Effect of reed canary grass cultivation on greenhouse gas emission from peat soil at controlled rewetting

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Abstract

Cultivation of bioenergy crops in rewetted peatland (paludiculture) is considered as a possible land use option to mitigate greenhouse gas (GHG) emissions. However, bioenergy crops like reed canary grass (RCG) can have a complex influence on GHG fluxes. Here we determined the effect of RCG cultivation on GHG emission from peatland rewetted to various extents. Mesocosms were manipulated to three different ground water levels (GWL), i.e., 0, -10 and -20 cm below the soil surface in a controlled semi-field facility. Emissions of CO_2 (ecosystem respiration, ER), CH_4 and N_2O from mesocosms with RCG and bare soil were measured at weekly to fortnightly intervals with static chamber techniques for a period of one year. Cultivation of RCG increased both ER and CH_4 emissions, but decreased the N_2O emissions. The presence of RCG gave rise to 69, 75 and 85% of total ER at -20, -10 and 0 cm GWL, respectively However, this difference was due to decreased soil respiration at the rising GWL as the plant-derived CO_2 flux was similar at all three GWL. For methane, 70-95% of the total emission was due to presence of RCG, with the highest contribution at -20 cm GWL. In contrast, cultivation of RCG decreased N_2O emission by 33-86% with the major reductions at -10 and -20 cm GWL. In terms of global warming potential, the increase in CH_4 emissions due to RCG cultivation was more than off-set by the decrease in N_2O emissions at -10 and -20 cm GWL; at 0 cm GWL the CH_4 emissions was offset only by 23%. CO_2 emissions from ER obviously were the dominant RCG-derived GHG flux, but above-ground biomass yields, and preliminary measurements of gross photosynthetic production, showed that ER could be more than balanced due to the photosynthetic uptake of CO_2 by RCG. Our results support that RCG cultivation could be a good land use option in terms of mitigating GHG emission from rewetted peatlands, potentially turning these ecosystems into a sink of atmospheric CO_2.

Keywords: Ecosystem respiration; Ground water level, Methane; Nitrous oxide; Paludiculture

1 Introduction

Peatlands cover 3% of the world's area but contain 30% of the soil organic carbon (Parish et al., 2008), signifying an important role in the global carbon cycle. About 15% of the world's peatlands have been drained for different human purposes mostly for agriculture and forestry and to a lesser extent for peat extraction (Joosten, 2009). Drained peatlands are major sources of CO_2 emissions and estimated to account for about 6% of the total anthropogenic CO_2 emission (Joosten, 2009). In order to reduce the large emissions of CO_2 from drained peatlands, extensive rewetting projects have been implemented in Europe and North America (Höper et al., 2008), and rewetted organic soils have been included in the guidelines for national greenhouse gas (GHG) inventories by the Intergovernmental Panel on Climate Change (IPCC, 2014). In addition, agricultural use of wet and rewetted peatlands for crop growth (paludiculture) is considered as a possible land use option that may indirectly reduce the CO_2 emissions by biomass production for energy purposes (Joosten et al., 2012; Günther et al., 2014).

Reed canary grass (RCG) (*Phalaris arundinacea*) is one of the suitable biomass crops for paludiculture (Wichtmann and Tanneberger, 2011). It can be established from seeds as normal agricultural grass (Kandel et al., 2013b) but in some countries it is considered as an invasive species (Maurer et al., 2003).The plants thrive in wet soils due to aerenchyma tissues (Kercher and Zedler, 2004; Askaer et al., 2011) that transport oxygen to the roots in otherwise anaerobic soil compartments. However, cultivating wetland plants like RCG may influence the overall GHG balance by a combination of contrasting effects. First of all, RCG can stimulate the processes of GHG production by increasing the labile soil organic carbon pool, e.g., via root exudates (Ström et al., 2003; Bastviken et al., 2005). Next, the transport of oxygen to anaerobic zones stimulates heterotrophic degradation of organic matter, but at the same time stimulates oxidation of CH_4 (Kao-

Kniffin et al., 2010) and suppress CH_4 production due to increase in redox potential (Laanbroek, 2010; Sutton-Grier and Megonigal, 2011). RCG may further increase the emissions of reduced soil gasses as the aerenchyma tissues act as a conduit for the direct transport of, e.g., CH_4 and N_2O produced in soil (Joabsson et al., 1999; Jørgensen et al., 2012). Also, RCG can decrease N_2O emissions by assimilation of mineral N which reduces the availability of electron acceptors (nitrate) for denitrifying microorganisms (Roobroeck et al., 2010). In summary, the introduction of RCG at rewetted peatlands may cause a change in the patterns and underlying mechanisms of GHG emission, which is rather complex.

In the natural state, GHG emissions from peatlands are predominantly controlled by the position (depth) of the water table (IPCC, 2014). Basically, due to slow diffusion of oxygen in water (10,000 times slower than in air), ground water level (GWL) has a strong control on the oxic/anoxic soil boundary and thereby on the biogeochemical processes involved in GHG fluxes (Dinsmore et al., 2009; Karki et al., 2014). However, the presence of aerenchymatous plants may strongly interact with GWL in being decisive for the resulting GHG emissions from wet peatlands. The objective of the present study was to quantify the role of RCG cultivation on the resulting GHG emissions of CO_2, N_2O and CH_4 from peat soils rewetted to various extents. Such type of information is very important to understand the total GHG balance from paludiculture and improve the basis for modelling future climate. To accomplish this, the GHG emissions of all three gases were measured in an annual study with peat soil mesocosms with RCG and bare soil rewetted to constant GWL of 0, -10 and -20 cm in a controlled semi-field facility.

2 Materials and methods

2.1 Site description

Soil cores were collected from a fen peatland in the Nørre Å river valley, Denmark (56°44’N, 9°68’E). The peatland was drained to a depth of 60-70 cm early in the 20th century and since then used for agricultural purposes. RCG experimental plots were established at the site in 2009 (Kandel et al., 2013b). The top soil layer (0-20 cm) at the study site had the following main properties: highly decomposed peat soil corresponding to H9 on the von Post scale; bulk density, 0.29 g cm^{-3}; total organic carbon, 37.8% and total nitrogen, 3.2% (Karki et al., 2014).

2.2 Experimental design

A total of 30 intact soil cores for the mesocosm study were collected in May 2012 by inserting PVC pipes of 60 cm depth and 30 cm diameter into the soil. Half of the soil cores were collected from RCG plots and the other half were collected from a grass field surrounding the RCG plots. The upper 5 cm of the soil and litter layer was removed from the grass field before inserting the PVC pipes and these soil cores were kept bare during the experiment. The soil cores were retrieved with help of a mini excavator and transported to semi-field facilities at AU-Foulum (Karki et al., 2014). The bottom of the PVC pipes were covered with net to allow for free water movement and the pipes were then installed in plastic cylinders (diameter, 37 cm; height, 70 cm). The plastic cylinders were filled with gravel at the bottom 10 cm and the space between the PVC pipes and the wall of the cylinders (ca. 3 cm) was filled with sand. The whole set up was then installed in a trench at the semi-field facility with the soil surface at ground level.

Mesocosms with bare soil and RCG were randomly divided into three groups and were manipulated to three different GWL of 0, -10 and -20 cm below the soil surface. Water table was adjusted by fitting a rubber tubing (diameter, 1 cm) to the bottom of each plastic cylinder and placing the other end of the rubber tubing at different heights corresponding to the level of GWL treatment. Water was supplied in the space between the PVC pipes and the wall of the cylinders every day for one hour by a drip irrigation system. Further details on mesocosm incubations and the semi-field facility were given by Karki et al. (2014).

Due to poor regrowth of RCG (both under mesocosm and field conditions), initial weed biomass was uprooted and new RCG seeds were spread on 21 June 2012. RCG was fertilized with surface application of 0.6 g N, 0.1 g P and 0.5 g K per mesocosm on 23 July 2012 (corresponding to 80 kg N, 13 kg P and 77 kg K ha^{-1}). This fertilization rate corresponded to the rate applied in a previous study at the RCG field site from where the mesocosms were collected (Kandel et al., 2013a), except that the nitrogen rate was slightly increases in the mesocosm study as lower N mineralization was expected at higher GWLs. After the regrowth of RCG in spring 2013, RCG was fertilized with the same amount of fertilizer on 30 April and again in 28 June 2013. RCG plants were harvested twice, first on 29 October 2012 and then on 27 June 2013.In bare soil mesocosms, emerging weeds were uprooted and mosses were eliminated by application of iron sulphate ($FeSO_4$) on 29 August 2012. No fertilizer was added to bare soil mesocosms.

2.3 Gas measurements and flux calculation

Dark PVC chambers (diameter, 30 cm; height, 50 cm) equipped with fans and pressure equilibration vents were used for the measurement of CO_2, CH_4 and N_2O (Karki et al., 2014). Gas measurements were carried out between 10:00 and 13:00 at weekly to fortnightly intervals during July 2012 to July 2013. Four gas samples (10 mL) were drawn from the chamber headspace with polypropylene syringes during 45 minutes of chamber enclosure and transferred to evacuated 6-mL Exetainers. Gas samples were analysed with an Agilent 7890 gas chromatograph connected to a CTC CombiPAL automatic sample injection system (Agilent, Nærum, Denmark). Fluxes were calculated with the HMR method (Pedersen et al., 2010) in the statistical software R version 3.0.2 (R Core Team, 2013) as non-linear increase in GHG concentration over time was often observed during the non-steady state chambers measurements (Davidson et al., 2002; Petersen et al., 2012). Thus according to statistical HMR analysis fluxes were calculated either by non-linear or linear models (Pedersen et al., 2010). Out of the total of 435 fluxes for each GHG, the non-linear approach was applied for 41, 40 and 18% of CO_2, CH_4 and N_2O fluxes from RCG mesocosms, respectively and 22, 16 and 22% of CO_2, CH_4 and N_2O fluxes from bare soil mesocosms, respectively. In bare soil at 0 cm GWL, approximately 3% of the CH_4 fluxes were discarded due to episodic release of CH_4 presumably by ebullition.

2.4 Biomass measurement

Biomass development was monitored through the non-destructive measurement of ratio vegetation index (RVI). RVI was determined for each mesocosm using a SpectroSense 2+ fitted with SKR1800 sensors (Skype Instruments, Powys, UK). The sensors measured the incident and reflected red light (R) at 656 nm and the incident and reflected infrared light (NIR) at 778 nm. RVI was then calculated as $(NIR_r/NIR_i)/(R_r/R_i)$ where the subscripts i and r denote the incident and reflected radiation. RVI has already been used as a useful predicting factor for modelling ER and CH_4 fluxes (Kandel et al., 2013a; Kandel et al., 2013b; Görres et al., 2014; Karki et al., 2014)

RVI measurements were done on the same days as GHG sampling except in winter when the soil was covered with snow or frozen. The total above ground dry biomass from each mesocosm was also determined after each harvest by oven drying the plant material at 60°C to constant weight. After the harvest in 2013, species composition from each mesocosm was determined on dry weight basis to quantify the contribution of volunteer weeds in the total biomass.

2.5 Environmental parameters and pore water analysis

Soil temperature at 5 cm depth and soil moisture was measured by temperature and time domain reflectometry (TDR) probes installed permanently in one of the five replicates for each GWL treatment. Soil temperature was measured automatically every hour while soil moisture measurements with TDR (volumetric water content, VWC) were done on every gas sampling occasion. The instrumented mesocosms also had Pt probes installed at 20 cm depth to measure soil redox potential. Soil redox potential was measured at fortnightly intervals from mid-April to July 2013 with a portable pH meter (PHM220, Radiometer) by gently pushing a double junction calomel reference electrode (REF251, Hach Lange) into the soil. Measured redox potential were converted to standard hydrogen electrode potential (Eh) by addition of +245 mV (Kjaergaard et al., 2012).

A piezometer (length, 65 cm; diameter, 2 cm) with the screen all the way down was installed in the instrumented mesocosms. Approximately 30 mL of soil water was sampled monthly from these piezometers except for February to April 2013 when water inside the piezometers was frozen. Water samples were analysed for ammonium, nitrate, and sulphate content. Ammonia and nitrate content were measured using an auto-analyzer (Bran+Luebbe GmbH; Norderstedt, Germany) and sulphate was determined by ion chromatography on a Dionex ICS-1500 IC-system (Dionex Corp.; Sunnyvale, CA, USA).

2.6 Cumulative GHG fluxes

For the mesocosms with RCG, CO_2 emissions from ecosystem respiration (ER) were modelled as a function of GWL, temperature, and biomass (RVI) by Model 1 (Karki et al. 2014); for bare soil mesocosms Model 2 excluding RVI was applied:

$$ER = (b_1 + b_2\mathrm{GWL}) \times \exp\left(b_3\left(\frac{1}{10 - T_0} - \frac{1}{T - T_0}\right)\right) \times (b_4 + RVI) \qquad [1]$$

$$ER = (b_1 + b_2\mathrm{GWL}) \times \exp\left(b_3\left(\frac{1}{10 - T_0} - \frac{1}{T - T_0}\right)\right) \qquad [2]$$

where T_0 is a notional zero respiration temperature, here fixed to -46.02°C (Lloyd and Taylor, 1994), T is the air or soil temperature (°C), RVI is the ratio vegetation index, GWL is water table depth below the soil surface (cm) and b_1, b_2, b_3 and b_4 are model parameters.

All model parameters were estimated by non-linear regression in SigmaPlot 11 (Systat Software, Chicago, IL, USA). Using the obtained model parameters, continuous temperature data and linearly interpolated RVI data, hourly rates of CO_2 emissions were reconstructed for each GWL. These hourly emissions values were summed to yield the annual flux from 10 July 2012 to 9 July 2013. The uncertainty of annual fluxes were addressed by deriving the minimum and maximum cumulative fluxes from upper and lower values of model parameters ± standard errors (SE) (Elsgaard et al., 2012). For model evaluation the Nash–Sutcliffe modelling efficiency (ME) was calculated according to:

$$ME = 1 - \frac{\sum_{i=1}^{n}\left(Mes_i - Mod_i\right)^2}{\sum_{i=1}^{n}\left(Mes_i - \overline{Mes}\right)^2} \qquad [3]$$

where Mes_i and Mod_i are measured and modelled values, respectively, and $\overline{Mes}$ is the mean of measured vales (Haefner, 2005).

Cumulative CH_4 and N_2O fluxes were calculated by linear interpolation between the sampling dates using the trapezoidal rule (Petersen et al., 2012). The linear interpolation method was used as there were no common models to predict CH_4 and N_2O fluxes for vegetated and bare soil plots. Cumulative fluxes were calculated for each individual mesocosm and then averaged for each GWL treatment ($n = 5$). Total GHG emissions were calculated by summing annual CO_2, CH_4 and N_2O emissions at each GWL; CH_4 and N_2O emissions were converted to CO_2 equivalents by multiplying with 28 and 265, respectively, according to the revised global warming potential (GWP) of the three GHG (Myhre et al., 2013). The plant-derived total GHG emission at each GWL was estimated as the difference between the total GHG emissions from RCG mesocosms and bare soil mesocosms. The uncertainty of annual plant-derived GHG emissions was derived from the uncertainty of plant and bare soil emissions added in quadrature.

2.7 Statistical analysis

Statistical analyses were done using R version 3.0.2 (R Core Team, 2013). Data were analyzed using a linear mixed model including the fixed effect of vegetation (bare soil/RCG), GWL, date and their two-way interactions. The model also included the random effect of each experimental unit. Prior to analysis, CH_4 and N_2O flux data were log-transformed after addition of a constant (minimum fluxes of CH_4 and N_2O) to obtain normal distribution and variance homogeneity. Dates were treated as repeated measurements by applying either compound symmetry structure (each dependent variable have constant covariance independent of time) or autocorrelation structure of order 1(errors at adjacent time points are correlated) (Maxwell and Delaney, 2004). Best model was selected by use of Akaike´s Information Criterion (AIC). For CH_4 and N_2O, autocorrelation structure was selected while compound symmetry was selected for CO_2 fluxes.

A similar linear mixed model was run to determine the effect of GWL on RVI development. One-way ANOVA was used to test the difference in mean yield between the treatments. Significance of all tests was accepted at $P<0.05$.

3 Results

3.1 Environmental conditions

The average air temperature during the study period was 6.9°C and total precipitation was 667 mm (Fig. 1). Snowfall started in early December 2012 and was observed till end of March 2013 with intermittent freezing and thawing events. The soil was frozen and covered with ice till mid-April 2013. The annual average soil temperature (5 cm depth) in RCG treatments was 7.4, 7.7 and 7.6°C at 0, -10 and -20 cm GWL, respectively; for bare soil treatments it was 7.5, 7.4 and 7.9°C at 0, -10 and -20 cm GWL, respectively. The average volumetric soil water content during the measurement period was 82 ± 5%, 67 ± 3%, and 58 ± 3% from RCG treatments at 0, -10 and -20 cm GWL, respectively, and 83 ± 4%, 62 ± 6%, and 55 ± 7% from bare soil treatments at 0, -10 and -20 cm GWL, respectively (mean ± standard deviation, $n = 22$). Average soil redox potential was -115, -27, and 40 mV from RCG treatments at 0, -10 and -20 cm GWL, respectively, and -118, -51, 151 mV from bare soil treatments at 0, -10, and -20 cm GWL, respectively (Fig. 2).

3.2 Biomass yield and RVI

The mean biomass yield was 6.0 and 6.6 Mg ha^{-1} across all GWL in 2012 and 2013, respectively (Fig. 3). During the first year there was a good stand of RCG but during the second year weed biomass became established especially at 0 cm GWL; this was notably meadow foxtail (*Alopecurus pratensis* L.) and grasses (*Poa* sp.) which made an important contribution to the total biomass at the time of harvest.

The pattern of RVI development was similar among the different GWL treatments; peak values of RVI occurred in late August 2012, where after RVI started to decline due to plant senescence. RVI started to increase again during the regrowth of biomass in spring 2013 (Fig. 4).

3.3 *Pore water properties*

The annual variation in soil water sulphate concentrations ranged from 1.3 to 56.9 mg L^{-1}. Generally, similar SO_4^{2-} concentrations were found in bare soil and RCG mesocosms at 0 and -10 cm GWL, but at -20 cm GWL consistently higher SO_4^{2-} concentrations were found in the bare soil mesocosms (Table 1). For ammonium the concentrations ranged from 0.1 to 10.2 mg L^{-1} and higher NH_4^+ concentrations were generally found in bare soil mesocosms than in RCG mesocosms at 0 and -10 cm GWL. In the bare soil treatments the level of NH_4^+ was lower at -20 cm GWL than at 0 and -10 cm GWL, but in RCG treatments NH_4^+ concentrations were similar at all the three GWLs (Table 1). The concentration of nitrate was low (<3.1 mg L^{-1}) across all treatments; the highest NO_3^- levels were generally seen at bare soil treatments at -20 cm GWL (Table 1).

3.4 *Measured GHG fluxes*

The emission of CO_2 was measured as ER in RCG and bare-soil treatments in order to evaluate the contribution of RCG in the total ER at the different GWLs. The emissions of CO_2 were different between RCG and bare soil mesocosms ($p < 0.001$) and also between the three GWL treatments ($p < 0.001$) (Table 2). CO_2 emissions decreased consistently with higher GWL both from RCG and bare soil mesocosms. The emissions showed expected seasonal variation with highest CO_2 fluxes during summer time ($p < 0.001$) (Fig. 5a, b). CO_2 emissions ranged from 20 to 485 mg m^{-2} h^{-1} across all GWL in bare soil and from 55 to 1700 mg m^{-2} h^{-1} in RCG treatments. Among the air and soil temperature at 5 cm, CO_2 emissions were better correlated with soil temperature in bare soil, but with the air temperature in RCG treatments.

Methane fluxes were significantly affected both by vegetation and GWL (Table 2). CH_4 emissions were highest at 0 cm GWL both from RCG and bare soil treatments (Fig. 5c, d). CH_4 emissions from RCG treatments showed temporal variation ($P < 0.001$) with highest emissions during summer time (Fig. 5c). Peak emissions of CH_4 from RCG treatments were observed in August 2012 across all GWL levels, ranging from 4.4 to 8.9 mg CH_4 m^{-2} h^{-1}. During November to early April (i.e., winter season), CH_4 emissions from RCG treatments were below 0.1 mg m^{-2} h^{-1} and even occasional uptake (25% of total fluxes measured) of CH_4 was recorded. From bare soil treatments, CH_4 fluxes were generally low and fluctuated between apparent net emission and net uptake except for few episodic peak events, generally from 0 cm GWL. These peak events were considered to represent unsystematic ebullition events.

N_2O fluxes from RCG treatments were generally low, fluctuating in a range between -0.02 to 0.07 mg m^{-2} h^{-1} except for peak events after fertilizer application (Fig. 5e). Emission peaks of 0.4, 0.7 and 0.4 mg N_2O m^{-2} h^{-1} were observed at 0 cm GWL immediately after the first, second and third fertilization events, respectively. Smaller peak emissions of 0.4 and 0.2 mg N_2O m^{-2} h^{-1} were observed at -10 cm GWL after the first and second fertilization event, but at -20 cm GWL, peak emission after the fertilizer application was absent. N_2O emissions from bare soil treatments generally were higher and ranged from -0.02 to 1.9 mg m^{-2} h^{-1}. Most of the N_2O emission in bare soil mesocosms was measured during the winter period from November 2012 to April 2013 accounting for more than 70% of the cumulative emission at 0 and -10 cm GWL and more than 50% at -20 cm GWL.

3.5 Annual GHG emissions and contribution of plants to annual GHG emissions

The estimated parameters for CO_2 flux models are presented in Table 3 showing also that the modelling efficiency was considerably higher for the RCG treatments than the bare soil treatments. Annual CO_2 emissions decreased consistently with raising GWL towards the soil surface both in RCG and bare soil treatments (Fig. 6). In contrast, CH_4 emissions increased systematically both from RCG and bare soil treatments in response to raising GWL (Fig. 6). The annual N_2O emissions showed a contrasting response to raising GWL in bare soil and RCG treatments; in bare soil treatments lower N_2O emissions occurred in response to raised GWL, but in RCG treatments there was a tendency of higher N_2O emissions in response to raised GWL (Fig. 6).

The presence of plants contributed 69-85% of the total CO_2 emissions from the RCG mesocosms (Fig. 6). The highest contribution was observed at 0 cm GWL and the contribution decreased at lower GWL. RCG likewise accounted for more than 70% of total CH_4 emissions with the highest contribution of 95% observed at -20 cm GWL. Thus at this GWL (-20 cm) CH_4 emission was negligible from bare soil treatments (0.2 g CH_4 m^{-2} yr^{-1}) whereas the emissions was substantial from RCG treatments (4.1 g CH_4 m^{-2} yr^{-1}). In contrast to CO_2 and CH_4 emissions, cultivation of RCG reduced the annual N_2O emissions despite the application of mineral N fertilizer in RCG mesocosms (Fig. 6). At -10 and -20 cm GWL, RCG eliminated 82-86% of the N_2O emissions as compared to bare soil treatments; from 0 cm GWL the reduction corresponded to 33% of the N_2O emissions. In terms of GWP, the increase in CH_4 emissions due to RCG cultivation was more than off-set by the decrease in N_2O emissions at -10 and -20 cm GWL, but apparently not at 0 cm GWL where CH_4 emissions were off-set by only 23% by the decreased N_2O emission (Fig. 7). CO_2 emissions from ER, though, were the dominant RCG-derived GHG fluxes (Fig. 7).

4 Discussion

During the present study, the effects of RCG cultivation on GHG emission from rewetted peatland was evaluated by comparison of planted and unplanted (bare-soil) mesocosms. Mesocosms with RCG and bare soil (rather than, e.g mesocosms with native grasses) were compared in order to tentatively isolate the contribution of RCG in the measured GHG fluxes. One concern of using this plant exclusion method for GHG studies is the difference in soil moisture regime and temperature that may develop between planted and bare soil treatments which may result in different decomposition rates of soil organic matter (Kuzyakov, 2006). With our experimental setup, we were able to control the GWL throughout the measurement period and this resulted in soil moisture contents (VWC) that were similar between RCG and bare soil treatments at each GWL; this was generally also seen for the soil redox potential and pore water sulphate concentration at least at 0 and -10 cm GWL. The average soil temperature difference between the RCG and bare soil treatments was found to be less than 1°C; however during the annual study we observed some seasonal difference in soil temperature especially during spring days (higher temperature in RCG treatments) and summer days (lower temperature in RCG treatments) which was attributed to the RCG cultivation. Yet, the differences in moisture and temperature regime between the planted and bare-soil mesocosms were considered to be modest and pertinent for an evaluation of the effects of RCG on total GHG emissions.

Monitoring of environmental variables was achieved by instrumentation of one out of five replicate mesocosms at each GWL. We assumed that the measured variables were representative for all replicates and that the instrumentation did not lead to any bias. This was substantiated by the absence of any systematic deviations in measured GHG fluxes from the instrumented and noninstrumented replicates. Thus, the average difference in annual fluxes with and without instrumentation was less than 15%.

4.1 CO_2 emissions

Plants can enhance CO_2 flux from ER directly by above- and below-ground respiration and indirectly by enhancing the decomposition of soil organic matter by the supply of easily degradable root exudates to the soil (priming effect) (Kuzyakov et al., 2001; Van Huissteden et al., 2006). In vegetated soils ER is essentially balanced by photosynthetic CO_2 uptake, and therefore CO_2 emissions from ER does not represent the net ecosystem exchange (NEE) of CO_2. Rather than quantifying NEE, an important result of the present study was that plant-derived ER from RCG mesocosms (the major part of total CO_2 emissions) was similar at all three GWL (Fig. 7) substantiating the results of Lafleur et al. (2005) and Riutta et al. (2007) who reported autotrophic respiration to be independent of water table depth. Thus, the observed increase (from 69 to 85%) in total ER with rising GWL was promoted mainly by decreasing soil respiration at the higher GWL (Fig. 6). The observed contribution of RCG to total CO_2 emissions was higher than the values of 55% previously reported by Shurpali et al. (2008). Yet, the results of Shurpali et al. (2008) were obtained for a drained peatland with an average GWL of -65 cm which would favor aerobic soil respiration to a larger extent than in our soils with GWL no deeper than -20 cm. In accordance with this we also observed a larger soil respiration at -20 cm than at 0 cm GWL.

4.2 Methane emissions

Methane fluxes from soil is the result of CH_4 production, consumption and transport (Lai, 2009). Plants play a key role on CH_4 fluxes as they have potential to influence all three processes (Joabsson et al., 1999) . CH_4 emissions were higher from RCG than bare soil treatments even though the GWL was raised to the soil surface. Plant roots release organic compounds to soil, which are easily available carbon sources to anaerobic microbial consortia eventually producing the precursors (acetate or H_2/CO_2) for methanogenesis (Ström et al., 2003). Such fresh organic carbon is suggested to be important substrates for methanogenesis as peat carbon is shown to be more recalcitrant to anaerobic decomposition (Tuittila et al., 2000; Hahn-Schöfl et al., 2011).

Methane produced in soil can be emitted to the atmosphere by diffusion, ebullition (release of gas bubbles) and plant-mediated transport (Whalen, 2005; Lai, 2009). Indeed, RCG can transport CH_4 from soil to the atmosphere directly through its aerenchyma tissue, thereby bypassing the microbial methane oxidation layer in the soil. On an annual basis it has been estimated that RCG may actually transport 70% of the total CH_4 emissions from a natural wetland in Denmark (Askaer et al., 2011). In the absence of plant-mediated transport, diffusion expectedly would be the dominant pathway of CH_4 emissions in bare soil treatments. CH_4 transport through diffusion is a slow but important process for bringing CH_4 in contact with the CH_4 oxidizing microbial community (Whalen 2005; Lai 2009). In our study there was negligible CH_4 emissions from bare soil at -20 cm GWL, aligning with the results of Schäfer et al. (2012) who reported this drainage depth to be sufficient to suppress diffusive CH_4 emissions due to methane oxidation and reduced methanogenesis.

The transport of oxygen by aerenchyma plants to anoxic soil compartments has been reported to increase the redox potential which could suppress CH_4 emission (Sutton-Grier and Megonigal, 2011). However, in our study neither the redox potential nor the sulphate content was consistently increased by the presence of plants suggesting the role of substrate availability and transport of CH_4 through RCG to be the important factors for controlling CH_4 emissions from the RCG treatments.

It is possible that we could have underestimated the total CH_4 emission from bare soil treatments at 0 cm GWL as episodic CH_4 release through ebullition was not taken into account in the annual balance. Ebullition events were identified by occasional erratic time courses of CH_4 concentrations during the flux measurements; however as these events were generally associated with the initial (time 0 and 15 min) chamber gas samplings it was believed to represent artifacts created during chamber deployment. Yet, episodic release of CH_4 may be more important in bare soil than in vegetated soil as plants may reduce the soil concentration of CH_4 by mediating CH_4 transport and also by rhizospheric oxidation of CH_4; these processes reduce the potential formation of CH_4 bubbles (Chanton, 2005). Tentatively accounting for the observed episodic CH_4 release, a total of 0.04 g m^{-2} of CH_4 was released during the study; this was a negligible contribution (< 1%) to the annual CH_4 flux from bare soil at 0 cm GWL. However, as ebullition events are short-lived and unsystematic they could easily be missed by the chamber measurements (Coulthard et al., 2009).

4.3 N_2O emissions

Annual fluxes of N_2O (0.2 to 0.4 g N_2O m^{-2} yr^{-1}) from RCG mesocosms were within the range (-0.4 to 0.8 g N_2O m^{-2} yr^{-1}) reported for undisturbed Danish riparian wetland (Audet et al., 2014). However, annual fluxes were higher in bare soil (0.6 to 2.0 g N_2O m^{-2} yr^{-1}) as compared to RCG treatments. Thus, RCG decreased the annual N_2O emissions, contradictory to the finding of Hyvönen et al. (2009) where fertilization in RCG increased the N_2O emissions by 90% as compared to bare soil. However, in the study by Hyvönen et al. (2009) N_2O emissions were quite low (0.01 g N_2O m^{-2} yr^{-1}) from the soil without vegetation. Their site was an abandoned peatland (Hyvönen et al. (2009) probably with limited nitrification because of a high C/N ratio (42.3) (Klemedtsson et al., 2005) compared to our peat soil with rich N content (3.2%) and a low C/N ratio (11.6). Thus, the ecosystem studied by Hyvönen et al. (2009) might have been more N limited at the unfertilized sites than was the case for our study site.

The effect of RCG cultivation on N_2O emissions was highly dependent upon the GWL. At 0 cm GWL, there was least effect of RCG cultivation on N_2O emissions due to peak emissions observed after fertilization. Peak emission observed after fertilization events suggest that N_2O emission was limited by mineral N content at 0 cm GWL. Saari et al. (2013) and Silvan et al. (2002) also reported a significant increase in N_2O emission after addition of inorganic nitrogen in riparian wetland due to favorable conditions for denitrification.

Previous studies have reported that winter emissions significantly contributed to annual N_2O emissions (Maljanen et al., 2004; Regina et al., 2004). Such emissions in winter have been related to the physical release of N_2O that is produced and trapped under frozen surface layers as well as the emissions of newly produced N_2O (*de novo* emissions) at the onset of thaw stimulated by increased biological activity and changes in physical and chemical soil conditions (Risk et al., 2013). Significant emissions at all GWL were observed in winter from bare soil treatments, but not from RCG treatments. After harvesting, there was regrowth of RCG and also other volunteer grasses which survived throughout the winter and which may have competed with microorganisms for available N. Maljanen et al. (2004) also observed higher N_2O emissions from bare soil as compared to vegetated plots during winter and likewise related the low emission in vegetated plots to low mineral N content due to uptake of nitrate by plants. Bare soil treatments indeed had a higher availability of mineral N (Table 1), and could be more prone to physical damage by freeze and thaw cycles due to lack of plant cover; both these factors stimulate the biological activities related to N_2O emissions as substantiated by the observed slight increase also in CO_2 emissions coinciding with increased N_2O emission especially at 0 and -10 cm GWL.

4.4 Effect of RCG cultivation on GHG balance from rewetted peatland

Two of the major concerns of growing wetland plants like RCG in rewetted peatland are the possible increase in CH_4 emissions due to supply of fresh plant material and transport of CH_4 by aerenchyma tissue (Ström et al., 2003; Askaer et al., 2011) and the possible increase in N_2O due to application of N fertilizers (Maljanen et al., 2010). However, in the present experiment, cultivation of RCG decreased N_2O emission to an extent that could offset the increase in CH_4 emission at -10 and -20 cm GWL, but apparently not at 0 cm GWL; the latter case being due to peak emissions in N_2O after fertilization events in RCG. This result suggests that emissions at 0 cm GWL can be reduced by reducing the N fertilization rate. Further studies are needed to assess the optimum amount and timing of fertilization required for optimum growth of RCG with acceptable N_2O emissions. Emissions of N_2O caused by N fertilization should not off-set the benefit of fossil fuel substitution obtained by the fertilizer induced increase of biomass production (Kandel et al. 2013a). Regarding the overall GHG emission, the CO_2 emissions from ER was clearly the dominant RCG-derived GHG flux. Yet, CO_2 flux from ER would to a large extent be counterbalanced by gross photosynthesis which expectedly was similar at all GWL treatments (based on the similar biomass yields), though CO_2 flux from photosynthesis was not measured in this annual study. Yet, a photosynthetic uptake of 6.2 kg CO_2 m^{-2} was measured from RCG mesocosms at 0 cm GWL during the growing season from May to September 2013 (S. Karki, unpublished results) reflecting that RCG potentially can turn the rewetted ecosystem into a sink of CO_2 from an atmospheric perspective. Adaptation or selection of RCG varieties that thrive especially well under distinct climate and shallow GWL conditions could further help to improve the GHG balance of paludiculture with RCG.

5 Conclusion

The present study to our knowledge is the first to compare the annual GHG emission from RCG and bare soil treatments of rewetted peatland at controlled GWL. The following conclusions were derived: (i) soil respiration decreased with increasing GWL from -20 to -10 to 0 cm, but RCG-derived ER was similar at all three GWL resulting in the highest contribution of RCG to total ER (85%) at 0 cm GWL, (ii) cultivation of RCG increased CH_4 emission at all GWLs, but relatively most at -20 cm GWL, (iii) N_2O emissions decreased due to RCG cultivation especially during winter; winter emissions were more important component of annual emission from bare soil than RCG, (iv) in terms of GWP, the increase in CH_4 emissions due to RCG cultivation was more than off-set by the decrease in N_2O emissions at -10 and -20 cm GWL, (v) CO_2 emissions from ER (the dominant RCG-derived GHG flux) could be balanced by photosynthetic CO_2 uptake at all three GWL as indicated by the large and similar above-ground biomass yields at all GWL, signifying a potential of RCG cultivation to turn the rewetted peatland into a sink of atmospheric CO_2.

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Table 1. Concentration of sulphate, ammonium, and nitrate (mg L^{-1}) in ground water samples collected from piezometers from bare soil and reed canary grass (RGC) mesocosms at different ground water levels (GWL).

Treatment	SO_4^{2-} (mg L^{-1}) at GWL			NH_4^+ (mg L^{-1}) at GWL			NO_3^- (mg L^{-1}) at GWL		
and date	0 cm	-10 cm	-20 cm	0 cm	-10 cm	-20 cm	0 cm	-10 cm	-20 cm
Bare soil									
26/07/2012	20.7	33.4	54.2	0.9	0.7	0.0	0.4	0.6	0.7
24/08/2012	6.6	10.0	56.9	1.4	2.0	0.1	0.2	0.1	2.0
26/09/2012	41.7	13.5	52.9	3.4	4.3	0.3	0.1	<0.1	1.8
05/11/2012	2.7	1.8	46.2	3.6	3.5	0.7	<0.1	0.1	0.5
30/11/2012	2.1	4.7	41.9	4.0	3.2	0.8	<0.1	<0.1	0.1
04/01/2013	2.3	4.0	29.7	2.9	3.8	0.4	0.1	0.3	1.0
06/05/2013	1.3	1.4	22.7	5.1	4.6	0.7	0.7	0.1	0.6
11/06/2013	2.0	2.1	18.1	5.8	3.8	0.2	<0.1	<0.1	0.6
16/07/2013	1.3	1.4	17.2	10.2	5.9	0.6	<0.1	<0.1	2.5
RCG									
26/07/2012	10.4	11.4	9.9	2.9	1.2	2.0	2.9	0.4	0.1
24/08/2012	3.8	2.4	9.2	0.2	0.1	0.5	<0.1	<0.1	0.1
26/09/2012	2.0	10.6	3.9	0.6	0.2	0.9	0.1	0.1	0.1
05/11/2012	5.6	3.6	2.3	0.8	0.1	1.0	<0.1	<0.1	<0.1
30/11/2012	4.1	3.2	3.9	0.6	0.4	0.8	0.5	0.2	0.1
04/01/2013	2.3	3.5	5.3	1.5	0.1	0.7	0.1	0.5	0.1
06/05/2013	2.0	1.8	3.5	1.7	0.4	0.2	<0.1	3.1	<0.1
11/06/2013	3.4	4.5	4.7	0.3	0.8	0.1	<0.1	0.1	0.3
16/07/2013	3.3	1.8	10.6	1.5	1.5	0.1	0.1	<0.1	0.3

Table 2. Statistical main effects of vegetation (i.e., reed canary grass cultivation or bare soil), ground water level (GWL), and date on fluxes of CO_2, CH_4 and N_2O as explored with linear mixed models.

Variables	CO_2			CH_4			N_2O		
	DF	*F* value	*P* value	*DF*	*F* value	*P* value	*DF*	*F* value	*P* value
Vegetation	1	956.2	<0.001	1	165.8	<0.001	1	0.5	<0.001
GWL	2	32.2	<0.001	2	15.4	<0.001	2	3.1	0.02
Date	28	75.6	<0.001	28	25.8	<0.001	28	<0.1	<0.001

Table 3. Parameter estimates (b_1, b_2, b_3 and b_4) for CO_2 flux (ecosystem respiration) models. Uncertainties shown in parentheses are standard error of parameter estimates. Also shown are correlation coefficients (r) between observed and modelled data and modeling efficiencies (ME).

Treatment	CO_2 flux model	b_1 (mg CO_2 m^{-2} h^{-1})	b_2 (mg CO_2 m^{-2} h^{-1} cm^{-1})	b_3 (K)	b_4	r	ME
Reed canary grass	Model 1	49.6 (3.8)	0.4 (0.1)	259.1 (15.5)	5.0 (0.7)	0.90	0.82
Bare soil	Model 2	79.1 (6.9)	5.7 (0.5)	286.4 (24.1)	na	0.68	0.46

na, not applicable

Figure captions

Fig. 1. **(a)** Hourly air temperature at 2 m height at the semi-field facility and hourly average soil temperature at 5 cm depth across all mesocosm treatments, and **(b)** daily precipitation at the semi-field facility during the study period (July 2012 to July 2013).

Fig. 2. Redox potential (Eh) at different ground water levels (GWL) from reed canary grass (RCG) and bare soil mesocosms. Eh was measured at 20 cm soil depth from April to July 2013.

Fig. 3. Mean dry biomass yield (Mg ha^{-1}) from mesocosms at different ground water level in 2012 and 2013. Error bars show standard error (*n* = 5).

Fig. 4. Average ratio vegetation index (RVI) development during the measurement period across all ground water levels. Error bars show standard error (*n* = 15). Dotted line represent the winter period when RVI was not measured due to ice and snow.

Fig. 5. Time course of greenhouse gas fluxes from the rewetted peat soil mesocosms during July 2012 to July 2013 in treatments with RCG cultivation (left panels) and bare soil (right panels). Data are shown for (a, b) CO_2 fluxes from ecosystem respiration, (c, d) CH_4 fluxes, and (e, f) N_2O fluxes. All data are mean and standard error of five replicates from each of the three ground water levels (GWL) at 0, -10 and -20 cm. Arrows marked by H indicate the times of harvest and arrows marked by F indicate the times of mineral fertilization.

Fig. 6. Annual fluxes of (a) CO_2 from ecosystem respiration, (b) CH_4, (c) N_2O and (d) total global warming potential (∑ GWP) from the rewetted peat soil mesocosms from July 2012 to July 2013 in treatments with RCG cultivation (gray bars) and bare soil (white bars) at ground water levels of 0, -

10 and -20 cm. Error bars for CO_2 data show the standard error (SE) derived from SE of model parameters. For CH_4 and N_2O, data are shown as mean and SE of individual mesocosms ($n = 5$). Numbers in parentheses indicate the contribution of RCG in total emission at the different GWLs.

Fig. 7. Plant-derived CO_2, CH_4 and N_2O emissions at different ground water levels as compared in terms of CO_2 equivalents (CO_2 eq.). Plant derived emissions were estimated as the difference between the total emissions from RCG treatments and bare soil treatments.

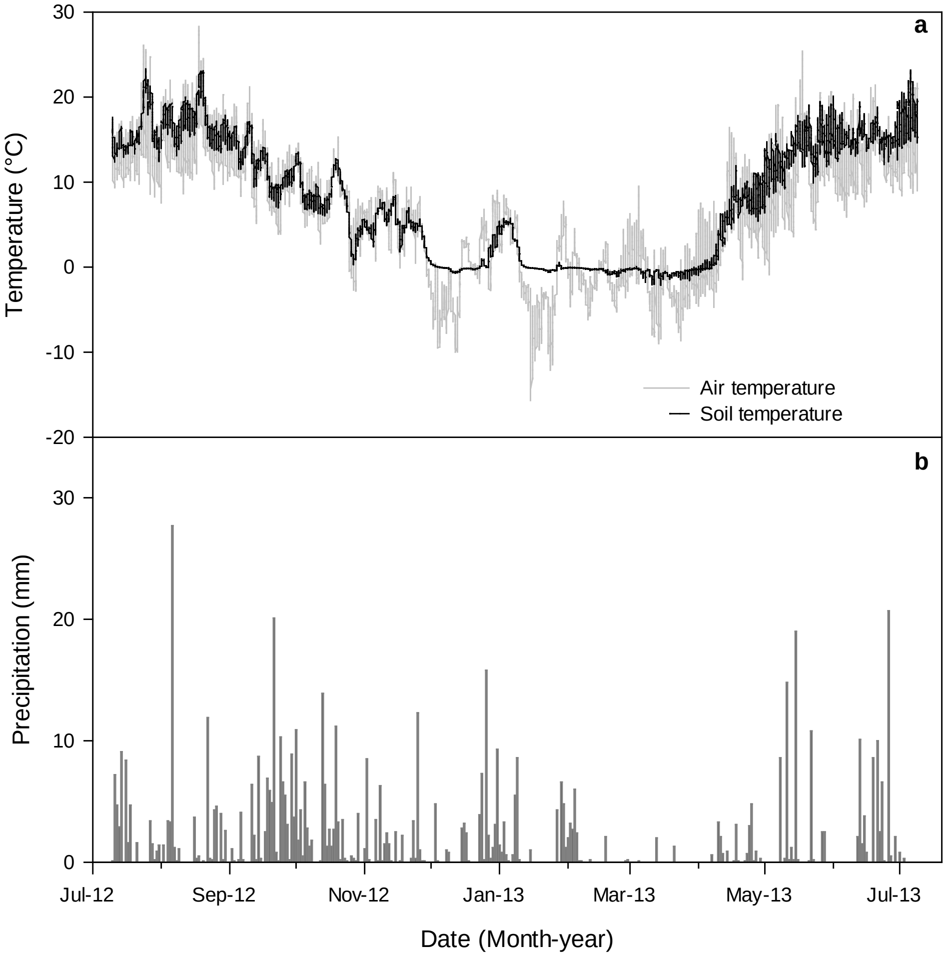
a
Temperature (°C)
30
20
10
0
-10
-20
Air temperature
Soil temperature
b
Precipitation (mm)
30
20
10
0
Jul-12
Sep-12
Nov-12
Jan-13
Mar-13
May-13
Jul-13
Date (Month-year)

Fig. 1.

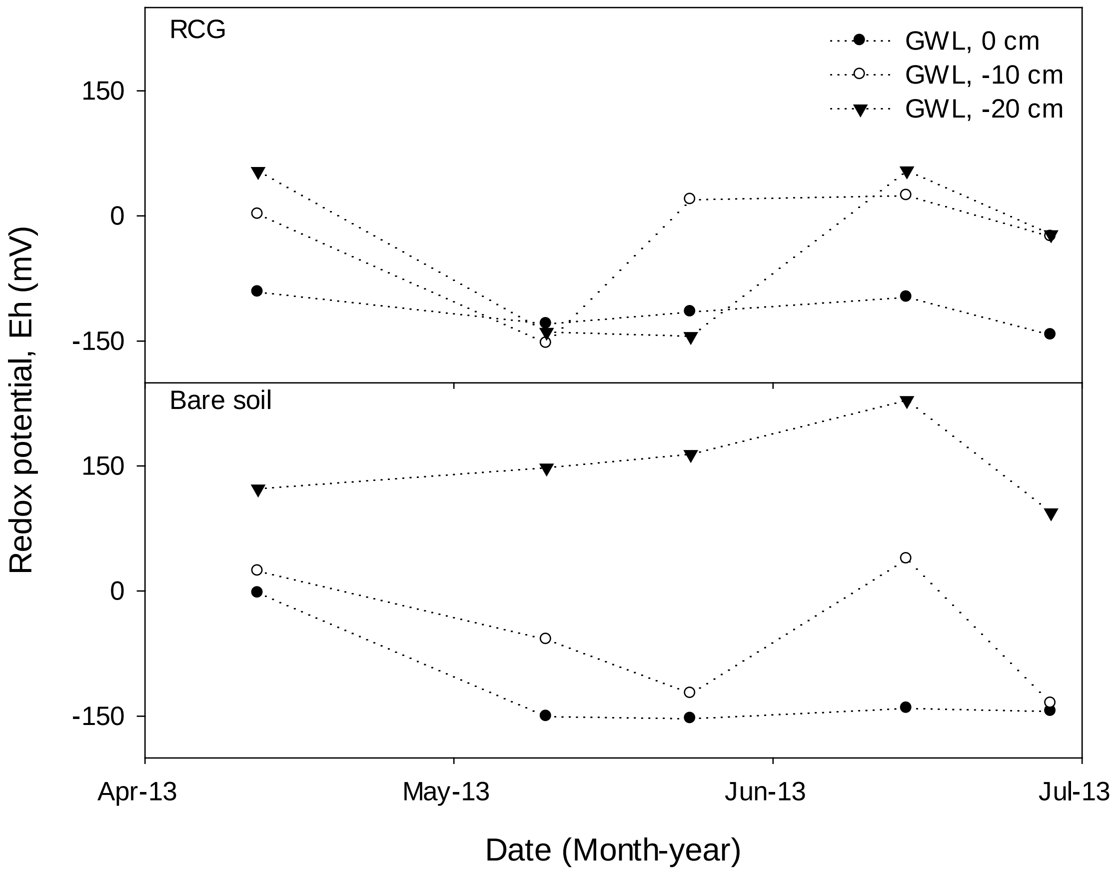
RCG
Bare soil
GWL, 0 cm
GWL, -10 cm
GWL, -20 cm
150
0
-150
Redox potential, Eh (mV)
Apr-13
May-13
Jun-13
Jul-13
Date (Month-year)

Fig. 2.

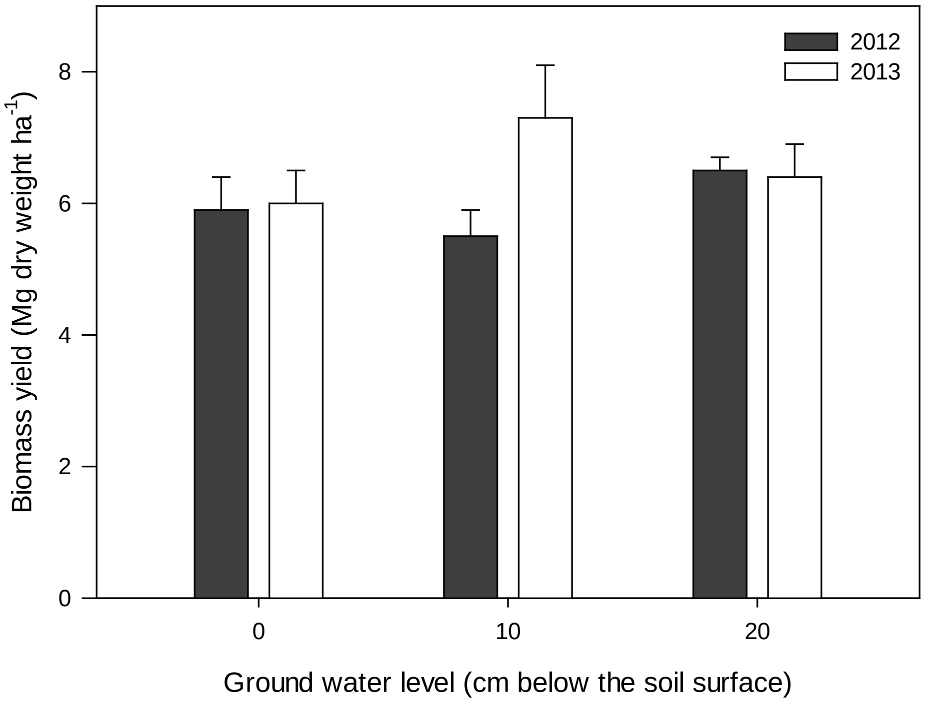
2012
2013
Biomass yield (Mg dry weight ha^{-1})
0
2
4
6
8
0
10
20
Ground water level (cm below the soil surface)

Fig. 3.

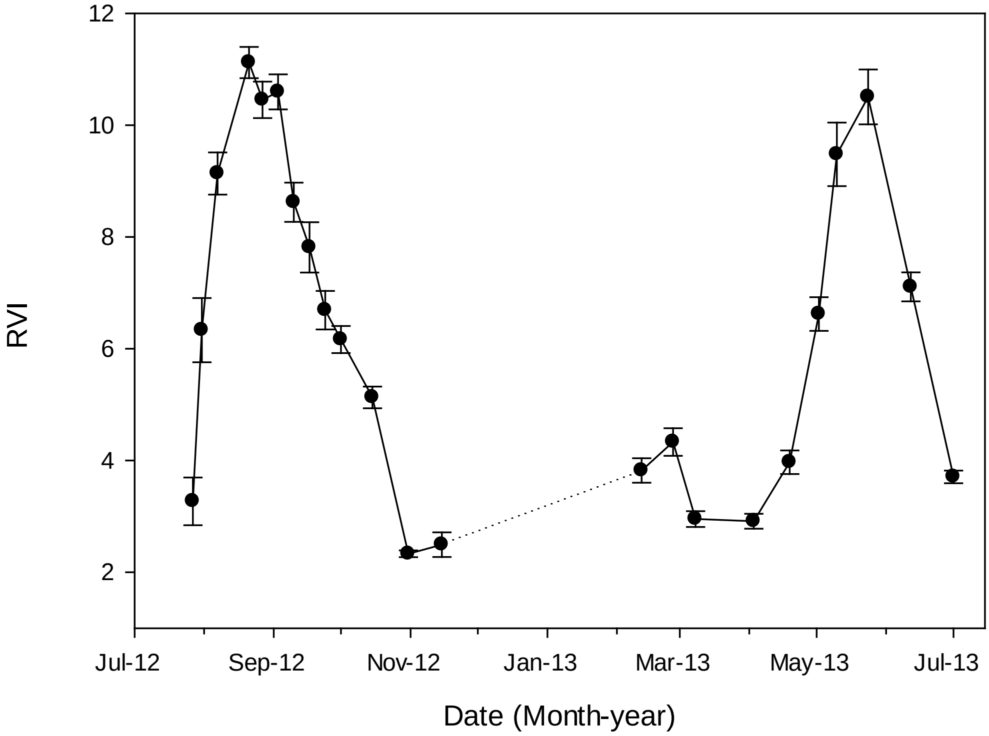
12
10
8
6
4
2
RVI
Jul-12
Sep-12
Nov-12
Jan-13
Mar-13
May-13
Jul-13
Date (Month-year)

Fig. 4.

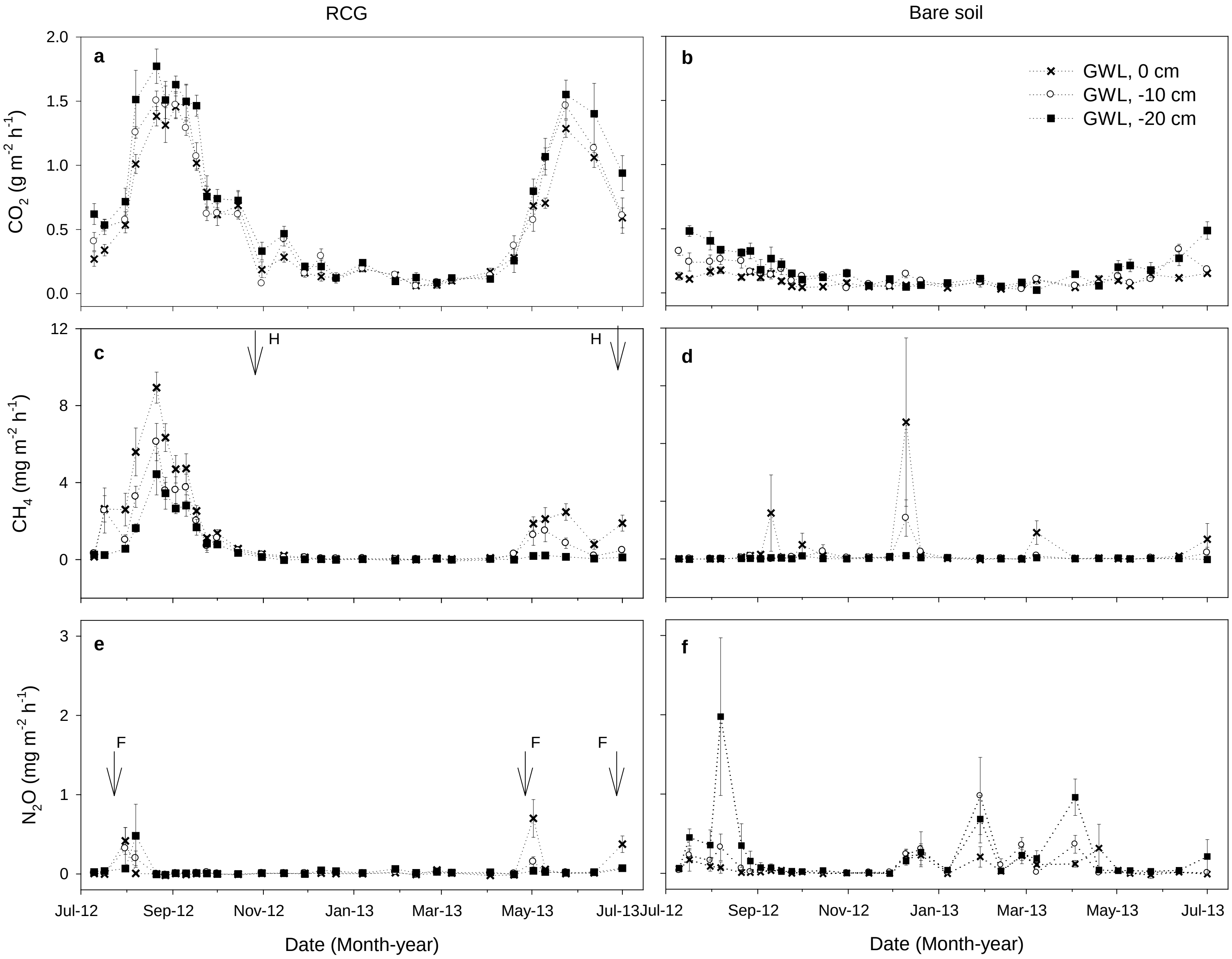
RCG
Bare soil
a
b
c
d
e
f
GWL, 0 cm
GWL, -10 cm
GWL, -20 cm
CO_2 (g m^{-2} h^{-1})
CH_4 (mg m^{-2} h^{-1})
N_2O (mg m^{-2} h^{-1})
0.0
0.5
1.0
1.5
2.0
0
4
8
12
0
1
2
3
H
H
F
F
F
Jul-12
Sep-12
Nov-12
Jan-13
Mar-13
May-13
Jul-13
Date (Month-year)
Date (Month-year)

Fig. 5.

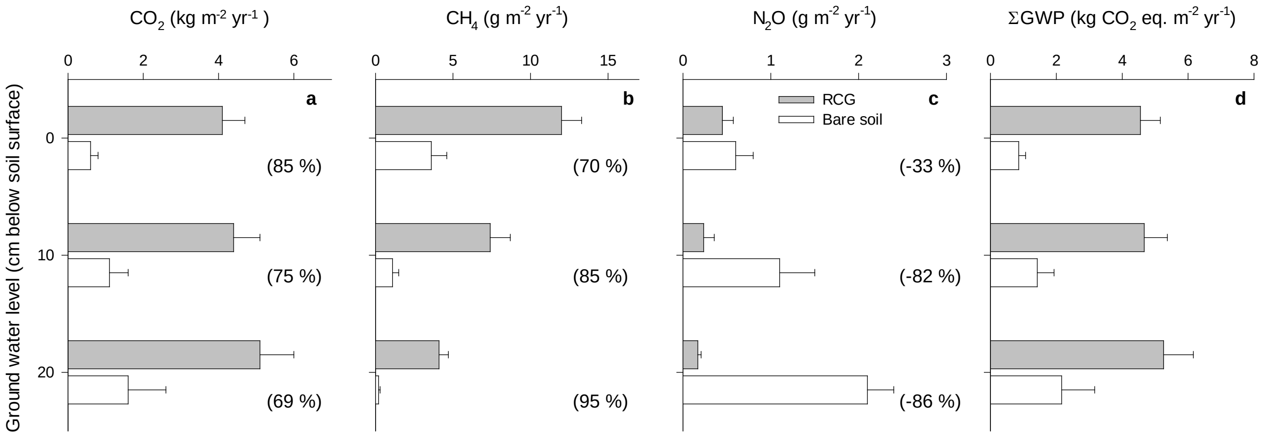
CO2 (kg m-2 yr-1)
0
2
4
6
a
(85 %)
(75 %)
(69 %)
CH4 (g m-2 yr-1)
0
5
10
15
b
(70 %)
(85 %)
(95 %)
N2O (g m-2 yr-1)
0
1
2
3
c
RCG
Bare soil
(-33 %)
(-82 %)
(-86 %)
ΣGWP (kg CO2 eq. m-2 yr-1)
0
2
4
6
8
d
Ground water level (cm below soil surface)
0
10
20

Fig. 6.

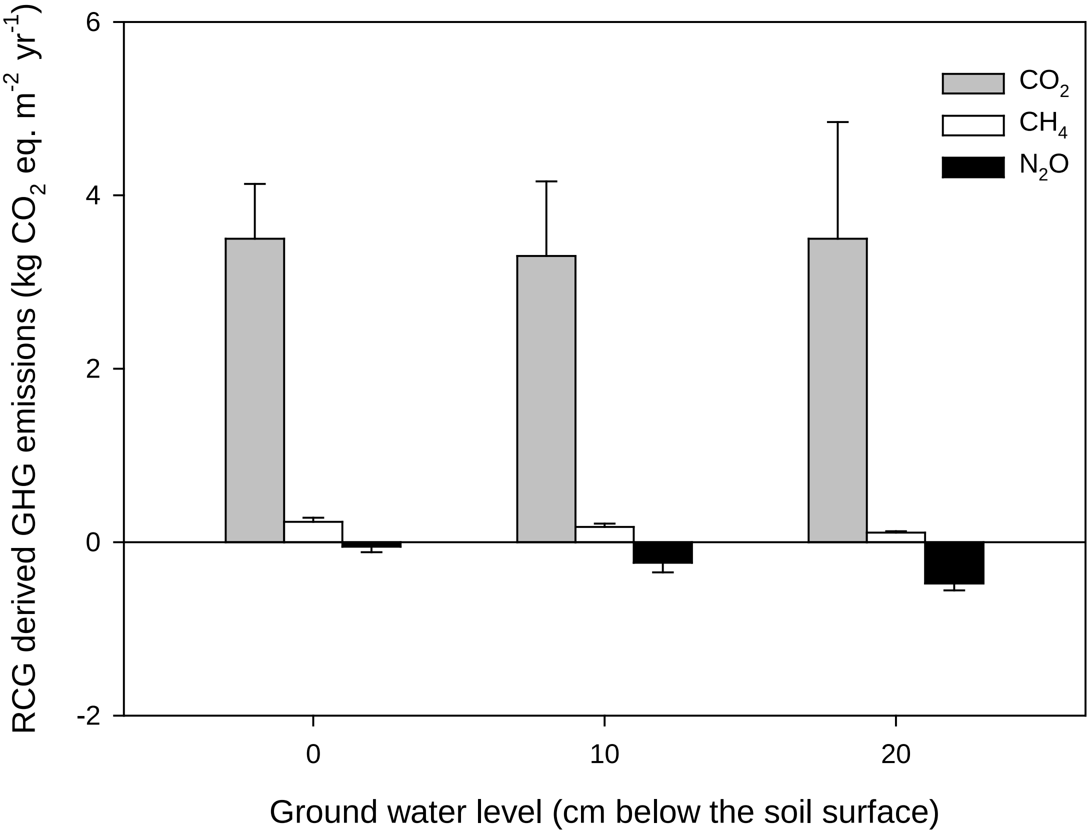
6
4
2
0
-2
RCG derived GHG emissions (kg CO_2 eq. m^{-2} yr^{-1})
CO_2
CH_4
N_2O
0
10
20
Ground water level (cm below the soil surface)

Fig. 7.