) at estimated rates of 0.074–0.094 GtC yr^{-1} while contributing approximately 2–10% to the global release of methane into the atmosphere (Bousquet et al., 2006; Mikaloff Fletcher et al., 2004). These processes are important in the global carbon cycle and sensitive to climate change, such as 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 temperatures and changes in the hydrologic regime (Moore, 2002). This may result in an increasing decomposition and an 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 effects of vegetation (Shannon and White, 1994). Understanding methane cycling and respiration pathways under changing environmental conditions is crucial 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 the depletion of electron acceptors and the 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). Some methanogens were suggested 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). Methane produced by acetate cleavage was found to be not as depleted in ^{13}C as CH_4 produced from CO_2 reduction with H_2. Fractionation factors for acetoclastic methanogenesis range from 1.000–1.032. while fractionation factors of hydrogenotrophic methanogenesis range from 1.045–1.082 (Conrad, 2005; 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 and the lower profile by hydrogenotrophic methanogenesis (Hornibrook et al., 2000a; Popp et al., 1999). A smaller depletion of ^{13}C in 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 mostly 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, controlling 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, water table position, and vegetation have been identified (e.g. Granberg et al., 1997; Strom et al., 2003; Roulet et al., 1992; Updegraff et al., 2001) but the impact of short term disturbances is still uncertain. This research deficiency is addressed in this study by analyzing CO_2 and CH_4 dynamics as well as 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 the presented effects occur. To this end we used intact peat monoliths (mesocosms), allowing 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. A simulated drought was expected to result in prolonged periods of low or absent methane production 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 and (vi) thermodynamic calculations.

2 Material and methods

2.1 Treatments and sampling

Three intact peat cores ("mesocosms"), with a diameter of 60 cm and a depth of 60 cm each, were collected in September 2005 at the Schlöppnerbrunnen fen site in northeastern Bavaria (50°08′38″ N, 11°51′41″ E, Fichtelgebirge, Germany). The site can be described as an acidic (pH 3.5–4.5), minerotrophic fen with highly decomposed peat soils rich in sulphur and iron. The mean water table level is located at 19±22 cm below surface (Paul et al., 2006; Knorr et al., 2008). The mesocosms were incubated in the laboratory for ∼300 days in a climate chamber at 15°C (∼60% rH, 12 h light/dark cycles, 660 μmol s^{-1} photosynthetic photon flux). The vegetation was left intact in two mesocosms. One mesocosm was kept wet at a 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 was kept devoid of vegetation ("drying/wetting-defoliated" or "DW-D") to study vegetation effects.

The vegetation on DW-V, and prior to defoliation also on DW-D, mainly comprised *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 *Carex rostrata* occurred. The permanently wet conditions presumably promoted the predominance of *Carex* in W-V with increasing incubation time, thus an increasing effect of *Carex* on soil processes is probable.

After 40 days with a water table of about 30 cm below surface (phase I), the water table of all mesocosms was adjusted to 10 cm below surface. To this end, 30 (DW-V, DW-D) or 40 mm (W-V) of irrigation were applied within two days, until the water table level was reached. 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 50 days, the water table dropped to approximately 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, the water table was rapidly raised 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+/−1.8 (DW-V) or 9.8+/−1.8 cm (DW-D) below surface until the end of the experiment.

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

In the drought phase (III), just before rewetting, maximum VGCs in the treatment DW-V reached 12, 6 and 2% in depths of 10, 20 and 30 cm. Only three days after readjusting the high water table, VGCs again decreased to 2–3%. In the treatment DW-D, VGCs of 12, 13 and 9% in 10, 20 and 30 cm in depth, respectively, were measured. Approximately 30 days after rewetting, VGCs decreased to below 4% in this treatment. 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. This was a value typically observed at the uppermost sensor in 10 cm when the water table was 5 cm below that sensor, i.e. at 15 cm depth. It has to be noted that a VGC of 1% would halve and a VGC of 3% double calculated fluxes at the surface, but leaving general trends of changes in turnover unaffected.

The irrigation water was prepared according to field measurements (Lischeid, personal communication) and was evenly distributed using a sprinkler. 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^+, NO_3^- (40 μmol L^{-1}) and DIC (∼15 μmol L^{-1}). Sulphuric acid was used to adjust the pH to ∼4.8 (included in SO_4^{2-} concentration). The contribution of the irrigation water to electron acceptors in the peat was calculated to be negligible (<1%).

Soil solution was sampled, at least weekly, from Rhizon® samplers (microporous polymer, <0.2 μm pore size, fibre glass support) at 5, 10, 15, 20, 30, 40 and 50 cm depth. As measurement of dissolved gases from suction samplers may be biased due to under-pressure derived degassing, soil gases were sampled from horizontally inserted silicon tubes at the same spatial and temporal resolution as the solutes. With this passive diffusion technique, the gas phase in equilibrium with the solution is measured; thus it can be applied in saturated and unsaturated soil (Kammann et al., 2001). Due to the short equilibration time (5–50 h), isotope fractionation through the samplers can be expected to be negligible. Methane emission from the mesocosms was measured weekly in duplicate, using shrouded chambers on previously inserted collars of 20 cm in diameter (one collar per treatment). Five to eight 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). A schematic drawing of the mesocosms is provided in Fig. 1.

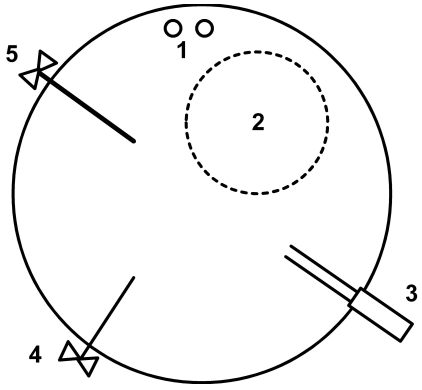

Fig. 1. Schematic drawing of a mesocosm (top view, 60 cm total diameter). 1: piezometers for water table levels (2.5 cm diam.); 2: collar for methane surface flux measurements (20 cm diam.); 3: TDR soil moisture probes (length of rods ∼15 cm) at 10, 20, 30, and 40 cm depth; 4: Rhizon® soil solution samplers (10 cm length) at 5, 10, 15, 20, 30, 40, and 50 cm depth; 5: Passive diffusion gas samplers (silicon tubes of 20 cm length) at same depths as Rhizons®.

At the end of the incubation, a ^{13}C-CO_2 pulse label was applied to each mesocosm to identify the zone of main root activity in the soil. A transparent chamber was placed on each mesocosm and a ∼900 ppm, ∼63% ^{13}C-CO_2 atmosphere was adjusted by dissolving 250 mg of 95% ^{13}C Na_2CO_3 in 6N HCl and manual mixing of the gas phase. Each mesocosm was exposed to the label for 60 min. Subsequently, the label was traced in the upper soil gas for the following 90 h. Stable isotopic composition was analyzed as outlined below.

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

2.2 Analytical techniques

Concentrations of CO_2 and CH_4 in gas samples were measured with a SRI 8610C gas chromatograph, equipped with FID and a CO_2 methanizer. Hydrogen was analyzed on a TA 3000 H_2-analyzer (Trace Analytical). Stable C isotope measurements of CO_2 and CH_4 were performed using a GC-Combustion-Isotope ratio mass spectrometer (GC-C-IRMS, 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. Isotopic signatures were expressed in the common δ-notation in ‰ versus the VPDB-standard (Eq. 1).

$$\delta = \left[\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right] \times 1000 \quad (1)$$

The $\delta^{13}C$ measurements were calibrated twice a day, using $NaCO_3$, with a known isotopic 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‰). For multiple measurements of CO_2 and CH_4, the standard deviation was below 0.5‰. For CH_4 samples with a very low isotopic signature of −80 to −110‰ a standard deviation of ∼2.5‰ was observed.

Carbon and nitrogen content along with the isotopic 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, the pH was determined using a glass electrode (WTW), and the levels of acetate were measured using a GC equipped with FID (Varian).

2.3 Calculations

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_{CO_2}=0.0463 mol L^{-1} atm^{-1}, K_{CH_4}=0.0017 mol L^{-1} atm^{-1}). DIC speciation was calculated using pH values obtained from Rhizon® samples and equilibrium constants 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).

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

The variable R_N is defined as the net turnover rate of a species A (nmol cm^{-3} d^{-1}), with $\Delta S_A/\Delta t$ representing 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 right 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 the time intervals between samplings were obtained by calculating the mean 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 a 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, an apparent isotope fractionation factor α_C between CO_2 and CH_4 was calculated, using Eq. (3) (Conrad, 2005; Whiticar, 1999).

$$\alpha_C = \frac{\delta^{13}C_{CO_2} + 1000}{\delta^{13}C_{CH_4} + 1000} \quad (3)$$

Assuming there was no significant fractionation during breakdown of organic matter (Boehme et al., 1996) and no carbon losses from the system except from CO_2 and CH_4, an isotope mass balance for different soil layers was calculated (Eq. 4). With this data and using methane fluxes from chamber measurements, an anaerobically generated CO_2 flux was calculated (Eqs. 5 and 6) (Lansdown et al., 1992). This approach was compared to anaerobic CO_2 production estimated from CO_2 evolvement in levels below the water table. As a result, a range of estimates of the effect of drought and rewetting on anaerobic respiration was obtained.

$$C_{\text{tot}} \cdot R_{\text{OM}} = C_{CO_2} \cdot R_{CO_2} + C_{CH_4} \cdot R_{CH_4} \quad (4)$$

$$F_{\text{tot}} = F_{CO_2} + F_{CH_4} \quad (5)$$

$$F_{\text{tot}} \cdot R_{\text{OM}} = F_{CO_2} \cdot R_{CO_2} + F_{CH_4} \cdot R_{CH_4} \quad (6)$$

Respectively, C_{CO_2} and C_{CH_4} represent the concentrations of CO_2 and CH_4, and R_{CO_2}, R_{CH_4} and R_{OM} represent the isotope ratios of CO_2, CH_4, and the soil organic matter. C_{tot} equals the measured sum of the assumed mineralization end products CO_2 and CH_4. The terms F_{CO_2}, and F_{CH_4} are the diffusive fluxes of CO_2 and CH_4, resulting in F_{tot}, the total diffusive C flux.

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

$$U_{CO_2} = \frac{\Delta \left[{}^{13}C\right]_{\text{soil}}}{\Delta t \cdot f({}^{13}C)_{\text{label}} \cdot A_{\text{mesocosm}}} \quad (7)$$

The term $\Delta[^{13}C]_{\text{soil}}$ is the change in ^{13}C content in the total soil CO_2, Δt is the time interval of labelling (1 h), $f(^{13}C)_{\text{label}}$ is the fraction of ^{13}C in the total labelling gas phase (62.9 %) and A_{mesocosm} is the area of the mesocosm in m^2. This results in a rate of uptake of CO_2, U_{CO_2}, in mmol m^{-2} h^{-1}.

The thermodynamic energy yield from hydrogenotrophic and acetoclastic methanogenesis, as well as from homoacetogenesis, was calculated using the reactions given in Table 1 (Eqs. 8–10). Thermodynamic data was taken from Nordstrom and Munoz (1994) along with measured concentrations of CH_4, CO_2, acetate and H_2.

As hydrogen measurements in environmental samples may be biased by clustered distribution of hydrogen producers and consumers (Hoehler et al., 2001), 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), was applied (Eq. 11).

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

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

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:	$CO_2(aq) + 4H_2(aq) \rightarrow CH_4(aq) + 2H_2O(l)$	ΔG_{hm}=−194.3	(8)
Acetoclastic methanogenesis:	$CH_3COO^-(aq) + H^+(aq) \rightarrow CO_2(aq) + CH_4(aq)$	ΔG_{am}=−49.8	(9)
Homoacetogenesis	$2\ CO_2(aq) + 4H_2(aq) \rightarrow CH_3COO^-(aq) + 2H_2O(l) + H^+(aq)$	ΔG_{ha}=−144.5	(10)

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)

3 Results

3.1 Solid phase data

Soil carbon content (w/w) varied with 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 level of carbon content in the upper profile was similar among treatments, treatment DW-V contained less carbon below 25 cm depth than W-V and DW-D.

The measured δ^{13}C in the total soil organic matter of the top soil ranged from −27.1‰ (DW-D) to −27.7‰ (DW-V) (Table 2). In DW-V and DW-D, δ^{13}C values decreased to −27.9 and −28.3‰, respectively. Maximum values of −26.8 to −27.3‰ occurred in ∼10–15 cm depth.

3.2 Concentration and isotope signature of dissolved CO_2 (DIC) and CH_4

At a constantly high water table in the wet treatment W-V, concentrations of DIC increased from below 0.5 mmol L^{-1} for about 140 days to levels of 1–2 mmol L^{-1} in the unsaturated zone and up to 7.6 mmol L^{-1} at 30 cm depth. In the treatments DW-V and DW-D, the highest DIC concentrations occurred just below the water table and just prior to the beginning of the drought phase. The concentrations reached 4.5 mmol L^{-1} around day 100 in 15 cm depth in DW-V, and 3.5 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 thereafter (DIC data not shown).

Values of δ^{13}C of dissolved CO_2 ($\delta^{13}C_{CO_2}$) showed a similar pattern in all mesocosms (Fig. 2). Values of −26 to −27.5‰ occurred in the upper profile or shortly after

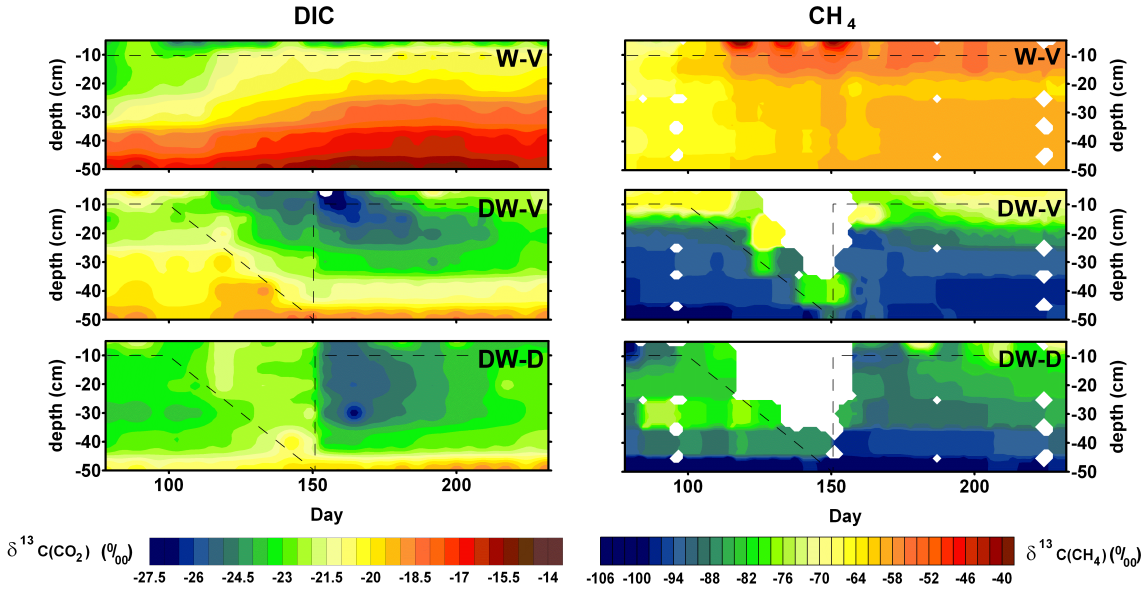

Fig. 2. Values of δ^{13}C of CO_2 (left) and δ^{13}C of CH_4 (right) (‰ vs. V-PDB) measured in the soil gas phase (saturated and unsaturated) of W-V (top), DW-V (middle) and DW-D (bottom). Colour scales are similar for all treatments. Dashed lines depict the approximate water table. For corresponding CO_2 concentrations, see text, for CH_4 concentrations see Fig. 3. If no isotopic signature could be determined for methane due to low concentrations, the points were left white.

rewetting, and maximum values of −18 to −14‰ occurred below 30 cm depth, particularly in the permanently wet treatment. A smaller maximum of $\delta^{13}C_{CO_2}$ occurred around 5 cm depth in DW-V during wet conditions. Only after rewetting did $\delta^{13}C_{CO_2}$ approximately match δ^{13}C measured in the soil solid phase ($\delta^{13}C_{OM}$). Drying and rewetting thus decreased $\delta^{13}C_{CO_2}$ in the soil DIC pool.

Concentrations of CH_4 peaked at 460 μmol L^{-1} at 50 cm depth in W-V, 150 μmol L^{-1} at 30 cm depth in DW-V and 100 μmol L^{-1} at 50 cm depth in DW-D (Fig. 3). In both mesoscocms with vegetation a secondary concentration maximum of 50–150 μmol L^{-1}in W-V and 40–100 μmol L^{-1} in DW-V (phases II and IV) occurred at (W-V) or above (DW-V) the water table. This depth segment was densely rooted and showed the strongest changes in $\delta^{13}C_{CO_2}$ and $\delta^{13}C_{CH_4}$ following the ^{13}C-CO_2 labelling pulse (see below). During water table drawdown, CH_4 concentrations strongly diminished in the newly unsaturated peat. Methane pools were restored following rewetting within approximately 40 (DW-V) and 50 (DW-D) days (Fig. 3). In the densely rooted upper 10 cm of the DW-V treatment, methanogenesis re-established more rapidly within 10 days.

The $\delta^{13}C_{CH_4}$ 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. 2). In DW-V, values of −65 to −75‰ were higher in the upper 15 cm. The carbon isotopic composition of methane in W-V differed substantially, as $\delta^{13}C_{CH_4}$ 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 $\delta^{13}C_{CH_4}$ in DW-V and DW-D (Fig. 2). The methanotrophic zone migrated downwards with the declining water table level because $\delta^{13}C_{CH_4}$ increased by ∼10–20‰ in DW-V and ∼5–10 in DW-D when the water table passed. After rewetting, methane in DW-D had again a consistently higher $\delta^{13}C_{CH_4}$ than in DW-V in the upper 30 cm but in each treatment values were similar as before drying. In terms of $\delta^{13}C_{CH_4}$, the predominating CH_4 production pathway was thus not affected by drying and rewetting.

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. Values of $\delta^{13}C_{CO_2}$ changed up to 3‰ in DW-V and 8‰ in W-V, compared to before labelling (Fig. 4). Also considering the shifts in $\delta^{13}C_{CH_4}$, 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. Within 90 hours, 1.3 and 1.7% of the incorporated label had already been transformed into methane in W-V and DW-V. In summary, a given 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_{CO_2} of 0.00, 0.67 and 1.80 mmol C m^{-2} d^{-1} for DWD, DW-V and W-V.

3.3 Methane efflux and turnover

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

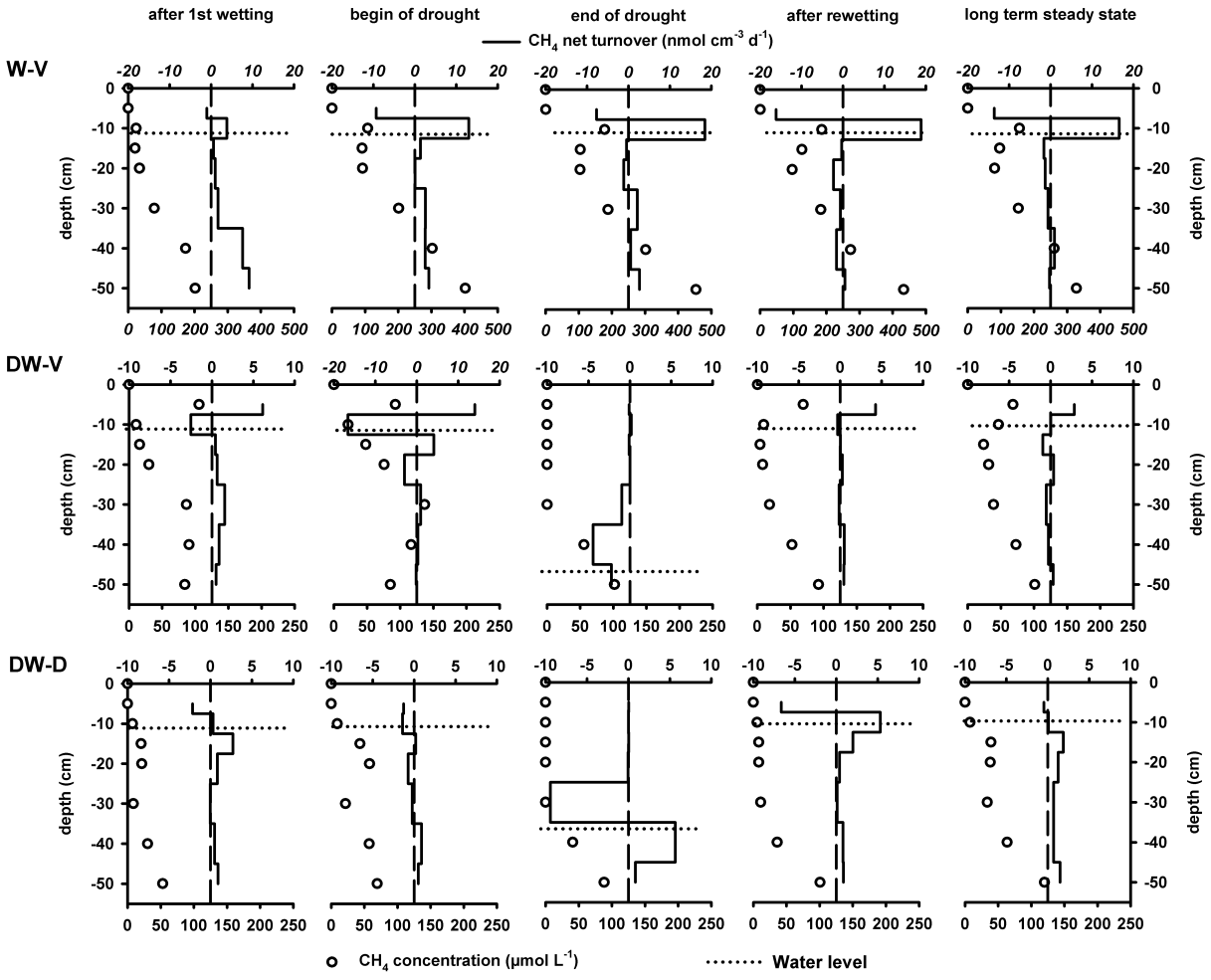

Fig. 3. Concentrations (lower x-axis), and calculated net turnover rates (upper x-axis) of CH_4 in the three treatments W-V, DW-V, and DW-D. Corresponding days are day 64 (after first wetting), day 108 (begin of drought), day 141 (end of drought), day 176 (after rewetting), and day 211 (long term steady state). Different turnover and concentration scales on the x-axis are indicated by letters in italic. For calculation of turnover rates, see methods section.

second half of the experiment (Fig. 5). These fluxes were maintained despite decreasing soil water concentrations 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}).

Under wet conditions in W-V, calculated methane net turnover (Fig. 3) reached 2 to 8 nmol cm^{-3} d^{-1} and peaked at the depth where the water table was located. 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 a high water table. This coincided with a local maximum in $\delta^{13}C_{CO_2}$, suggesting CO_2 to be the precursor. A second 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 and followed the water table downward in DW-V and DW-D. Negative turnover rates occurred above the water table. This may, however, not be solely interpreted as methane oxidation, as also degassing from previously stored pools contributes to turnover using the mass balance approach. Therefore these numbers will not be discussed. After rewetting, methane production rebounded 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.

3.4 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, with isotopic signatures of -21.8 ± 9.3‰ (W-V),

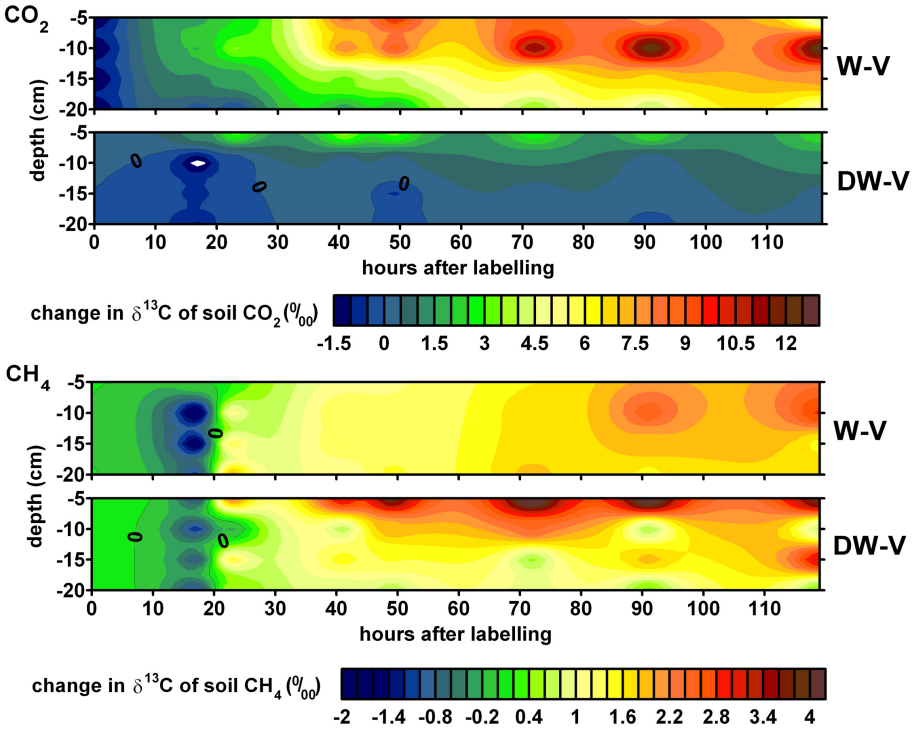

Fig. 4. Absolute changes in δ^{13}C (‰ vs. V-PDB) of soil CO_2 and CH_4 in the vegetated wet treatment W-V and drying/wetting treatment DW-V after application of the ^{13}C-CO_2 pulse label (time=0 h).

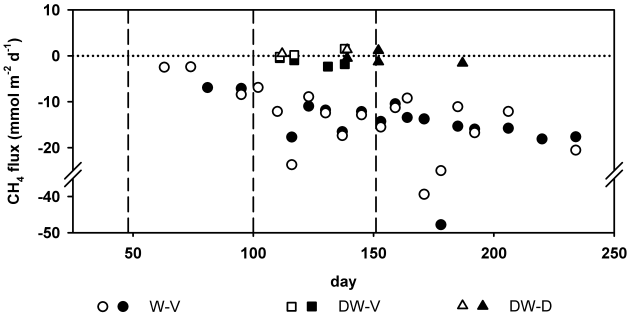

Fig. 5. Methane exchange of W-V, DW-V and DW-D measured with static chambers. Open and solid symbols denote two replicate measurements in one collar per treatment. Fluxes were calculated from concentration over time through linear regression (r^2>0.9). Vertical dashed lines separate the different phases (I: initial dry, II: first wet, III: dry and IV: rewetted phase).

−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, a suppression of methanogenic activity, leading to less residual ^{13}C enrichment in the released CO_2 is supported.

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‰, which was thus comparable to values observed in W-V. During the dry phase, treatment DW-V emitted CH_4 with lower δ^{13}C values, a probable cause being the release of previously stored highly ^{13}C depleted CH_4. After rewetting, the treatments W-V and DW-V again emitted 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 isotopic balance, however, these ratios were much smaller, i.e. 5.4 (W-V), 9.7 (DW-V) and 7.2 (DW-D). 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 Eq. (4)–(6), the contribution of anaerobic respiration to CO_2 fluxes was 64.0 (W-V), 12.8 (DW-V) and 9.8 mmol m^{-2} d^{-1} (DW-D). These fluxes compare to a measured soil CO_2 flux in DW-D of 94 mmol m^{-2} d^{-1}. The mentioned fluxes from concentration gradients and isotope mass balances were taken as lower and upper estimate of anaerobic CO_2 fluxes, and the 94 mmol m^{-2} d^{-1} CO_2 flux of DW-D was used as the total soil CO_2 flux reference for all treatments. With these assumptions the aerobic CO_2 fluxes

from the soil accounted for 32–96% (W-V), 86–99% (DW-V), and 89–92% (DW-D) of the total CO_2 flux.

3.5 Isotope ratio cross plot and apparent fractionation factors

As depicted in the isotope ratio cross-plot (Fig. 6) for DW-V and DW-D, most $\delta^{13}C_{CH_4}$ and $\delta^{13}C_{CO_2}$ 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 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 of α_C=1.055 and α_C=1.04. Above the water table $\alpha_C<1.04$ was calculated (open circles). An increasing contribution of acetoclastic methanogenesis or methanotrophy thus seemed likely (Fig. 6).

3.6 Concentrations of acetate and hydrogen and thermodynamic calculations

Acetate concentrations generally ranged from 50 to 100 μmol L^{-1} (Fig. 7) but increased to about 300–350 μmol L^{-1} in the unsaturated peat of DW-V and DW-D prior to rewetting. Subsequently, acetate concentrations decreased below 50 μmol L^{-1} and finally readjusted to pre-drought levels in about 30 days. Concentrations were higher in W-V, especially in 5–10 cm and 50 cm depth, where concentrations often exceeded 350 μmol L^{-1}.

Hydrogen concentrations were mostly below 1 nmol L^{-1} (Fig. 7). In W-V and DW-V, concentration reached 2.5–5 nmol L^{-1} at 5–10 cm depth during wet periods. The concentration maximum of H_2 thus coincided with a maximum in 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 the maximum in CH_4 concentrations was also measured.

The Gibbs free energy yield from hydrogenotrophic methanogenesis ΔG_{hm} was mostly positive (Fig. 8). This finding was primarily caused by low hydrogen concentrations (see Eq. 8). Concentrations of >4 nmol L^{-1} would be necessary for methanogens to gain energy. This result is an apparent contradiction to the predominance of hydrogenotrophic methanogenesis as derived from $\delta^{13}C$ 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 coinciding with a production maximum of CH_4. Acetoclastic methanogenesis (Eq. 9) 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. 10) required $9->70$ kJ mol^{-1} in all treatments. To make the process exergonic, H_2 concentrations of >50 nmol L^{-1} would have been needed.

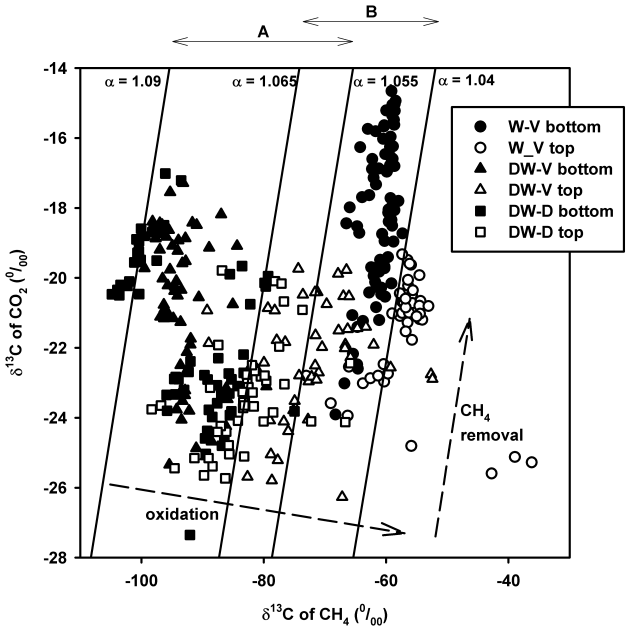

Fig. 6. Cross-plot of corresponding $\delta^{13}C_{CH_4}$ and $\delta^{13}C_{CO_2}$ values (‰) in the soil gas of the three treatments W-V, DW-V, and DW-D. Diagonal lines for different fractionation factors α_C (Whiticar, 1999; Conrad, 2005) are also given. Arrows A and B indicate ranges of fractionation factors indicative of hydrogenotrophic and acetoclastic methanogenesis, respectively. 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.

Using the relationship of ΔG_{hm} for hydrogenotrophic methanogenesis and the apparent fractionation factor α_C (Eq. 3) given in Penning et al. (2005) (Eq. 11), this process was viable in all layers where the isotopic composition of CH_4 could be quantified. Values of ΔG_{hm} shifted from positive values, as calculated using the measured H_2 concentrations, to values ranging from -2 to -80 kJ mol^{-1} H_2 following the relationship derived in Penning et al. (2005).

4 Discussion

4.1 Respiration and methanogenesis

Generally, methane concentrations measured in this study were lower than previously observed in bog mesocosms (Blodau and Moore, 2003a) but were comparable to other fen soils (Chasar et al., 2000; Smemo and Yavitt, 2006). The

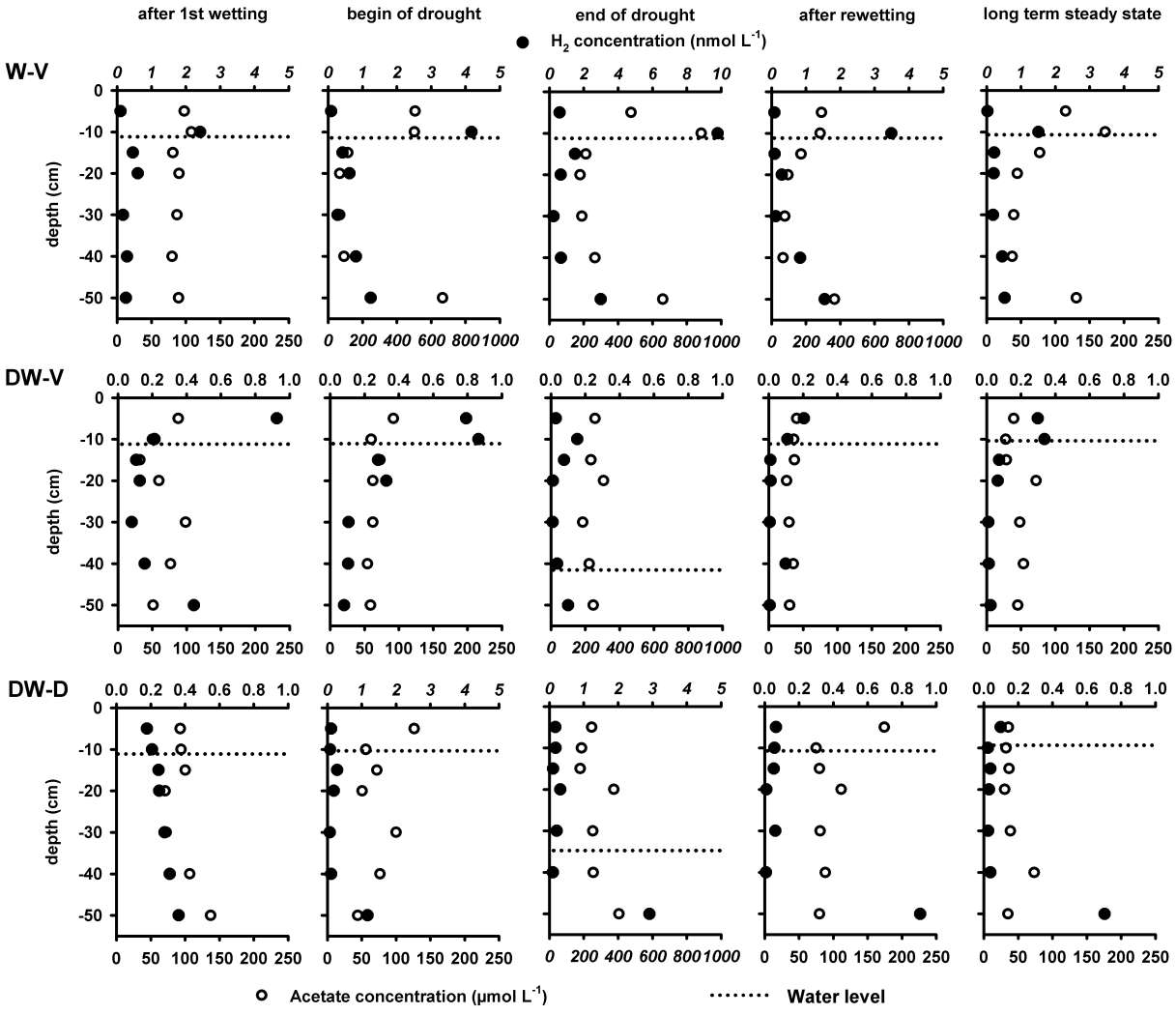

Fig. 7. Concentrations of hydrogen (upper x-axis) and acetate (lower x-axis) in the three treatments W-V, DW-V, and DW-D. Corresponding days are day 52 (after first wetting), day 101 (begin of drought), day 136 (end of drought), day 176 (after rewetting), and day 216 (long term steady state). Different concentration scales on the x-axis are indicated by letters in italic.

isotopic composition of methane in W-V was in accordance with $\delta^{13}C_{CH_4}$ reported in other studies, particularly if sedges were present. Values observed in DW-V and DW-D were considerably lighter in isotopic composition than previously reported (Chasar et al., 2000; Lansdown et al., 1992; Popp et al., 1999; Waldron et al., 1999). 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. *Carex* roots can access deeper soil layers, which may lead to CH_4 bypassing 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). Slowly declining CH_4 concentrations, during the growing season at *Carex* dominated sites, were already reported by Joabsson and Christensen (2001) who hypothesized that increased rooting would raise rates of methane oxidation and emission from the rhizosphere.

The C content of the fen soil under study was, in some parts of the profile, low compared to other organic soils (Hornibrook et al., 2000c), but the isotopic signature of the soil organic matter was more or less consistently −27‰. Only small differences in $\delta^{13}C$ in this peat suggested that the isotopic signature of CO_2 formed by respiration should not vary much with depth. Major effects on $\delta^{13}C$ in CO_2 should thus be due to methanogenic activity (Whiticar, 1999). A residual enrichment of ^{13}C in CO_2 as observed in this study is consistent with prior investigations (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). Therefore, it was frequently found that $\delta^{13}C_{CO_2}$ does

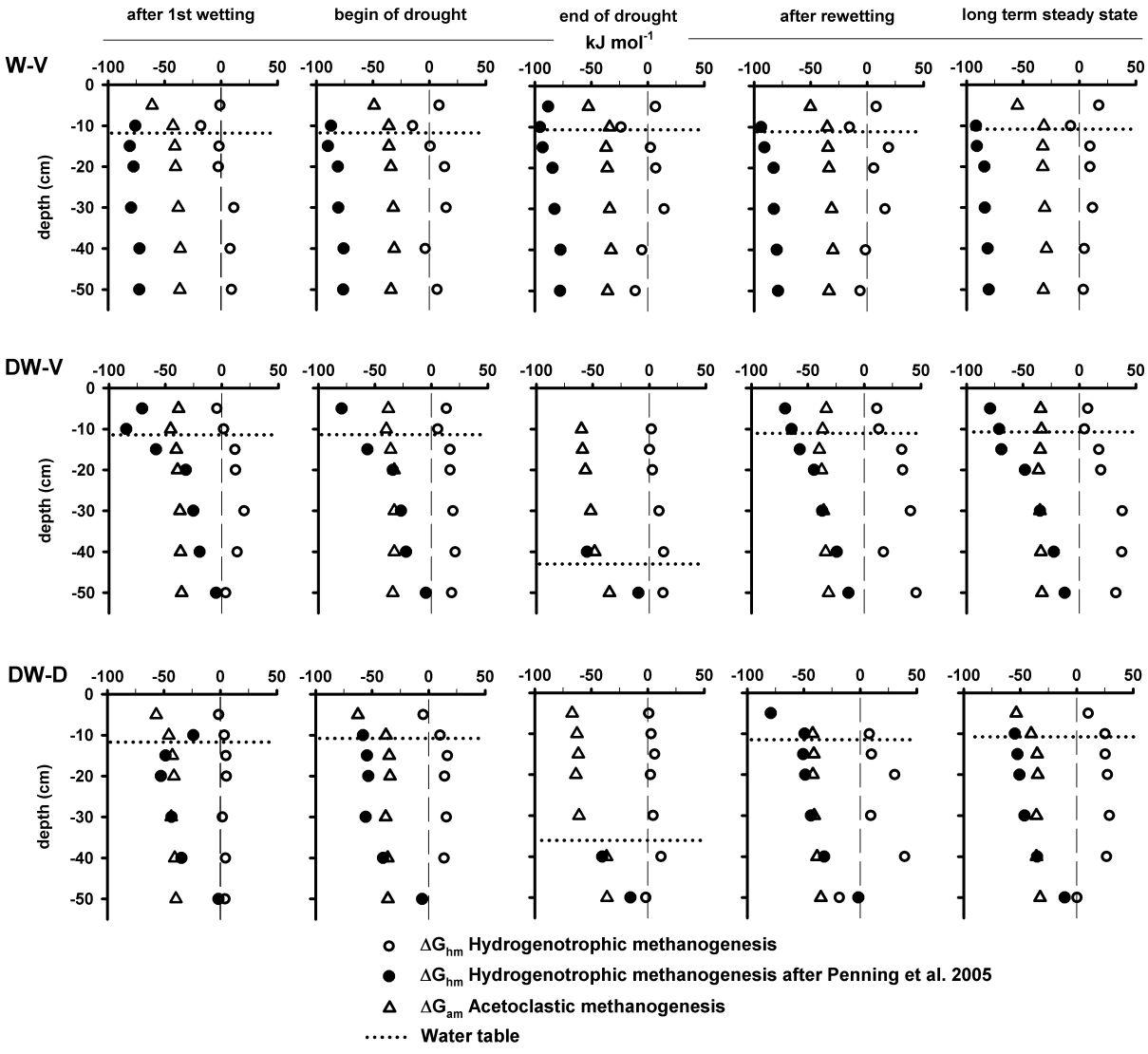

Fig. 8. Values of ΔG for hydrogenotrophic (ΔG_{hm}) and acetoclastic methanogenesis (ΔG_{am}) over depth and selected time points as calculated according to the stoichiometry given in Table 1. Corresponding days are day 52 (after first wetting), day 101 (begin of drought), day 136 (end of drought), day 176 (after rewetting), and day 216 (long term steady state). 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.

not match δ^{13}C of the solid phase (Hornibrook et al., 2000a; Waldron et al., 1999). At greater depths in W-V, $\delta^{13}C_{CO_2}$ reached values of around −15‰ compared to a $\delta^{13}C_{SOM}$ of −28‰. The diffusive CO_2 fluxes from below the water table were also considerably less depleted in ^{13}C (−20 to −23‰) than the soil organic matter and in contrast to the lighter values reported by Lansdown et al. (1992).

Following the ^{13}C-CO_2 label experiment, the signal was quickly transferred into $\delta^{13}C_{CO_2}$ within the soil, in a period of 12 hours. Although less than one percent of the tracer amount had been taken up, calculated rates of CO_2 incorporation were 0.7–1.8 mmol C m^{-2} d^{-1} under vegetation, and thus in the same range as reported for arctic wet sedge tundra (King and Reeburgh, 2002). The labelling experiment demonstrated that in our peat, the rhizosphere associated respiration was mainly limited to the upper 10–20 cm. Fresh organic matter input through plants may therefore fuel anaerobic microbial activity in these layers to a great extent, as proposed by Coles and Yavitt (2004). Accordingly, changes in δ^{13}C of the soil CH_4 pool were detected after approximately 24 h. Within 90 h, about 1.3 to 1.7% of the label that had been taken up had been transformed into methane. Thus,

in the studied mesocosms, recent photosynthetates and root associated CO_2 may contribute considerably to CH_4 production, coinciding with previous studies in which methanogenesis was found to depend on input of fresh and labile carbon compounds provided by vegetation (Whiting and Chanton, 1993; Popp et al., 1999). According to Chimner and Cooper (2003), one may thus expect that for the peat used in this study manipulating the water table would have most impact on soil respiration when manipulated within the range of the most active surficial zone. Interestingly, the CO_2 incorporation was the same order of magnitude as the depth integrated CH_4 production in the upper 20 cm. It is plausible to 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.

4.2 Impact of drying and rewetting on methane dynamics and isotopic composition of CO_2 and CH_4

The drying/rewetting cycle had substantial effects on methane production and dynamics in the studied mesocosms, as was expected from previous work (Aerts and Ludwig, 1997; Blodau and Moore, 2003a; Shannon and White, 1994; Updegraff et al., 2001). Drought successfully suppressed methanogenic activity. This suppressive effect persisted on a time scale of days to weeks after wetting, with response times depending on depth. In this study, experimental drought lowered the water table by 30 - 40 cm. This treatments closely resembles natural patterns observed in the field site (Paul et al., 2006). VGCs of up to >12% were considered high, as 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 observed effects are relevant on the field scale. A direct extrapolation of the results to the field is, however, limited by the higher incubation temperature and the absence of advective flow in the mesocosms.

During dry phases in DW-V and DW-D, methane concentrations rapidly decreased with the peat becoming unsaturated. After rewetting, methane production was retarded, likely because electron acceptors were preferentially used for respiration (Peters and Conrad, 1996; Roden and Wetzel, 1996). Methane concentrations in the lower profile gradually increased after rewetting. In subsequent days, the concentrations increased more rapidly in the shallow and rooted peat of DW-V. Thus, methanogenesis recovered more quickly in this case in comparison with the mesocosm experiments with peat from a dry, ombrotrophic bog (Blodau and Moore, 2003a). In this study, methane was even produced above the water table (Knorr et al., 2008). This rapid production of methane at shallow depths of DW-V coincided with the results from the labelling experiment. Methane production above the water table has so far only been documented with respect to potential methane production in laboratory incubations (Coles and Yavitt, 2004), but this study illustrated the possible detection of methane production above the water table in intact soils.

Concerning the temporal dynamics of $\delta^{13}C_{CO_2}$, 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, thus differing substantially from the isotopic composition before drought. Upon interpretation, the suggested origin of this result is caused by the drought-induced, 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 $\delta^{13}C_{CO_2}$ was temporarily suppressed, and $\delta^{13}C_{CO_2}$ approached the isotopic signature of the solid phase. It is plausible that the elevated acetate concentrations had contributed to this post-rewetting respiration pulse, as this is a commonly used substrate (Achtnich et al., 1995). These results further 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.

As the zone of higher $\delta^{13}C_{CH_4}$ values closely followed the water table drawdown and re-elevation, this ^{13}C enrichment in the CH_4 pool is suggested, to a great extent, to be 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 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.

The defoliated treatment DW-D had lowest observed $\delta^{13}C$ in the CH_4 diffusive flux. This number reflected the strongly ^{13}C-depleted methane from bottom layers. Treatment DW-V and especially W-V emitted methane less depleted in ^{13}C. This methane was near the surface presumably produced from fresh plant material as according to Popp et al. (1999), at non-vegetated sites methane was found to be more depleted in ^{13}C than at vegetated sites and the authors attributed this 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. Probably, because roots did not penetrate below 15 cm in DW-V, a lower contribution of fresh plant derived compounds may have caused methane to be

produced at lower rates and to have a different isotopic signature in the lower profile.

4.3 Impact of drying and rewetting on anaerobic and aerobic respiration

Ratios of CO_2/CH_4 of diffusive fluxes were high compared to other studies in methanogenic environments (e.g. 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 shifting respiratory activity away from methanogenesis as found in previous work (Achtnich et al., 1995; Dettling et al., 2006). Calculated from the isotope mass balance (Eqs. 4–6), these numbers were much smaller, however, 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 and that produced under 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, an anaerobic CO_2 flux of 64 mmol m^{-2} d^{-1} for this treatment was calculated. This flux was much higher than that reported for a bog in the study of Lansdown et al. (1992). Assuming CH_4 fluxes at the detection limit of our chamber technique, calculated anaerobic CO_2 fluxes for DW-V and DW-D of 10–13 mmol m^{-2} d^{-1} would approach the numbers calculated by Lansdown et al. (1992), although the values would be higher by a factor of 2–4. This may be due to the higher temperature used for incubation, in comparison to field site temperatures. There were obviously too many differences among the mesocosms, such as in the vegetation, which probably obscured increased anaerobic respiration due to the drought and subsequent rewetting. We speculate that fresh carbon and electron accepting capacity input at greater depths through *Carex* roots in W-V may have contributed to this exceptionally high anaerobic CO_2 production under constantly wet conditions.

Minding uncertainty due to a lack of replicates, one may furthermore assume the non-vegetated treatment to represent soil respiration for the other treatments. This estimate allowed calculating 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 somewhat speculative, these numbers supported the importance of the few centimetres of aerobic layer above the water table of similar fen sites. This layer consisted of most easily degradable fresh organic carbon (Chimner and Cooper, 2003; Coles and Yavitt, 2004) and thus supports high rates of respiration (Knorr et al., 2008).

4.4 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 previous studies (Conrad, 2005; Whiticar, 1999), and therefore suggest that CH_4 was, to a great extent, 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, which resulted in low hydrogen concentrations due to the presence of other electron acceptors in the bulk peat.

Most α_C values calculated for levels above the water table were in an overlap range of α_C from hydrogenotrophic and acetoclastic methanogenesis (Whiticar, 1999; Chasar et al., 2000), and most values of α_C for the latter pathway summarized by Conrad (2005) were still lower. A $\delta^{13}C_{CH_4}$ of approximately -70‰ of the methane formed in the shallow depths of DW-V and α_C=1.05–1.07 thus supported that the methane to a great extent was formed by hydrogenotrophic methanogens and not by acetotrophs (Whiticar, 1999). This is in contrast to prior studies, reporting a predominance of acetotrophs in shallow peats (Chasar et al., 2000; Popp et al., 1999; Hornibrook et al., 2000a). Predominance of hydrogenotrophs would further be supported by higher H_2 concentrations at shallow depths in this treatment. Methanotrophic activity at the aerobic/anaerobic interface may have shifted $\delta^{13}C_{CH_4}$ to less negative values as observed in greater depths during drought (Whiticar, 1999). This would along with the net turnover calculations explain why methane efflux could not be measured in this study.

After rewetting of DW-V and DW-D, as soon as methane concentrations were high enough to measure the isotopic composition, high α_C values similar to that which were observed before the drought period were found. Drying and rewetting thus did not shift methanogenesis away from CO_2-reduction, as this would have been indicated by lower apparent fractionation factors α_C (Whiticar, 1999; Conrad, 2005). The inverse pattern of $\delta^{13}C_{CO_2}$ and $\delta^{13}C_{CH_4}$, referring to an enrichment of ^{13}C in CO_2 in zones of production of CH_4 poor in ^{13}C, therefore suggests that in this peat hydrogenotrophic methanogens dominated also under transient conditions of soil moisture. Up to now it has been speculated that the predominance of acetoclastic methanogens in surficial peat may be related to the temporary occurrence of aerated conditions (Hornibrook et al., 2000a; Popp et al., 1999). Due to the high water content in the upper profile, even at a water table of 50 cm below surface, one may assume that the aeration of the peat was still poor (Lafleur et al., 2005). Thus anoxic microenvironments likely provided a suitable habitat for methanogens during drought (Wachinger et al., 2000). Furthermore, some hydrogenotrophs were demonstrated to have a capacity for iron reduction and had possibly shifted

their metabolic pathway (van Bodegom et al., 2004).

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. Considering the results mentioned above and the postulates of Penning et al. (2005), it is still reasonable to assume CO_2 as the precursor of CH_4 in the peat. Only in the permanently wet treatment W-V acetoclastic methanogenesis may have been more important. Strongly negative values calculated for 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 concentrations of hydrogen. 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 (Hoehler et al., 2001).

Hydrogen measurements serve as an indicator on the scale of the measuring device. Larger sampling devices may thus reflect hydrogen concentrations, which are not representative for processes occurring in microenvironments (Hoehler et al., 2001). Minding the results from mass balance considerations, i.e. methane production at considerable rates, measured hydrogen concentrations were likely underestimated by about two orders of magnitude. 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 this point cannot be clarified without further analysis of e.g. hydrogen isotopes or isotope analysis of acetate (Conrad, 2005), a dominance of acetoclastic methanogenesis from our point of view seems unlikely. Such high values of α_C as observed in DW-V and DW-D have never been reported for acetoclastic methanogens 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 likely. Discrepancies in results derived from thermodynamic calculations and isotope fractionation factors may eventually be used to study biogeochemical heterogeneity in wetland soils.

A process combination that also needs to be mentioned with regard to closing isotope mass balances (Hornibrook et al., 2000b) is the conversion of CO_2 to acetate (homoacetogenesis) followed by disproportionating acetate into CO_2 and methane (acetoclastic methanogenesis). This combination seems unlikely to be important, however, as ΔG_{ha} for homoacetogenesis was always positive. For homoacetogenesis to become viable, even higher H_2 concentrations of $>50\,\mathrm{nmol\,L^{-1}}$ would have been needed. The arguments about thermodynamic calculations and soil heterogeneity also apply in this case, though.

The observed range of fractionation factors in the wet treatment W-V would lead to the conclusion that a significant part of methane was produced via acetoclastic methanogenesis. On the basis of the comprehensive data set, however, we did not follow this interpretation of values of α_C. Due to the inverse pattern of $\delta^{13}C_{CO_2}$ and $\delta^{13}C_{CH_4}$, also in this case, and isotope mass balance considerations, a dominant contribution of hydrogenotrophic methanogens must have occurred. Additionally, values of α_C were still in the overlap range of fractionation factors from both processes (Whiticar, 1999; Conrad, 2005). The measured $\delta^{13}C_{CH_4}$ values also coincide well with data from other fens where *Carex* species were found (Chasar et al., 2000; Popp et al., 1999), as was the case in W-V.

The apparently low fractionation in the W-V treatment was, in our opinion, 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 become clear that *Carex* roots had grown throughout the mesocosm down to 60 cm. The effects of *Carex* roots are represented in the isotope ratio cross-plot (Fig. 6). The arrow shifting $\delta^{13}C_{CH_4}$ towards less negative values, but correspondingly decreasing $\delta^{13}C_{CO_2}$ 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 $\delta^{13}C_{CH_4}-\delta^{13}C_{CO_2}$ pairs along the lines of constant α_C towards both less negative $\delta^{13}C_{CH_4}$ and $\delta^{13}C_{CO_2}$, 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 has already been described for lake sediments, where the lighter isotope tends to escape from methanogenic sediments by ebullition (Gu et al., 2004). Transport through roots can cause the same effect.

5 Conclusions

A number of key patterns of respiration and responses to drying and rewetting in a fen soil could be identified. Estimating heterotrophic respiration from the defoliated treatment, aerobic CO_2 production could be calculated via isotope balancing. Based on this approach, aerobic respiration predominated overall C fluxes even when the unsaturated zone was shallow. This finding is in agreement with earlier work suggesting a very small contribution of anaerobic respiration to C fluxes in a bog ecosystem and the investigated fen (Blodau et al., 2007; Knorr et al., 2008). Methanogenesis in the

soils was dominated by the hydrogenotrophic pathway according to isotopic fractionation factors and must have occurred in microenvironments, as in most of the peat matrix hydrogen concentrations were too low to support this process thermodynamically. According to this finding, similar peat can probably be conceptualized as a mosaic of environments that, as a whole, can sustain different anaerobic respiration processes under conditions that appear adverse on the soil horizon scale. This concept may eventually lead to a better understanding of variable responses of respiration and methanogenesis to changes in soil moisture and temperature in peat soils. The vegetation likely had an effect on $\delta^{13}C$ of CH_4, as we observed consistently higher values in the permanently wet treatment W-V, which was the only treatment containing *Carex* species. Mass balance considerations and isotope budgets supported a selective CH_4 removal, especially under *Carex*. The study thus demonstrated that the chosen combination of mass balance and isotope budgets can serve as a useful approach to analyze processes patterns and rates under in situ conditions. The study also showed that rates and depth distribution of methanogenesis and methanotrophy were strongly impacted in the short term, which has often been reported. The most prominent effect was a shift in the zone of isotopically heavier CH_4 following the water table level, indicating that CH_4-oxidation followed the water table level. The predominant hydrogenotrophic methanogenic pathway remained stable through the drying/rewetting period, however. Even strong changes in redox conditions, coupled to changing availability of organic substrates, do not necessarily entail shifts from hydogenotrophic to acetoclastic methanogenesis in peat soils.

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