Ice age wetland biogeochemistry: The influence of carbon dioxide starvation on wetland methane emissions

Thesis

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Ice Age Wetland Biogeochemistry: The Influence of Carbon Dioxide Starvation on Wetland Methane Emissions

A thesis submitted for the degree of Doctor of Philosophy

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March 2010

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DATE OF AWARD: 3 NOV 2010

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Ice core records show that the atmospheric concentration of methane (CH$_4$) during the Last Glacial Maximum (LGM) was 40-50% lower than during the preindustrial Holocene. To understand this natural variation it is important to know how the sources and sinks of CH$_4$ change over time. Natural wetlands were the single largest contributor of CH$_4$ to the atmosphere in glacial times, yet models used to estimate their behaviour and CH$_4$ flux are largely based around relationships derived under modern day conditions. This thesis responds to this issue by exposing wetland mesocosms with contrasting nutrient availability, to the atmospheric concentration of carbon dioxide (CO$_2$) present at the LGM for 2 years.

At the end of this experiment, total CH$_4$ flux was suppressed by an average of 29% in the nutrient rich fen (P < 0.05). In contrast, the nutrient poor bog showed no response to the treatment (P > 0.05). Further exploring the effects of CO$_2$ starvation showed that the fen ecosystem exhibited notable reductions in dissolved organic carbon, dissolved CH$_4$ and a change in the response of CH$_4$ flux to changing temperature, variables and relationships which all remained unchanged in the bog. The contrasting response of the two ecosystems to CO$_2$ starvation could be largely explained by differences in nutrient status, species composition and dominant CH$_4$ production pathways. In particular, it is hypothesised that bog plants under LGM CO$_2$ concentrations supplemented photosynthesis through the use of subsurface derived CO$_2$, thus counteracting the treatment effect.

The results from this thesis suggest that the CH$_4$ source strength of late-glacial and early Holocene wetlands may currently be over-estimated because fen ecosystems are a far smaller CH$_4$ source under low atmospheric [CO$_2$] than they are today. Furthermore, the results provide new insights into the role of glacial atmospheric CO$_2$ concentrations in influencing CH$_4$ emissions from terrestrial ecosystems and provide empirical evidence for a connection between glacial-interglacial changes in atmospheric CH$_4$ and CO$_2$ concentrations observed in ice cores.
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<table>
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<th>Abbreviation</th>
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<th>Description</th>
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<tr>
<td>[]</td>
<td>Concentration</td>
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<td>[CH₄]ₐm</td>
<td>Atmospheric Methane Concentration</td>
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<tr>
<td>[CO₂]ₐm</td>
<td>Atmospheric Carbon Dioxide Concentration</td>
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</tr>
<tr>
<td>B/A</td>
<td>Boling-Allemd</td>
<td></td>
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<tr>
<td>CCDS</td>
<td>CO₂ Control and Distribution System</td>
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<td>CEU(s)</td>
<td>Controlled Environment Unit(s)</td>
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<td>CH₄</td>
<td>Methane</td>
<td></td>
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<tr>
<td>CO₂</td>
<td>Carbon Dioxide</td>
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<td>CRDLS</td>
<td>Cavity Ring Down Laser Spectroscopy</td>
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<td>DIC</td>
<td>Dissolved Inorganic Carbon</td>
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<td>DM</td>
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<tr>
<td>DOC</td>
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<tr>
<td>FID</td>
<td>Flame Ionisation Detector</td>
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<td>FMA</td>
<td>Los Gatos Research RMA-200 Fast Methane Analyser</td>
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</tr>
<tr>
<td>FTIR</td>
<td>Fourier Transform Infrared Spectroscopy</td>
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<td>Greenhouse Gas(es)</td>
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<td>GPP</td>
<td>Gross Primary Production</td>
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<td>Nondispersive Infrared</td>
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<td>Net Ecosystem Exchange</td>
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</tr>
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<td>NH</td>
<td>Northern Hemisphere</td>
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<td>NPOC</td>
<td>Non-Purgeable Organic Carbon</td>
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<td>PIH</td>
<td>Pre-Industrial Holocene</td>
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<tr>
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<td>Abbreviation</td>
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<tr>
<td>--------------</td>
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<tr>
<td>RF</td>
<td>Radiative Forcing</td>
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<td>Standard Error</td>
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<tr>
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<td>Total Carbon</td>
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<tr>
<td>TDL</td>
<td>Tuneable Diode Laser</td>
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<td>TOC</td>
<td>Shimadzu Total Organic Carbon VCSN analyser</td>
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<tr>
<td>T\text{opt}</td>
<td>Thermal Optimum</td>
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<tr>
<td>UV</td>
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<tr>
<td>VFA</td>
<td>Volatile Fatty Acid</td>
<td></td>
</tr>
<tr>
<td>WSL</td>
<td>West Siberian Lowland</td>
<td></td>
</tr>
<tr>
<td>YD</td>
<td>Younger Dryas</td>
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CHAPTER ONE

Introduction

1.1 Overview

Atmospheric CH₄ plays an important role in the radiative balance of the Earth's atmosphere. Over a 100-year time scale, the global warming potential of 1 kg of CH₄ in the atmosphere is ~33 times greater than for the same mass of CO₂ (Shindell et al., 2009). Therefore, to accurately model conditions in the past and the effect of global warming in the future, it is crucial to understand how the sources and sinks of CH₄ can vary over time. CH₄ is produced from a variety of natural environments and anthropogenic activities (Denman et al., 2007). Wetlands are the largest natural source of CH₄, and their global extent and productivity can dramatically influence atmospheric [CH₄] (hereafter referred to as [CH₄]ₘ₉) (Brook et al., 2000). Wetlands are characterised by high water tables where carbon accumulates due to low decomposition rates (Gorham, 1991). Within these ecosystems CH₄ is produced by a group of microbes called methanogens during the terminal stages of anaerobic decomposition (Whalen, 2005).

Ice core records have repeatedly shown that during glacial maxima the [CH₄]ₘ₉ is reduced by ~50% compared to peak interglacial concentrations (Petit et al., 1999, Spahni et al., 2008).
This natural phenomenon may be explained in part by an increased atmospheric CH$_4$ sink caused by a global reduction in biogenic volatile organic carbon compounds (BVOC) from forest (Valdes et al., 2005, Kaplan et al., 2006), but what remains uncertain is the contribution of natural wetlands to the [CH$_4$]$_{am}$ during glacial maxima. It has been suggested that both wetland extent and global wetland CH$_4$ emissions did not vary significantly at the LGM to account for the observed changes in [CH$_4$]$_{am}$ (Kaplan et al., 2006). However, the exact source strength of wetlands during glacial maxima remains unknown and is currently simply estimated using wetland emission models that utilise relationships derived under modern day conditions. One controlling variable on wetland CH$_4$ emission that has received insufficient attention to quantify its effect, is the influence of the sub-ambient atmospheric [CO$_2$] ([CO$_2$]$_{am}$) present at the time.

Glacial maxima are characterised by low (~180 ppmv) [CO$_2$]$_{am}$ (Luthi et al., 2008). Studies have shown that the [CO$_2$]$_{am}$ could potentially be one of the largest controls on CH$_4$ emissions from wetlands (Dacey et al., 1994, Hutchin et al., 1995). Increasing the [CO$_2$]$_{am}$ is hypothesised to increase plant derived methanogen substrates as a consequence of increased plant productivity and biomass. Given that the LGM was characterised by exceptionally low CO$_2$ concentrations that would have limited photosynthesis and the export of carbon into the rhizosphere (Dippery et al., 1995, Sage, 1995), this thesis explores the idea that the LGM [CO$_2$]$_{am}$ would have had an important limiting effect on CH$_4$ flux in a way that contrasts with those observed in CO$_2$ enrichment studies (Hutchin et al., 1995, Megonigal & Schlesinger, 1997), i.e. a decrease in flux would be observed. To test this hypothesis, a two growing season controlled environment experiment was designed to investigate how LGM CO$_2$ concentrations might have influenced CH$_4$ flux from two contrasting natural temperate wetland ecosystems.
The following sections in this Chapter outline the role of greenhouse gases on climate (1.2) and the natural variations in \([\text{CH}_4]_{\text{atm}}\) over the last 800,000 years (1.3). The Chapter then focuses on the largest natural source of \(\text{CH}_4\) (wetlands) and the major biological processes behind the release of \(\text{CH}_4\) from it to the atmosphere (1.4). The Chapter then provides background information on the LGM and the global conditions at the time, before highlighting the most likely mechanisms for creating high atmospheric \(\text{CH}_4\) concentrations during interstadials (~700-800 ppbv) and low concentrations (~350 ppbv) during glacials (1.5). Sections 1.6 and 1.7 deal with the main issue investigated in this thesis, the response of plants and wetland environments to changes in \([\text{CO}_2]_{\text{atm}}\). The chapter finishes with a summary of the aims and layout of the thesis.

1.2 Climate Change and the Greenhouse Effect

1.2.1 Radiative Forcing (RF) and \(\text{CH}_4\)

Global temperature on Earth is determined by incoming solar radiation from the Sun, the properties of Earth’s surface and the surrounding atmosphere (Soloman et al., 2007). The top of the atmosphere reflects approximately one-third of the short wavelength radiation received from the Sun, with the remaining two-thirds of the energy either absorbed by the surface of the planet or reflected. Approximately half the solar radiation reaching Earth’s surface is absorbed and radiated back into the atmosphere as infrared radiation (IR). Some of this IR passes through the atmosphere, but most is absorbed and reradiated in all directions by molecules in the atmosphere and clouds. This is called the greenhouse effect and causes the warming of Earth’s surface and the lower atmosphere (Le Treut et al., 2007). Increases in the atmospheric abundance of molecules that absorb radiation in this spectral region (~7 to 12 µm) contribute to the greenhouse effect. Molecules such as these
are called greenhouse gases (GHGs) and can be generally categorised as either long-lived (e.g. CO₂ and CH₄) or short-lived (e.g. CO, NOₓ) GHGs based on their residence time in the atmosphere. On a molecular basis, water vapour is the most potent GHG followed by CO₂, CH₄, nitrous oxide (N₂O) and ozone (O₃) are other gases present in the atmosphere that also contribute to the greenhouse effect. Changes in the concentration of atmospheric GHGs is a natural phenomenon, however since the industrial revolution, concentrations of GHGs such as CO₂, CH₄ and N₂O have increased to levels unprecedented during the last 800,000 years (Loulouergue et al., 2008, Luthi et al., 2008).

![Figure 1.1 Radiative forcing values for carbon dioxide (CO₂), methane (CH₄), tropospheric ozone (O₃), sulphate (SO₄), nitrate (NO₃), water (H₂O) carbon monoxide and volatile organic compounds (CO+VOC) mono-nitrogen oxides (NOx) sulphur dioxide (SO₂) and ammonia (NH₃) from the year 1750 to 2000. Original diagram by Shindell et al., (2009).]
RF is used to assess the contribution of a perturbation (in most cases, the increase since 1750 A.D., figure 1.1) to the net irradiance at the top of the tropopause after allowing the stratosphere to adjust to radiative equilibrium. The direct RF of a greenhouse gas is determined by the increase in abundance from its pre-industrial value to present day concentration. In the case of atmospheric CH$_4$, its pre-industrial value was 700 ppbv (Etheridge et al., 1998, Petit et al., 1999), its modern day concentration is $\sim$1800 ppbv (Dlugokencky et al., 2009) with a calculated abundance based RF value of 0.48 W m$^{-2}$ (Forster et al., 2007, Shindell et al., 2009). The concentrations of other atmospheric compounds can indirectly contribute to the RF of greenhouse gases because certain species are linked through atmospheric chemistry (e.g. CH$_4$, O$_3$ and aerosols). The sum of the forcings that take place via response of a particular species can be calculated in an emission based RF assessment (Shindell et al., 2009, figure 1.1). An emission based assessment shows that CH$_4$ emissions provide the second largest contribution to historical warming after CO$_2$ and places the combined direct and indirect RF value of CH$_4$ close to 1 W m$^{-2}$ (Shindell et al., 2009).

1.2.2 Glacial-Interglacial Cycles

RF can also occur naturally over glacial-interglacial cycles through periodic changes in Earth’s orbit around the Sun, which controls the seasonal and latitudinal distribution of incoming solar radiation (insolation). The Milankovitch (orbital) theory describes how precession, obliquity and eccentricity changes in Earth’s orbit and axial tilt can cause ice ages to develop. Precessional changes moderate the time of the year Earth is closest to the Sun with quasi-periodicities of approximately 19,000 and 23,000 years. Precessional changes alter the position and duration of the seasons and strongly modulate the latitudinal
and seasonal distribution of insolation. The obliquity (tilt) of Earth's axis varies between 22° and 24.5° with a strong quasi-periodicity around 41,000 years. A change in angle of Earth modulates seasonal contrasts as well as changes in mean annual insolation. The eccentricity of Earth's orbit around the Sun has a longer quasi-periodicity at 400,000 and 100,000 years. Changes in eccentricity alone have a limited impact on insolation. However, eccentricity interacts with obliquity and precessional changes to significantly modulate the effects associated with each of them. For a more detailed description of orbital forcing and glacial-interglacial transition mechanisms see the Technical Summary (Soloman et al., 2007) and Chapter six (Jansen et al., 2007) of the Climate Change: The Physical Basis, 2007, Intergovernmental Panel on Climate Change (IPCC) Report.

The start of ice ages (figure 1.2) coincide with reduced summer insolation at high latitudes (near 65°N) in the northern hemisphere (NH) that enables winter snow fall and ice sheets to persist all year round (Jansen et al., 2007). Orbital insolation changes alone are not enough to allow perennial snow cover. Shifts in the northern treeline position, expansion of sea ice at high latitudes and warmer low-latitude oceans as a source of moisture for the ice sheets, provide feedbacks that amplify the local insolation forcing over the high-latitude continents and allow for the growth of ice sheets (Crucifix & Loutre, 2002, Jackson & Brooccoli, 2003, Meissner et al., 2003, Kohler et al., 2005). Ice age terminations are thought to be consistent with an increase in NH summer insolation that causes a retreat in northern ice sheets (Cheng et al., 2009). Meltwater is thought to enter the North Atlantic and alter the oceanic and atmospheric circulation and associated fluxes of heat and carbon, which leads to increases in atmospheric \([\text{CO}_2]_{\text{am}}\) and Antarctic temperatures that drive the termination in the Southern Hemisphere (Cheng et al., 2009).
During glacial times ice core records show that the $[\text{CO}_2]_{\text{atm}}$ varied in the range of 180 to 200 ppmv (Petit et al., 1999, Siegenthaler et al., 2005, Luthi et al., 2008) (figure 1.2). Milankovitch cycles are thought to be the fundamental driving force behind glacial-interglacial oscillations in $[\text{CO}_2]_{\text{atm}}$, however the direct energy changes associated with orbital cycles alone is not enough to account for large scale changes (Archer et al., 2000, Sigman & Boyle, 2000, Skinner, 2009). Positive feedbacks within Earth's climate system amplify orbital forcing to produce glacial cycles, but the operation of these internal feedbacks is poorly understood. On glacial-interglacial timescales $[\text{CO}_2]_{\text{atm}}$ is mainly governed by the interplay between ocean circulation, marine biological activity, ocean-sedimentation interactions, sea water carbonate chemistry and air-sea exchange (Jansen et al., 2007). Glacial $[\text{CO}_2]_{\text{atm}}$ would be reduced by 30 ppmv due to the increased solubility of CO$_2$ in colder glacial oceans, however changes of this magnitude would be counteracted
by reduced solubility of CO₂ in a more saline global ocean and a large reduction in the terrestrial biosphere under glacial conditions (Sigman & Boyle, 2000).

More complex inter-reservoir mechanisms are required to explain glacial-interglacial changes. Hypotheses generally fall into three main categories: (1) those involving an increase in the export rate of organic carbon to the deep sea (Broecker, 1982b, Broecker, 1982a); (2) those involving a reduction in the ‘ventilation’ of water exported to the deep Southern Ocean (Keeling & Stephens, 2001, Watson & Garabato, 2006); and (3) those involving changes in whole ocean chemistry and ‘carbonate compensation’, possibly promoted by changes in the ratio of organic carbon and carbonate fluxes to the deep sea (Archer & Maierreimer, 1994). It appears likely that a range of mechanisms act in unison to create lower [CO₂] atm during glacial times compared to interglacial times (Kohler et al., 2005).

1.3 Atmospheric CH₄ Through Time

1.3.1 Atmospheric [CH₄] in the Last 800 kyr

As with CO₂, the analysis of air bubbles trapped inside ice provides an accurate measurement of [CH₄] atm during the late Pleistocene and early Holocene (e.g. Petit et al., 1999, Spahni et al., 2005). This technique has shown that the concentration of CH₄ in the atmosphere (pre-anthropogenic influence) over the last 800,000 years has varied between ~350 and ~700-800 ppbv during glacial and interglacial periods, respectively (Loulergue et al., 2008, figure 1.3).
Records from both Greenland and Antarctica provide a consistent pattern of CH$_4$ levels that are dominated by ~100,000 year glacial-interglacial cycles (Chappellaz et al., 1990, Brook et al., 1996, Petit et al., 1999, Spahni et al., 2005). Combining the influence of all the orbital periodicities (100, 41, 23 and 19 kyr) using spectral analysis, shows that Earth’s orbital pattern dictates the [CH$_4$]$_{atm}$ (Loulergue et al., 2008). The orbital pattern affects the magnitude of CH$_4$ sources and sinks in a way that creates a low concentration during glacial maxima and higher concentrations during interstadials (figure 1.2 and 1.3). The main CH$_4$ sources and sinks are described in section 1.3.4 and the current understanding regarding the causes of glacial maxima low [CH$_4$]$_{atm}$ is explained in section 1.5.2. The background atmospheric CH$_4$ level is thought to be mainly modulated by tropical wetlands and/or volatile organic compound emissions from tropical forests during the late Quaternary, with overshoots every 100 kyr associated with varying extents of northern ice sheets and periglacial wetlands (Adams et al., 2001, Valdes et al., 2005, Kaplan et al., 2006, Loulergue et al., 2008). Tropical monsoon patterns could play an important role in determining the CH$_4$ level, particularly at precessional periodicities (Clement et al., 2004,
Loulergue et al., 2008). This is because orbital forcing modulates tropical monsoon patterns (Liu et al., 2003) and the position of the intertropical convergence zone (Chiang et al., 2003). These two factors affect precipitation rates and influence wetland extent and OH radical atmospheric chemistry by altering volatile organic compounds emissions from forests.

1.3.2 Late Pleistocene/Early Holocene Concentrations

Ice core records show that \([\text{CH}_4]_{\text{am}} \) rose from \(-350\) to \(650\) ppbv between the LGM and the Bølling-Allerød (B/A) warm period (\(-15\) to \(13\) ka) as shown in figure 1.4. The concentration then declined during the Younger Dryas (YD) stadial (\(-13\) to \(11.5\) ka) by \(200\) ppbv (figure 1.4). The YD was a rapid return to glacial conditions in the higher latitudes of the NH, which may have been caused by the shutdown of the North Atlantic thermohaline circulation in response to a sudden influx of fresh water from deglaciation in North America (Alley, 2000, Broecker, 2006a, Broecker, 2006b). After the YD, the atmospheric concentration rose rapidly to over \(700\) ppbv in the early Holocene (\(11\) to \(8\) ka) and then declined again between \(8\) and \(6\) ka (Blunier et al., 1995). The sudden rise of \(\text{CH}_4\) at the beginning of the B/A and at the end of the YD has been subject to intense research and speculation. Modelling studies have shown that changes in the atmospheric concentration of OH radicals during this time are unable to account for the increase (Thompson et al., 1993, Martinerie et al., 1995); therefore the observed changes are likely to have been driven by increases in \(\text{CH}_4\) sources. Several hypotheses have been suggested regarding possible sources. These include increased \(\text{CH}_4\) emissions from: circumarctic peatlands (MacDonald et al., 2006), Russia’s West Siberian Lowlands (Smith et al., 2004), tropical wetlands (Chappellaz et al., 1993), marine clathrates (Kennett et al., 2000) and thermokarst lakes (Walter et al., 2007). Recent isotope studies indicate that a low latitude
wetland source is likely to be responsible for the majority of the observed rise, rather than marine clathrates (Schaefer et al., 2006, Sowers, 2006, Petrenko et al., 2009).

1.3.3 Pre-Industrial to Present Concentrations

Atmospheric CH₄ levels steadily increased from 5 ka until the start of the industrial revolution. This steady rise has recently provoked the question; when did humans begin to influence the atmospheric concentrations of GHGs? Ruddiman et al., (2003) argue that the natural trend during previous interglacials was downwards, therefore by definition the observed trend is anomalous by comparison. There remains some scepticism surrounding this claim, with particular focus on the importance of precessional forcing on glacial-
interglacial CH$_4$ excursions (Schmidt et al., 2004). However, if this theory is correct, it is possible that anthropogenic activity could have offset an incipient glaciation. Ruddiman (2008) proposes that the early increase in CH$_4$$_{atm}$ was caused by an expansion in irrigated rice agriculture in China, at a time when the Asian population was rapidly increasing (Li et al., 2007). Alternative explanations for this rise in CH$_4$$_{atm}$ include: a reduction in ice sheet volumes, higher wetland emissions from northern latitudes and the growth of large river delta CH$_4$ emitting systems (Schmidt et al., 2004). An increase in the NH wetland CH$_4$ flux is a plausible alternative, as this is supported by the finding of significant increases in peat growth rates from 3 ka to 1 ka in Canada (Zoltai & Vitt, 1990) and Sweden (Franzen, 1994).

Present day CH$_4$$_{atm}$ is ~1800 ppbv (Denman et al., 2007, Dlugokencky et al., 2009). Ice core records and global monitoring networks show that the CH$_4$ in the atmosphere has more than doubled since the industrial revolution (Dlugokencky et al., 1998, Luthi et al., 2008). The majority of this increase was due to an increase in emissions from anthropogenic sources (Etheridge et al., 1998). More recently, the average global growth rate of atmospheric CH$_4$ has decreased from an average of ~14 ppbv yr$^{-1}$ (equivalent to an imbalance between emissions and losses of 40 Tg yr$^{-1}$) in the 1980s, to a recent average global growth rate of ~4 ppbv yr$^{-1}$ (Dlugokencky et al., 2009). A global growth rate as low as this is equivalent to a decrease in global emissions at a rate of 1.0 ± 0.2 Tg of CH$_4$ yr$^{-1}$ since 1993 (Bousquet et al., 2006). A recent (last 30 years) trend in the global atmospheric CH$_4$ growth rate has been of large fluctuations from year-to-year (Dlugokencky et al., 2009). Since emissions from anthropogenic sources change gradually, it is likely that the interannual variability in CH$_4$ growth rate is caused by changes in emissions from biomass burning, wetlands and changes to the atmospheric OH radical concentration (Dlugokencky et al., 1996). The origin of these changes is likely to be in the tropics as NH regions show
smoother variations with systematically less emissions in the 1990s compared to the 1980s (Bousquet et al., 2006). It is unclear whether a steady state has been achieved in the atmosphere where sources are equal to sinks, or whether this represents a temporary pause in the human-induced increase in atmospheric CH₄ (Bousquet et al., 2006).

1.3.4 Sources and Sinks

Modern day atmospheres are characterised by CH₄ released from a variety of natural and anthropogenic sources (Denman et al., 2007). Recent estimates using process-based and inverse modelling approximate the contemporary CH₄ yearly source at 503-610 Tg (CH₄) yr⁻¹ (Hein et al., 1997, Wuebbles & Hayhoe, 2002, Fletcher et al., 2004, Wang et al., 2004, Chen & Prinn, 2006). Anthropogenic sources, which account for 264-428 Tg (CH₄) yr⁻¹ (Hein et al., 1997, Wuebbles & Hayhoe, 2002, Fletcher et al., 2004, Wang et al., 2004, Chen & Prinn, 2006), are derived from biogenic emissions from agriculture and waste disposal. This includes landfill sites (17%), rice paddies (17%), biomass burning (14%), domestic ruminants (23%) and fossil fuel extraction (29%) (Wuebbles & Hayhoe, 2002).

Natural biogenic CH₄ emissions contribute 145-260 Tg (CH₄) yr⁻¹ to the atmosphere (Houweling et al., 2000, Wuebbles & Hayhoe, 2002, Fletcher et al., 2004, Wang et al., 2004, Chen & Prinn, 2006). The natural source includes emissions from wetlands (72%), termites (13%) and oceans (6%), with the remaining natural emissions (9%) made up from wild ruminants and hydrates (Wuebbles & Hayhoe, 2002). Significant amounts of CH₄ (40-60 Tg (CH₄) yr⁻¹) is also produced by bacterial and thermogenic processes from the Earth’s crust through faults, fractured rocks and geothermal gas seepage (Etiope & Klusman, 2002, Etiope, 2004). This source potentially accounts for 3 Tg (CH₄) yr⁻¹ from Europe, making it the second largest CH₄ source in this region behind natural wetlands.
(Etiope, 2009). Non-biogenic sources also include leaks that occur during natural gas processing, transmission and distribution.

The largest atmospheric abundances of CH$_4$ are found over the Gangetic plains of India, Southeast Asia and areas of China (Frankenberg et al., 2005, Frankenberg et al., 2008). These sources can be mostly attributed to rice cultivation, wetland emission and fossil fuel production (Frankenberg et al., 2005). Figure 1.5 shows areas in the world that are associated with enhanced CH$_4$ production. This image was created using data from the Scanning Imaging Absorption Spectrometer for Atmospheric Cartography (SCIAMACHY) instrument on board the European Space Agency’s Environmental Research Satellite (Frankenberg et al., 2005). Using a global chemistry model with modern day emission inventories, Frankenberg et al., (2005) found that there was a discrepancy between CH$_4$ recorded in the tropics from SCIAMACHY and their model predictions. This discrepancy could be explained by inaccurate tropical CH$_4$ emission projections from existing wetland sources, new CH$_4$ sources which are not fully accounted for, or a combination of both. Recent discoveries of new CH$_4$ sources, including aerobic CH$_4$ emission from terrestrial plants caused by UV radiation and other environmental stresses (Keppler et al., 2006, McLeod et al., 2008, Messenger et al., 2009), and CH$_4$ emission from tree trunks (Doronina et al., 2004, Mukhin & Voronin, 2008, Mukhin & Voronin, 2009), could help to explain an elevated tropical source. Since the original SCHIMACHY research by Frankenberg et al., (2005), the annual tropical emission estimates have been refined down from 260 to approximately 201 Tg CH$_4$, however, this still remains higher than previously anticipated (Bergamaschi et al., 2007, Frankenberg et al., 2008, Schneising et al., 2009).
Figure 1.5 SCHIMACHY measurements of column-averaged CH$_4$ Volume Mixing Ratio (VMR) in ppbv units. The measurements are averaged over the time period of August through to November 2003 on a $1^\circ$ horizontal grid. VMR is calculated using near-infrared spectrometers to calculate the column-averaged dry VMR of CH$_4$ in the atmosphere relative to the VMR of CO$_2$. For full details see Frankenberg et al., (2005).

The main removal mechanisms of atmospheric CH$_4$ are tropospheric degradation through reaction with the hydroxyl radical (OH), dry soil oxidation and transport to the stratosphere (Wuebbles & Hayhoe, 2002, Denman et al., 2007). Reaction with the OH radical (equation 1.1) is responsible for the removal of between 428-507 Tg (CH$_4$) yr$^{-1}$ (Hein et al., 1997, Wuebbles & Hayhoe, 2002, Fletcher et al., 2004, Wang et al., 2004), which accounts for 90% of atmospheric CH$_4$ (Lelieveld et al., 1998).

$$CH_4(g) + OH(g) \rightarrow CH_3(g) + H_2O(g)$$  \hspace{1cm} (Equation 1.1)

The hydroxyl radical is created through the photolysis of O$_3$ (equations 1.2-1.5), which is dependent on the solar ultraviolet (UV) radiation flux and water vapour concentration (Bahm & Khalil, 2004, Lelieveld et al., 2004). Photodissociation of O$_3$ at UV wavelengths...
produces electronically excited O($^1$D) atoms, which are reduced to the ground state O($^3$P) by air molecules in equation 1.3 (M represents air molecules N$_2$ and O$_2$). These molecules subsequently combine with O$_2$ to produce O$_3$, which feeds back into the cycle (equations 1.2-1.4). Only a small fraction of the O($^1$D) atoms form OH radicals, the exact amount depending on humidity (Lelieveld et al., 2004).

$$O_3 \xrightarrow{hv} O_2 + O(^1D) \quad (\lambda \leq 340 \text{ nm})$$  \hspace{1cm} (Equation 1.2)

$$O(^1D) + M \rightarrow O(^3P) + M$$ \hspace{1cm} (Equation 1.3)

$$O(^3P) + O_2 (+M) \rightarrow O_3 (+M)$$ \hspace{1cm} (Equation 1.4)

$$O(^1D) + H_2O \rightarrow 2OH$$ \hspace{1cm} (Equation 1.5)

There are two other atmospheric reactions that produce OH. The oxidation of CO produces atomic H that subsequently forms HO$_2$. HO$_2$ can react with both O$_3$ and NO to produce OH (equations 1.6 and 1.7). These reactions are generally known as radical recycling reactions (Lelieveld et al., 2002, Lelieveld et al., 2004). Equation 1.7 produces NO$_2$ which easily photodissociates and produces the ground state oxygen atoms that form O$_3$ through equation 1.4. This is the main tropospheric O$_3$ production mechanism (Lelieveld et al., 2004).

$$O_3 + HO_2 \rightarrow 2O_2 + OH$$ \hspace{1cm} (Equation 1.6)

$$NO + HO_2 \rightarrow NO_2 + OH$$ \hspace{1cm} (Equation 1.7)

OH can vary by up to an order of magnitude over single latitudes, which demonstrates the geographical dependence and the importance of local conditions in OH formation.
The OH radical is also the primary oxidant for most tropospheric pollutants, including carbon monoxide, nitrogen oxide species and organic compounds. Therefore any changes to these reactions can directly affect the oxidising capacity of the atmosphere and indirectly affect atmospheric CH$_4$ lifetime and atmospheric concentration.

The stratospheric loss (30-45 Tg (CH$_4$) yr$^{-1}$) and oxidation in soils (26-34 Tg (CH$_4$) yr$^{-1}$) account for ~10% of the global sink for atmospheric CH$_4$ (Hein et al., 1997, Wuebbles & Hayhoe, 2002, Fletcher et al., 2004, Wang et al., 2004). Consumption of atmospheric CH$_4$ by soils is an entirely biological process where methanotrophic bacteria oxidise CH$_4$ (Bender & Conrad, 1994). This sink is modified by environmental factors such as: temperature, soil moisture, soil nitrogen content, organic matter content and pH. Dry tropical ecosystems account for almost a third of this sink due to the high diffusivity of dry sandy soils and high temperature driven microbial activity (Ridgwell et al., 1999). An additional smaller CH$_4$ sink is oxidation by chlorine (Cl) atoms in the marine atmospheric boundary layer. This could possibly account for 19 Tg (CH$_4$) yr$^{-1}$ (Gupta et al., 1997, Tyler et al., 2000, Platt et al., 2004, Allan et al., 2005).

1.4 Wetlands as a CH$_4$ Source

1.4.1 Introduction

Wetlands are the largest individual source of CH$_4$ (Whalen, 2005). They can be defined as land where the water-table is close to or above the surface, or land which is saturated for a significant period of time (Charman, 2002). This would include most peatlands, but also ecosystems on mineral substrates where water is flowing or shallow. In this thesis, the ecosystems experimented with will be commonly referred to as either fen or bog. A fen is
classified as a peatland that is influenced by water from outside its own limits, typically by local geology and water movements in the form of upwellings or underground through-flow. A bog is also a peatland, yet this type of ecosystem only has access to water from rain and/or snow fall, therefore bogs tend to be nutrient limited. Access to contrasting nutrient supplies influences plant community composition and decomposition rate, and ultimately leads to different CH$_4$ production pathways (Galand et al., 2005) and rates (Hornibrook & Bowes, 2007) between fens and bogs.

Peat is the major constituent of most wetland soils and consists of accumulated plant remains which are slowly decomposing (Clymo, 1984). The decomposition rate is controlled by the quality of the litter (Latter et al., 1998), the abiotic conditions under which the litter decomposes (e.g. temperature, pH, oxygenation and moisture) (Brinson et al., 1981), along with the nature and abundance of decomposing organisms (Freeman et al., 2004b). A key feature of a wetland is that it exerts control over the movement and sequestration of carbon (Denman et al., 2007). Wetlands play an important role in the carbon cycle as they take up carbon in the form of CO$_2$ via photosynthesis from the atmosphere and lock it away in long-term stores (Gorham, 1991). Although wetlands provide a net sink for carbon (Christensen et al., 2003b), wetlands may actually elevate the warming capacity of the atmosphere due to the anoxic conditions promoting the production of CH$_4$ through methanogenesis (Bridgham et al., 2006). For example, northern peatlands have a net carbon accumulation rate of 76 Tg C yr$^{-1}$, however they release 46 Tg CH$_4$ yr$^{-1}$ (Gorham, 1991) which is equivalent to ~12% of the total global emission amount (Wuebbles & Hayhoe, 2002). Within wetlands, CH$_4$ is produced as a by-product to adenosine triphosphate (ATP) synthesis by methanogenic archaea. This specialised group of microbes inhabit anaerobic environments and utilise the end products of fermentation and hydrolysis in anaerobic soils to produce energy.
1.4.2 Global Distribution of Wetlands

The present global wetland area is estimated at between $5.2 \times 10^6$ km$^2$ (Chappellaz et al., 1993) and $11.0 \times 10^6$ km$^2$ (Kaplan, 2002), with the majority of wetlands (approximately one-half of the total area) located between 50°-70°N (Matthews, 2000). Defining what constitutes a wetland can be difficult considering the seasonal nature of some areas. Using satellite data, Prigent et al., (2001) were the first to quantify seasonality of global inundation with a clustering analysis of a suite of satellite observations covering a wide spectral range including passive and active microwaves, visible and near-IR observations, together with a linear mixing model to estimate inundated pixel fractions. Using this analysis, they estimated a maximum of $5.75 \times 10^6$ km$^2$ to a minimum of $2.16 \times 10^6$ km$^2$ for natural wetlands, irrigated rice fields and lakes/rivers. The largest wetland extents were found in boreal regions, with a second latitudinal belt between the tropics (Prigent et al., 2001).

1.4.3 Methane Production

Methanogens are the only group of microbes that produce methane or any other hydrocarbon as a major catabolic product. They are phylogenetically classified as Archaeobacteria which are distinct from eukaryotes and bacteria due to a number of characteristics (e.g. distinct ribosomal RNA sequences and membrane lipids (Boone et al., 1993)). They can be classified into five orders, namely Methanopyrales, Methanobacteriaceae, Methanococcales, Methanomicrobiales and Methanosarcinales (Garcia et al., 2000). Methanogenesis is the terminal step in carbon flow in many anaerobic habitats. Typically, methanogens utilise only one or two substrates usually
containing one carbon molecule, which means methanogens are dependent on other anaerobes for their substrates.

Methanogens can be found in the complete range of salinities from freshwater to hypersaline and from cold marine sediments (2°C) to geothermal areas above 100°C (Zinder, 1993, Garcia et al., 2000). Most methanogens have a pH optimum near neutrality, yet some species can exist in extreme pH environments such as peat bogs, which can be as low as pH 3-4 (Dunfield et al., 1993, Sjors & Gunnarsson, 2002). There are three different methanogenic ecosystems found in nature (Garcia et al., 2000). The first environment is where complex organic matter is completely degraded, which includes wetlands, rice paddy soils and marine sediments. The second is where the process of mineralisation is incomplete and the intermediate products which form (e.g. volatile fatty acids) are reabsorbed into the bloodstream of living creatures, e.g. ruminants. The third environment is the absence of organic matter where methanogenesis occurs only from geochemical hydrogen formed as part of the geological process.

The microbial decomposition of organic material and the production of CH₄ in wetlands are illustrated in figure 1.6. The production of CH₄ from anaerobic sediments is as a result of a syntrophic relationship between non-methanogenic bacteria and methanogen archaea. This can be divided into four major steps: hydrolysis, fermentation (or acidogenesis), syntrophic acetogenesis and ultimately methanogenesis (Boone, 2000, Garcia et al., 2000, Whalen, 2005). Hydrolytic and fermentative bacteria are responsible for degrading complex organic matter into short chain volatile fatty acids (carboxylic acids), alcohols, CO₂ and H₂ (Whalen, 2005). Only a small fraction of the substrate at this stage is available for methanogens to utilise and convert to energy, CH₄, CO₂ and H₂O. Volatile fatty acids (excluding acetate) and short carbon chain molecules cannot be broken down by
methanogens. They require a specialised group of bacteria called obligate proton reducing acetogens (Boone, 2000). This bacterium oxidises simple substrates (e.g. propionate, butyrate and aromatic compounds) to acetate and CO₂ using H⁺ as an electron acceptor to form H₂. Methanogenic archaea utilise these by-products by acting as living electron acceptors, reducing CO₂ to CH₄, with electrons provided by proton reducing acetogens via interspecies transfer. In wetlands where homoacetogenic bacteria replace methanogens as H₂ scavengers, acetate is produced and CH₄ production via acetate reduction is enhanced (Whalen, 2005).

The two major pathways of methanogenesis in wetland soils are acetotrophic and hydrogenotrophic (Chapelle, 2001). Acetotrophic methanogens reduce acetate according to
equation 1.8; hydrogentrophic methanogens reduce CO$_2$ using H$_2$ as an electron donor as shown in equation 1.9.

\[
CH_3COOH \rightarrow CH_4 + CO_2 \quad \Delta G^0 = -31 \text{ kcal} \quad \text{(Equation 1.8)}
\]
\[
4H_2 + CO_2 \rightarrow CH_4 + 2H_2O \quad \Delta G^0 = -32.4 \text{ kcal} \quad \text{(Equation 1.9)}
\]

In acidic wetlands, the main CH$_4$ production pathway is thought to be hydrogentrophic (Whiticar et al., 1986, Lansdown et al., 1992, Hornibrook et al., 1997, Horn et al., 2003), with acetoclastic methanogenesis predominating in upper vegetated zones and a shift to CO$_2$ reduction in deep peat layers (Hornibrook et al., 1997, Chasar et al., 2000b). Deeper subsurface peat is largely recalcitrant and a poor source of fresh labile substrates, hence the shift away from acetotrophic methanogenesis to CO$_2$ reduction. Where strong surface and groundwater flows are present, it is possible to find methanogen communities associated with surface peats (obligate acetoclastic methanogens) in deep peat bottom layers (Putkinen et al., 2009). Acetoclastic methanogens are generally linked to fresh organic matter, therefore it is possible that the sub-surface flow of water transports acetate deep into the peat.

Differences in dominant CH$_4$ production pathways have been reported between contrasting nutrient status wetlands. Minerotrophic fens (nutrient rich) and ombrotrophic bogs (nutrient deficient) exhibit contrasting dominant CH$_4$ production pathways with bogs exhibiting methanogen communities dominated by CO$_2$/H$_2$ utilisers and more nutrient rich fens inhabited by a greater presence of obligate acetotrophs (Galand et al., 2005, Juottonen et al., 2005). Differences in abiotic and biotic factors between fens and bogs, such as pH
and plant community composition, could explain this contrast in dominant CH₄ production pathways. For example, sphagnum dominated bogs tend to have lower pH values and shallow rhizoid systems with no aerenchyma tissues when compared to nutrient rich fens. This difference would limit acetotrophic methanogenesis and promote CO₂ reduction in the bog.

1.4.4 Methane Consumption

Oxidation of CH₄ in aerobic soils requires the presence of methanotrophic bacteria and suitable soil conditions that allow the bacteria to be active (Bender & Conrad, 1995). Methanotrophic capabilities are recognised in members of two bacterial phyla; the Proteobacteria and Verrucomicrobia (Dedysh, 2009). The latter are generally associated with geothermal habitats, however Verrucomicrobia-related 16S rRNA gene sequences have been observed in Sphagnum peat (Dedysh et al., 2006). Proteobacteria methanotrophes subsist on C-1 compounds for energy production and assimilate formaldehyde as a carbon source for growth (Hanson & Hanson, 1996). These obligate methanotrophes oxidise CH₄ sequentially to methanol, formaldehyde, formate and finally to CO₂ in wetlands (Whalen, 2005). Proteobacteria methanotrophes can be separated into three assemblages based on the criteria of phylogeny, formaldehyde assimilation pathway, cell morphology and other biochemical characteristics. Type 1 includes the genera Methylomonas and Methylobacter, type 2 includes the genera Methylosinus and Methylocystis, and type X includes the genera Methylococcus (Hanson & Hanson, 1996).

Oxidation of CH₄ by all aerobic methanotrophes is initiated by the enzyme methane monooxygenase (MMO). The use of MMO to convert CH₄ to methanol is a defining characteristic of methanotrophic bacteria. A membrane bound or particulate form
(pMMO) is found in all methanotrophes, while a soluble form (sMMO) is restricted to mainly type 2 methanotrophes (Hanson & Hanson, 1996). Methanotrophes that use sMMO have a broader substrate range compared to pMMO utilisers, however pMMO has a lower oxygen requirement and cells that contain pMMO obtain higher growth yields from CH₄ (Whalen, 2005). During the process of CH₄ oxidation, formaldehyde is used as an intermediate molecule. Type 1 and type 2 methanotrophes use different metabolic pathways to derive energy from formaldehyde. Type 1 methanotrophic bacteria use a Ribulose Monophosphate (RuMP) pathway whereas type 2 uses a Serine pathway. Type X methanotrophes are capable of acquiring energy via both pathways as they have enzymes associates with both mechanisms (Hanson & Hanson, 1996). Characteristic differences between methanotrophes can lead to environments that are more suitable for different assemblages. Type 1 methanotrophes dominate in acidic (pH 3.5-5) wetlands, whereas in less acidic (pH 5-6) and colder wetland tundra, type 1 and 2 are of equal proportion (Dedysh, 2009).

More than 90% of CH₄ produced in anaerobic soils is consumed in aerobic layers before it is released into the atmosphere (Yavitt & Lang, 1988, Frenzel et al., 1992, Oremland & Culbertson, 1992, Sass et al., 1992, Sundh et al., 1995, Frenzel & Karofeld, 2000). Within soils, methanotrophic activity is generally classified into two groups: high affinity (low atmospheric CH₄ concentrations) and low affinity (high atmospheric CH₄ concentrations) (Segers, 1998, Le Mer & Roger, 2001). The transition point between the two is between 100-1000 ppmv (gas phase) (Bender & Conrad, 1995). CH₄ oxidation in methanogenic environments (e.g. peatlands, rice paddies and landfills) is a low affinity activity (Bender & Conrad, 1995, Le Mer & Roger, 2001). In wetlands, methanotrophes develop in the oxidised soil layer, in the aerobic rhizosphere of plants processing aerenchyma tissues and in the roots of rice plants (Bosse & Frenzel, 1997, Watson et al., 1997). Recently, Dedysh
et al., (2009) suggested that pMMO processing methanotrophes in wetlands could also metabolise carbon compounds (e.g. acetate) in the absence of CH₄. This removes the assumption that methanotrophes are limited by the presence of CH₄. The exact nature of this switch in substrates is yet to be identified.

1.4.5 Methane Transport Mechanisms

For wetland produced CH₄ to have an influence on the Earth's atmosphere it must first be transported out of the rhizosphere. CH₄ produced in wetland soils is released into the atmosphere by diffusion, ebullition and plant mediated transport (Schutz et al., 1991, figure 1.7, Chanton, 2005). Anaerobic peat layers typically contain higher concentrations of CH₄ when compared to the atmosphere. This sets up a concentration gradient between the two, where according to Fick's first law of diffusion, CH₄ randomly moves from the region of high concentration (soil) to the region of low concentration (atmosphere), with a magnitude that is proportional to the concentration gradient in one (spatial) dimension. This is represented by equation 1.10:

\[
J = -D \frac{\partial \phi}{\partial X}
\]  
(Equation 1.10)

where \(J\) is the diffusive flux (mol cm⁻² s⁻¹), \(D\) is the diffusion coefficient (cm² s⁻¹), \(\phi\) is the \([\text{CH}_4]\) (mol cm⁻³) and \(X\) is the depth of peat (cm). The diffusive flux through the soil is a slow process that is dependent on the rate at which methanotrophic bacteria consume CH₄ in oxygenated layers. The diffusive pathway is an important pathway where the ground cover is mainly sphagnum (Chasar et al., 2000a, Chasar et al., 2000b).
Ebullition is a process that releases CH$_4$ into the atmosphere in the form of gas bubbles (Reynolds et al., 1992). Bubbles form in peat because CH$_4$ is only partially soluble in water (Yamamoto et al., 1976). When the partial pressure of CH$_4$ (and other gases) is greater than the hydrostatic pressure in the peat, gas bubbles are formed. Up to 60% of CH$_4$ formed in anaerobic wetland soils can accumulate in the form of bubbles (Tokida et al., 2005). Newly formed bubbles are not released instantly to the atmosphere, they often require a trigger to release them. Bubble release can be triggered by a drop in atmospheric pressure (Tokida et al., 2007, Waddington et al., 2009), falling hydrostatic pressure (Strack et al., 2005) and a rise in temperature (Tokida et al., 1999).
et al., 2005) and a rise in temperature (Fechner-Levy & Hemond, 1996, Waddington et al., 2009). Ebulbition of bubbles can produce rapid movements of CH$_4$ in peat and can account for up to 50% of the total CH$_4$ emission from wetlands (Christensen et al., 2003b).

Many emergent (vascular) plants have large interior spaces, termed aerenchyma or lacunae, which act as gas conduits allowing oxygen into the rhizosphere and CH$_4$ into the atmosphere (Armstrong et al., 1991). The release of oxygen into anoxic zones supports root respiration and also contributes to the oxidation of CH$_4$ (Watson et al., 1997). CH$_4$ transportation through aerenchyma tissues often bypasses methanotrophes in oxygenated surface layers (Bellisario et al., 1999). Clipping and sealing the ends of wetland plants (Carex spp.) reduces CH$_4$ flux (Schimel, 1995) and also shows that the majority of CH$_4$ is released in the first 10 cm of the plant (Kelker & Chanton, 1997). Due to the aerenchyma pathway, larger CH$_4$ fluxes are recorded in areas with a high density of vascular plants compared to bryophyte dominated areas (Saarnio & Silvola, 1999). Wetland plants also release CH$_4$ through leaf surface conductance, which include both stomata and cuticle exchange pathways (Morrissey et al., 1993). The stomata pathway is sensitive to changes in environmental variables such as light, temperature and water vapour pressure (Yang et al., 2005), therefore a strong diurnal pattern of CH$_4$ release through wetland plant stomata is frequently measured (Morrissey et al., 1993, Knapp & Yavitt, 1995, Garnet et al., 2005). Vascular plants can account for up to 90-97% of measured CH$_4$ flux from wetlands (Waddington et al., 1996, Kelker & Chanton, 1997, King et al., 1998, Frenzel & Karofeld, 2000) due to their internal structure and ability to export labile carbon into the rhizosphere (Strom et al., 2005).
1.4.6 Factors Affecting Methane Emissions

The controlling variables of wetland CH$_4$ emissions are a combination of environmental and biological parameters which affect the interplay between CH$_4$ production, oxidation and gaseous transport pathways. One of the most significant environmental parameters is temperature (Macdonald et al., 1998). Temperature variations influence CH$_4$ production rates by altering carbon mineralisation, substrate supply and the rate of methanogenesis (van Hulzen et al., 1999, Hoj et al., 2008). Wetlands are consistently shown to respond to linear increases in temperature (0 to 30°C) with an exponential increase in CH$_4$ flux (Thomas et al., 1996, Daulat & Clymo, 1998). Temperature coefficients ($Q_{10}$) show a large range of 1 to 35 for methanogenesis in wetland soils (Whalen, 2005). The wide range in values is likely to reflect the temperature sensitivity of microbial processes that precede methanogenesis, as these processes limit the temperature response of methanogens (Bergman et al., 1998). Furthermore, temperature increases can also enhance CH$_4$ transport by increasing ebullition (Waddington et al., 2009). Temperature variation can also affect CH$_4$ oxidation in aerated layers in wetland soils (Dunfield et al., 1993). Temperature coefficients for CH$_4$ oxidation are lower than CH$_4$ production (1.8-2.9) (Whalen, 2005), however relative to methanogenesis, limited data exits on the temperature sensitivity of CH$_4$ oxidation.

CH$_4$ emissions from wetlands are dependent upon the rate of production of CH$_4$ and the rate at which it is consumed by methanotrophic bacteria. The position of the water-table in a wetland ecosystem is therefore one of the fundamental controls on emissions as this defines the boundary between CH$_4$ production and oxidation. Water-table manipulations within wetlands have shown that a high water-table produces large CH$_4$ fluxes and low CO$_2$ emissions (Blodau & Moore, 2003). Drawing down the water-table increases the
aerobic area which causes an increase in organic decomposition that produces less CH₄ but more CO₂ (Moore & Dalva, 1993a, Daulat & Clymo, 1998, Blodau & Moore, 2003). Depth distributions of CH₄ oxidation and production show that they overlap and/or show close proximity to the local water-table height (Moore & Dalva, 1997). Methanotrophes are able to survive for extended periods in anoxia and resume activity within hours of the water-table falling (Roslev & King, 1996). In contrast, methanogens are not as tolerant to oxygen exposure, with CH₄ production severely suppressed after water-tables rise again (Whalen & Reeburgh, 2000).

The biggest influence on the magnitude of CH₄ emissions from wetlands is plant composition and productivity (Strom et al., 2005). The decomposition of roots, leaves and plants provide a long term source of carbon for methanogens, however radiocarbon analysis of CH₄ emitted from wetlands show that plant root exudates are the primary methanogenic substrate (Aravena et al., 1993, Chanton et al., 1995). Plants excrete a wide variety of compounds that allow them to influence the soil microbial community in their vicinity, manage herbivores, encourage beneficial symbiosis, change the chemical and physical properties of the soil and inhibit the growth of competing plant species (Walker et al., 2003). Root exudates released into the soil can account for up to 20% of all photosynthetically fixed carbon in plants (Hutsch et al., 2002). These organic species are often waste products of plant metabolism and include: mucilage, ectoenzymes, organic acids, sugars, phenolics and amino acids (Bais et al., 2006, Badri & Vivanco, 2009). The majority of root exudates tend to be lower molecular weight compounds (Walker et al., 2003), this making them readily available for obligate proton reducing acetogens and methanogens to utilise. The labile carbon exported by plants in the form of root exudates can be detected in CH₄ emission as soon as 2 to 12 hours after being radioactively labelled (King & Reeburgh, 2002, King et al., 2002, Strom et al., 2003).
The composition of wetland plants can produce contrasting CH₄ fluxes from wetlands. For example, Strom et al., (2005) found distinct differences in the functioning of wetland sedges in terms of their effects on CO₂ and CH₄ fluxes, bubble emission of CH₄, decomposition of ¹⁴C-labelled acetate into ¹⁴CH₄ and ¹⁴CO₂, rhizospheric oxidation of CH₄ to CO₂ and stimulation of methanogenesis through root exudation of substrate (e.g. acetate). Plant productivity also plays a significant role in determining CH₄ flux (Whiting & Chanton, 1993). A positive linear relationship exists between Net Ecosystem Exchange (NEE) and CH₄ flux in wetlands (Whiting & Chanton, 1993, Waddington et al., 1996). More recently, artificially induced shading has been shown to simultaneously lower NEE and CH₄ emission (Joabsson & Christensen, 2001). A similar experiment was performed by Strom et al., (2003), where during shading, they also measured a decrease in acetate in the soil which is likely to have been caused by a decrease in root exudation from the plants.

1.4.7 Fluvial Carbon Dynamics

It is important to monitor belowground variables in wetlands because CH₄ emissions are strongly correlated with the proportion of dissolved carbon in the rhizosphere. Where peatlands appear in the landscape they contribute significantly to the dissolved organic carbon (DOC), dissolved inorganic carbon (DIC) and particulate organic carbon (POC) content of rivers (Freeman et al., 2001). DOC mainly comprises of fulvic and humic acids (50-75%) and colloidal organic matter complexes (Hope et al., 1994). It also contains small quantities of fatty acids, carbohydrates, amino acids and hydrophilic acids which are important for methanogenesis. DIC is generally derived from carbonate sources such as the weathering of the underlying strata. DIC therefore comprises of HCO₃⁻, CO₂⁻, H₂CO₃ ions or exists as dissolved free CO₂ (Hope et al., 1994). Biological processes such as photosynthesis, respiration and decomposition can influence the flux of the free CO₂ in
stream water, altering the concentration of inorganically derived \( \text{HCO}_3^- \) ions (Strumm & Morgan, 1981). The distinction between POC and DOC is generally made on the basis of whether or not it can pass through a 0.45 \( \mu \text{m} \) filter. Concentrations of DOC within peat profiles can range from <10 to 120 mg L\(^{-1} \), with the highest concentrations measured in the summer at depths of 20 to 40 cm (Blodau et al., 2007).

The fluvial loss of carbon from peatlands is a significant pathway that accounts for ~10% of total carbon released by these types of ecosystems (Worrall et al., 2003). Research has shown that the area of peat cover in a catchment is directly linked to the [DOC] in rivers (Hope et al., 1994). The dominant pathway for water movement in peatlands is near surface flow and saturated overland flow. Sub-surface conduits can also form in peat (called macropores or pipes) which can account for over 30% of runoff in fens and blanket peats in the United Kingdom (Baird, 1997, Holden et al., 2001).

Over the last 20 years there has been more than a 90% increase in DOC in UK lakes and streams (Evans et al., 2006). Suggested reasons for this increase include: increases in temperature, changing hydrological factors, elevated net primary production caused by elevated CO\(_2\) and a reduction in sulphur pollution (Freeman et al., 2001, Evans et al., 2002, Freeman et al., 2004a, Evans et al., 2005, Evans et al., 2006). Evans et al., (2006) suggest that temperature increases causing an increase in organic matter decomposition rates could account for approximately 10-20% of the measured increase. Increased DOC in rivers associated with increased CO\(_2\) and net primary production may only account for 1-5% of the increase, and there appears to be no consistent pattern of hydrological changes. Decreases in SO\(_4\) and a recovery from acidification, combined with temperature increases, could therefore be the main reasons for the measured increase in DOC in rivers (Evans et al., 2006).
1.5 The Last Glacial Maximum (LGM)

1.5.1 Global Conditions

The LGM was ~21,000 years before the present and was characterised by an expansion and thickening of the ice sheets at high latitudes (Bonelli et al., 2009), a large reduction in both atmospheric CO₂ and CH₄ (Spahni et al., 2005, Luthi et al., 2008), and reduced vegetation cover (Henrot et al., 2009). The global annual mean surface air temperature was ~9°C, which is ~5-6°C colder than present day (Guilderson et al., 1994, Jahn et al., 2005, Jiang, 2008, Kim et al., 2008). Terrestrial annual global temperature was reduced on average by ~7°C, however terrestrial tropic temperatures were only on average ~2°C colder than today (Jiang, 2008). At high latitudes in the NH summer, temperatures would have remained below 0°C, with values over central parts of the Canadian plateau reaching as low as -25°C (Bonelli et al., 2009). The global decrease in temperature was mainly caused by an increased albedo in the NH caused by a growth in ice sheets and changing vegetation patterns. For example, the replacement of forest by herbaceous vegetation in response to cooling, increased the albedo of northern high latitudes, especially in winter and spring when the surface is covered in snow (Jahn et al., 2005). The ice sheet volume at the LGM is predicted to have been 52.5 x 10¹⁵ m³, with the Laurentide ice sheet having started to grow from 122 kyr BP when summer insolation started to decrease (Bonelli et al., 2009). In contrast, early Fennoscandian ice sheets were not stable enough to survive periods of increased summer insolation, and it was only after 75 kya BP (MIS5/MIS4) that both weak summer insolation and low [CO₂] atm allowed the Fennoscandian ice sheet to grow (Bonelli et al., 2009).
The LGM climate was drier, particularly around the Amazon and Congo basins (Kim et al., 2008), with global average precipitation and terrestrial annual precipitation ~10% and ~25% lower compared to present day figures, respectively (Jiang, 2008). Compared to the Pre-Industrial Holocene (PIH), the dry and cold conditions present at the LGM caused an expansion of grasslands and deserts (Henrot et al., 2009, figure 1.8). The boreal evergreen forest (taiga) occupied a far smaller area than today and temperate deciduous forest was extremely restricted during the LGM. The shift in biomes (figure 1.8) generally represents the response of vegetation to temperature reductions (Prentice et al., 2000, Henrot et al., 2009). There were only a few regions with the same biome at the LGM as today. Central Asia is an example of such a region, however the steppe vegetation present there may have altered in floristic composition (Prentice et al., 2000). LGM continental temperature and
precipitation mainly results from regional interactions with vegetation (Crucifix & Hewitt, 2005). For example, in Eurasia (particularly Siberia and Tibet) the response of the biosphere substantially enhances the glacial cooling through a positive feedback loop between vegetation, temperature and snow-cover (Crucifix & Hewitt, 2005).

1.5.2 Atmospheric [CH₄] and Wetlands

The main mechanisms for the high atmospheric CH₄ concentrations during interglacials (~700-800 ppbv) and low concentrations (~350 ppbv) during glacials are thought to be variations in productivity and extent of global wetlands (Chappellaz et al., 1993a, Chappellaz et al., 1997, Brook et al., 2000), and changes to the strength of the tropospheric sink (reaction with the OH radical) (Adams et al., 2001, Valdes et al., 2005, Kaplan et al., 2006, Harder et al., 2007). During the LGM the combination of colder and drier global conditions (Jahn et al., 2005), the presence of ice sheets across northern boreal latitudes (Abe-Ouchi et al., 2007), and the low [CO₂]ₐₑₘ (Petit et al., 1999) may have limited the global wetland CH₄ source. However, the exact behaviour of wetlands under glacial conditions is unclear and the magnitude of the global wetland CH₄ flux during glacial times remains uncertain.

The global contribution of natural wetlands to the [CH₄]ₐₑₘ at any point in time is a balance between the rate of CH₄ production and global extent. The latter is thought to have been larger at the LGM (6.8 x 10⁶ km²) compared to the PIH and of similar magnitude to present day (Kaplan et al., 2006). During LGM simulations by Kaplan et al., (2006), wetlands were greatly reduced from their current extent in North America, Europe and Western Siberia because of the presence of ice sheets and perennially frozen ground (figure 1.9), however the overall global wetland area is estimated to have been larger than present day
The increase in wetland area at the LGM may have been caused by a fall in global sea levels exposing low lying continental shelves, particularly in Beringia and the Gulf of Carpentaria (Kaplan et al., 2006, figure 1.9). Not all LGM wetland models however predict a larger wetland area at the LGM compared to the PIH or present day. Valdes et al., (2005) predicted an annual mean wetland area of 7.8 and \(6.0 \times 10^6\) km\(^2\) at the PIH and LGM respectively. The reduction at the LGM was attributed to a cooler, drier climate and the physical presence of ice sheets, however Valdes et al., (2005) also predicted that the removal of high latitude wetlands at the LGM may have been offset by lowered sea levels creating new wetland areas.

Although wetland area was potentially greater at the LGM compared to modern day, Kaplan et al., (2006) suggest that the global wetland CH\(_4\) flux was similar at the LGM and the PIH, with a value of \(\sim 110\) Tg yr\(^{-1}\) (figure 1.10). Valdes et al., (2005) predict a similar wetland contribution of \(108.4\) Tg yr\(^{-1}\) during the LGM, however they estimate PIH wetland CH\(_4\) emissions at \(\sim 150\) Tg yr\(^{-1}\). Models have consistently shown that wetland area and global emissions may not have varied substantially between the PIH and the LGM to affect \([\text{CH}_4]_{\text{atm}}\) alone (Valdes et al., 2005, Kaplan et al., 2006, Harder et al., 2007). Therefore, an additional mechanism is required to explain the low \([\text{CH}_4]_{\text{atm}}\) at the LGM. One possible explanation could have been a combination of lower global temperatures and a contraction of global forests that may have reduced atmospheric emissions of BVOC (Adams et al., 2001, Petron et al., 2001, Cinege et al., 2009) during the LGM. A reduced BVOC source would enhance the oxidative capacity of the atmosphere and reduce the photochemical lifetime of CH\(_4\), which would be reflected in a reduced \([\text{CH}_4]_{\text{atm}}\). It is estimated that BVOC may have been \(\sim 60-65\%\) less at the LGM compared to the PIH (Valdes et al., 2005, Kaplan et al., 2006) (figure 1.10). This theory is contested by Arneth et al., (2007) as they suggest only a 15% difference in isoprene and monoterpene emissions between the LGM.
Figure 1.9 Modelled Global distributions of total annual CH₄ emissions from wetlands at (a) the PIH, (b) 6 ka, (c) 10 ka and (d) the LGM. White areas indicate where no wetland area was simulated. Grey indicates ice sheet extent. Diagram produced by Kaplan et al., (2006).
Figure 1.10 Wetland area, CH$_4$ emissions and Biogenic Volatile Organic Compound (BVOC) emissions simulated by Kaplan et al., (2006) at 1000-year paleoclimate scenario time slices
and the PIH, which would have created a more stable [OH] during the Holocene. The observed increase in isoprene emissions at leaf area scale to LGM CO₂ concentrations compared to modern day values (Possell et al., 2005, Arneth et al., 2007b, Wilkinson et al., 2009), may have counteracted the effect of lower temperature and reduction in forested area at the LGM.

1.6 Plant Physiological Response to CO₂ Starvation

The atmospheric concentration of CO₂ has varied from minima of 170-200 ppmv during glacial periods, to maxima of 280-300 ppmv in recent interglacials (Luthi et al., 2008). The physiological response of ecosystems to CO₂ starvation present at glacial maxima is determined by whether the predominant species are either C₃ or C₄ (Prentice & Harrison, 2009). Plants can be divided broadly into two categories based on their photosynthetic pathway. The number of carbon atoms found in the first organic intermediate of photosynthesis (either 3 or 4) denotes whether a plant is categorised as either C₃ or C₄ (Pearcy & Ehleringer, 1984). These two different ways of harnessing energy contrast in their response to CO₂ starvation. A reduction in [CO₂]ₘₚ would cause C₃ plants to become substrate (CO₂) limited during the carboxylation reaction and causes an increase in the inhibitory process of photorespiration (Tissue et al., 1995, Cowling & Sykes, 1999, Sage & Coleman, 2001). Photorespiration happens when CO₂ concentrations are low and rubisco (the enzyme responsible for CO₂ fixation) binds O₂ instead of CO₂, a process which uses energy yet provides no sugars. C₃ plants exhibit a reduction of ~50% in photosynthetic capacity at optimal temperatures (20-30°C) as the [CO₂] is reduced from modern day to the LGM concentrations (Sage, 1995). Due to this decrease in photosynthetic activity, C₃ plants exhibit a decrease in root density and growth rates when starved of CO₂ (Dippery et
In contrast to the C₃ pathway, C₄ plants are less susceptible to low atmospheric CO₂ concentrations because they use mechanisms to concentrate CO₂ near chloroplasts and use enzymes that do not promote the use of photorespiration (Pearcy & Ehleringer, 1984). C₄ plants are therefore more efficient at utilising CO₂ when concentrations are low, however the same mechanisms that offer this advantage, also lead to CO₂ saturation at relatively low atmospheric CO₂ concentrations (Tissue et al., 1995).

Temperature plays a key role in plant physiology as it controls many enzyme driven processes, e.g. photosynthesis (Sage & Kubien, 2007). The temperature at which plants exhibit maximum rates of photosynthesis and growth (called thermal optimum, T₀pt), is determined by the effects of changing temperature on photosynthesis, mitochondrial (dark) respiration and photorespiration, processes that are all highly CO₂-dependent (Cowling & Sykes, 1999). A reduction in [CO₂] atm may reduce the T₀pt in C₃ plants (figure 1.11) because of enhanced rates of photorespiration, particularly at high temperatures (Cowling & Sykes, 1999). However, as the temperature falls below optimal, this reduces the effects of photorespiration and the effect of CO₂ starvation on C₃ photosynthesis (Sage & Coleman, 2001), as shown in figure 1.11. Therefore in glacial times, a colder global temperature would have reduced photorespiration, however low atmospheric CO₂ concentrations would have (in part) counteracted that process. C₄ plants in glacial times would have had a growth (Ward et al., 2008) and water-use efficiency (a good indicator of plant water stress) advantage over C₃ species (Cowling & Sage, 1998). The ratio of carbon assimilation per unit of transpiration (water-use efficiency) is sensitive to changes in CO₂ through effects on stomatal conductance (Farquhar & Sharkey, 1982). Cowling and Sage (1998) found that at glacial CO₂ concentrations, Phaseolus vulgaris (C₃) vegetation had a 62% lower water-use efficiency compared to the modern day control, however the
decrease in global temperatures during the LGM however would have reduced this advantage (Ward et al., 2008).

![Diagram of CO2 assimilation vs temperature for two CO2 concentrations](image)

**Figure 1.11** Modelled responses of light-saturated rates of CO2 fixation (µmol m$^{-2}$ s$^{-1}$) in C$_3$ plants to changes in atmospheric CO2. Dashed vertical lines indicate photosynthetic thermal optima (T$_{opt}$) defined as the temperature at which photosynthesis is at its maximum, for both low and ambient CO2 (180 and 360 µmol m$^{-1}$, relatively). Diagram by Cowling and Sykes (2000).

During the LGM the low [CO2]$_{am}$ would have favoured C$_4$ species because of an increase in photorespiration in C$_3$ plants. The C$_4$-C$_3$ transition temperature (point at which C$_4$ abundance drops below 50%) is estimated to be lower during the LGM; thus adding to the favourable conditions for C$_4$ species (Cowling & Sykes, 1999). Collatz et al., (1998) used climatological data sets to provide estimates of LGM mean monthly temperature to classify the globe into areas which should favour C$_4$ photosynthesis. Their model predicted that colder temperature and the reduced [CO2]$_{am}$ at the LGM would have caused a substantial expansion of C$_4$ vegetation, particularly in Asia and at high latitudes. This is a typical
finding that builds on the work of other modellers such as Ehleringer et al., (1997), who also predict a C₄ expansion during the LGM. Predictions by models are also verified by measured data. Using stable isotope ratios of carbon (¹⁴C) in peat, Rajagopalan et al., (1997) found that in the montane region of India, C₄ species were more prominent than C₃ during the LGM. The same stable isotope techniques have been used to find similar patterns all over the world. For example, Galy et al., (2008) showed that the Himalayan basin was colonised by C₄ plants during the LGM, but by the mid-Holocene this had switched to C₃. Understanding the composition of C₃ and C₄ species on LGM wetlands is crucial when trying to compare modern day wetlands to those during glacial times.

The partial pressure of CO₂ in the atmosphere is not however the only factor which dictates the C₃/C₄ balance in ecosystems. The composition is dependent upon both climatic conditions (particularly temperature) and the [CO₂]ₐₙₙ (Flores et al., 2009). This created a scenario during the LGM, where because ecosystem C₃/C₄ balance may be overidingly determined by climatic conditions (Huang et al., 2001), not all areas in the world experienced a proliferation of C₄ plants despite global [CO₂]ₐₙₙ selecting for it. For example, the intertropical highlands where characterised by C₄ plants during the LGM and then shifted to C₃ in the Holocene (Flores et al., 2009). Present day wetlands dominated by C₄ species may behave and produce similar amounts of CH₄ when compared to equivalent LGM wetlands. In contrast however, C₃ dominated wetlands are unlikely to release as much CH₄ because plant photosynthesis and the amount of carbon entering the rhizosphere is likely to be reduced due to CO₂ starvation. The ratio between C₃ and C₄ wetland plants at the LGM however, remains uncertain at this time. The experiments performed in this thesis used wetland mesocosms that contained vascular and bryophyte species that used the C₃ pathway.
1.7 Wetland Biogeochemistry in Different CO₂ Atmospheres

There is a considerable amount of evidence to suggest that predicted future rises in [CO₂]ₐₐm will increase CH₄ emissions from wetlands (Dacey et al., 1994, Hutchin et al., 1995, Megonigal & Schlesinger, 1997, Saarnio & Silvola, 1999, Saarnio et al., 2000, Vann & Megonigal, 2003, Marsh et al., 2005, Ellis et al., 2009). The first observation of stimulation of CH₄ emissions from wetlands by CO₂ enrichment was made by Dacey et al., (1994). Dacey et al., (1994) measured an 80% increase in CH₄ emission after only one week of exposing brackish tidal marsh vegetation to twice the present ambient concentration of atmospheric CO₂. Following this work, Hutchin et al., (1995) was the first to show an increase in CH₄ emission after CO₂ fertilisation in ombrotrophic peatland mesocosms removed from Migneint in North Wales. During 4 months of measurements (5 and a half months of treatment) CH₄ measurements were consistently 100% greater than the controls. A large increase (136%) in CH₄ emission post-CO₂ fertilisation was also reported by Megonigal and Schlesinger (1997) when exposing wetland soils containing aquatic flowing plants (Orontium aquaticum) to elevated CO₂. Since these early studies, Ellis et al., (2009), Saarino et al., (2000), and Saarnio and Silvola (1999) have all reported smaller increases in CH₄ emissions after CO₂ fertilisation of 58, 15-20 and 10-20% respectively.

The main hypothesis for increased CH₄ emission from wetlands exposed to elevated CO₂ is an increase in plant derived labile carbon as a consequence of increased plant productivity and biomass. Increases in plant photosynthetic rates after CO₂ fertilisation were measured by Hutchin et al., (1995) and Megonigal and Schlesinger (1997) in conjunction with elevated CH₄ emissions. Measurement of soil pore water after ¹⁴C pulse labelling shows
that wetland plants rapidly transfer photosynthetically fixed carbon to the rhizosphere in the form of root exudates, which is subsequently converted into CO₂ or CH₄ (King & Reeburgh, 2002, King et al., 2002). Altering photosynthetic rates and primary production in wetlands by CO₂ fertilisation also causes an increase in DOC in wetland soils (Kang et al., 2001, Freeman et al., 2004a, Kang et al., 2005, Marsh et al., 2005, Fenner et al., 2007, Kim & Kang, 2008). Increases in DOC can have a 'priming' effect where soil organic matter decomposition is enhanced as a result of a proliferation of microbes under carbon limitation (Freeman et al., 2004a). The impact of elevated CO₂ on 'priming' however remains unclear. Kim and Kang (2008) reported no changes in soil enzyme activity in wetland soils, despite measuring higher DOC concentrations. This could be because carbon flow through roots is essentially easily accessible carbon, or the priming effects of elevated CO₂ through an increase in DOC may be offset by inhibitory effects of phenolic compounds (Freeman et al., 2004b, Kim & Kang, 2008).

Elevating the atmospheric concentration of CO₂ could change the species composition of natural wetlands (Berendse et al., 2001, Fenner et al., 2007). If changing CO₂ concentrations were to offer particular species an advantage, this could ultimately change the amount of CH₄ emitted to the atmosphere. For example, Sphagnum species create conditions that strongly favour carbon sequestration (Van Breemen, 1995) and produce material that is rich in phenolics and therefore far more resistant to decomposition compared to vascular plant litter (Verhoeven & Toth, 1995). Altering vascular plant assemblages would also have implications for CH₄ transport in wetland soils (Bellisario et al., 1999). Fenner et al., (2007) found that peat monoliths maintained in an elevated [CO₂] increased both their above ground (115%) and below ground biomass (96%) during the experiment, and also experienced a shift in plant species composition. Sphagnum-dominated communities declined by 39% during the experiment, whereas Juncus effusus
significantly increased its percentage cover. Fenner et al., (2007) hypothesised that because of Sphagnum's close proximity to the surface, it is less likely to be CO₂ limited compared to vascular plants and therefore unlikely to respond to elevated CO₂. Another possible explanation is that Sphagnum and other wetland species may not respond to elevated CO₂ because their growth is limited by nutrient availability (Hoosbeek et al., 2001).

1.8 Thesis Aims and Layout

There has been no direct research into the effects of a sub-ambient CO₂ concentration on wetland biogeochemistry. The effects of elevated [CO₂]ₐₑₙ on wetland biogeochemistry does however provide insights into the likely outcomes of CO₂ starvation. For example, C₃ species are more likely to be adversely affected by a reduction in [CO₂] when compared to C₄ species, and a suppression of photosynthetic activity is likely to cause a decrease in root exudates and suppress methanogenesis. The main focus of this thesis is, therefore, to quantify the effect of the LGM [CO₂]ₐₑₙ (180 ppmv) on CH₄ flux from wetlands. This thesis aims to narrow the uncertainty associated with modelling the LGM CH₄ budget by addressing the physiological link between the LGM [CO₂]ₐₑₙ and wetland CH₄ emissions. The results may help to clarify whether changes to the OH radical sink, wetland productivity, wetland extent, or a combination of the latter, are the most likely causes of the low [CH₄]ₐₑₙ measured at the time.

The thesis is laid out across 7 chapters. Chapter 2 presents the principle methods used throughout the thesis, with particular emphasis on the equipment and protocol required to create an atmospheric [CO₂] of ~180 ppmv. Chapter 3 details the results of the 2 year
experiment investigating the impact of the LGM [CO₂] on CH₄ emissions from wetland mesocosms. Chapter 4 builds on the results of Chapter 3 by increasing the temporal resolution of sampling to provide a more in-depth examination of the variation in CH₄ flux over diurnal timescales under CO₂ starvation. Chapter 4 also analyses the potential differences in CH₄ flux pattern between wetlands dominated by either bryophytes or vascular plants. Chapter 5 isolates the influence of temperature on CH₄ emissions and investigates whether CH₄ temperature response curves are altered by the LGM [CO₂] atm. The results from Chapter 5 are further explored using the CH₄ production equation in the Cao et al., (1996) model. Chapter 6 details the effects of CO₂ starvation on a range of rhizosphere variables that are relevant to CH₄ flux. These include: DOC, DIC, dissolved CH₄ (DM) and acetate. Chapter 7 presents a general discussion of the combined findings and investigates the broader implications of the results. Conclusions and recommendations for future work are also presented.
CHAPTER TWO

Methods

2.1 Introduction

This chapter describes the methods and techniques used to investigate CH$_4$ emissions and pore water carbon content from two different temperate wetland ecosystems. This includes a detailed description of field sites, the sampling technique required for extracting intact peat mesocosms, the analytical theory and protocol used for CH$_4$ flux, DM, DOC and DIC determination. The statistical methods applied to ascertain significance are also described. Methods more specific to certain Chapters are discussed where relevant.

2.2 Field Site Description

2.2.1 Cors Goch

Cors Goch is situated on the Isle of Anglesey, North Wales, UK. It is a Site of Special Scientific Interest (SSI) located at UK grid reference SH 504 817 (figure 2.1). It forms
part of a group of four Alkaline Fens (Cors Goch, Cors Erddreiniog, Cors Bodeilio and Cors y Farl) which can be found in the area (JNCC, 2007a). All four base-rich wetlands occupy former lake basins which have been mostly in-filled with calcium carbonate lacustrine sediments and peat deposits. The Anglesey Alkaline Fens physio-chemical characteristics and nutrient status are strongly influenced by the underlying Carboniferous limestone and proximity to the sea (Kang & Freeman, 1999). The national vegetation classification (NVC) (a comprehensive phytosociological classification, which assesses vascular plant, bryophyte and macro-lichen species within a certain vegetation type)
describes Cors Goch as a site which supports M9 Carex rostrata – Calliergon cuspidatum/giganteum, M10 Carex dioica – Pinguicula vulgaris and M13 Schoenus nigricans – Juncus subnodulosus vegetation. The advantage of using the NVC is that it is based solely on plant species composition which can be used to indicate certain physio-chemical characteristics of the area.

Cors Goch has an open fen area of 0.25 km² ha with a dominant vegetation of both rushes and sedges. Notable plant species include: Carex riparia curtis (great pond sedge), Cladium mariscus (great fen sedge), and Juncus subnodulosus (blunt-flowered rush). The area also has the distinctive alkaline fen species of Carex lepidocarpa (long stalked yellow sedge) and Schoenus nigricans (Black Bog Rush). Sphagnum species can be found in localised patches on Cors Goch, usually colonising sections maintained as firebreaks where vegetation height is restricted. Areas such as the Anglesey Alkaline fens have declined dramatically in the past century in the UK because of anthropogenic pressure on the sites. Only small pockets of this kind of habitat can now be found in the UK. A photograph of a small, but representative section of Cors Goch can be seen in figure 2.2.

2.2.2 Migneint

Migneint is located in the Snowdonia National Park at UK grid reference SH 816 440 (central point) (figure 2.1). Migneint and the surrounding area of Arenig and Dduallt is designated as a Special Area of Conservation. It covers a total area of ~200 km², of which ~52% can be categorised as bog/marsh/water fringed vegetation (JNCC, 2007b). The site
Figure 2.2 Photograph taken at the boundary of Cors Goch in February 2006. The tall common reed (*Phragmites australis*) can be seen in the background and the distinct red/brown of bog-myrtle (*Myrica gale*) in the foreground.

Figure 2.3 Photograph of Migneint taken in February 2006 (SH 433 767). The photograph shows the meeting point of the B4406 and the B4407 roads. The darker patches of vegetation consist of drier heath vegetation (*Culluna vulgaris*) and the lighter, wetter areas consisting of blanket bog vegetation.
supports a large area of blanket bog and is particularly significant for the extent of *Sphagnum-*rich M19 *Calluna vulgaris - Eriophorum vaginatum* blanket mire. Also present is M18 *Erica tetralix - Sphagnum papillosum* blanket mire, with localised patches of the bog-moss *Sphagnum magellanicum*. Other notable species include *Carex magellanica* (tall bog-sedge) and *Carex pauciflora* (few-flowered sedge), which is towards the southern limit of its UK distribution. Certain parts of the area have a history of anthropogenic modification from burning and grazing that has resulted in M20 *Eriophorum vaginatum* blanket mire. Nutrients enter wetlands from a variety of sources including streams, drainage channels, ground water from other catchments, from the air in rainfall, spray drift and decomposition of plant litter (Sorrell, 2010). The nutrient source in the Migneint area is largely restricted to the low levels found in rainfall, hence its ombrotrophic classification (Freeman *et al*., 2004a). A photograph of Migneint can be seen in figure 2.3.

### 2.3 Selecting and Extracting Field Samples

A total of thirty two 11 x 40 cm peat mesocosms complete with pristine surface vegetation, were collected in autumn 2006 from Cors Goch and Migneint. Sampling was performed in locations with a near-surface water-table that was representative of the vegetation in the area. Hummocks and hollows were disregarded as the experimental design required a fixed near surface water-table (within 2-3 cm). Locations with rushes taller than 50 cm were avoided when sampling due to height restrictions in the Controlled Environment Units (CEUs) in which the mesocosm would be housed. Choosing mesocosms with plants adapted to dryer or wetter conditions may have caused unwanted mortality in the surface vegetation during the experiment. Wooden boards were used to minimise the impact of the excavation on the surrounding vegetation and to provide stability when working. Opaque
A custom made iron chisel (100 x 0.5 cm) was used to insert the PVC pipe through the aerobic acrotelm and into the anaerobic catotelm at both sites. The chisel was essential for cutting through the perimeter surface vegetation and cutting through horizontal roots in the rhizosphere. The pipes were submerged to a point where the surface vegetation was ~2 cm from the top of the pipe. Mesocosms with signs of compaction caused by the insertion were rejected. This insertion method caused minimal disturbance to the surrounding vegetation and caused no damage to the mesocosm plants (figure 2.4). Removing the mesocosm required a small trench to be created at the side of the pipe, where a spade was then inserted and used to lever it out of the ground. Once removed, a plastic cap (an
underground pipe end cap 11 cm in diameter) was immediately placed on the base to sustain the anaerobic condition of the peat. A 60 cm length of narrow hollow metal pipe was placed down the side of the core when fitting the end cap to stop air being forced up through the mesocosm. An airtight seal was created at the base of the mesocosms by using a silicone-based adhesive to secure the end caps. The silicone sealant created a seal that would not deteriorate during the experiment.

2.4 Controlled Environment LGM CO₂ Experiment

2.4.1 Controlled Environment Unit (CEU) Specification

The peat mesocosms were maintained in the Department of Earth and Environmental Sciences controlled environment facility at the Open University in Milton Keynes. Mesocosms were split between two Snijders Microclima 1750E CEUs for more than 1000 days. The internal capacity of each CEU comprised of a 1.75 m³ internal space with a growth area of 1.4 m² and a growth height of 1.2 m. The overall internal dimension measured 185 x 80 x 115 cm (L x W x H), and externally the units measured 242 x 105 x 202 cm (L x W x H). With the light turned off, the units have a temperature range of -15 to +50°C, with the light switched on, 0 to +50°C. Depending on the temperature, relative humidity is controlled at 55–95 (20°C) and 40–95 (40°C). The CEUs controlled humidity to ±5% and temperature to ±0.3°C of set values. The units use 20 x 54 W, 20 x 24 W and 6 x 58 W Brite Gro 2023 bulbs, which are capable of creating lighting up to ~1000 µm m⁻² s⁻¹. Each CEU had a Vaisala CARBOCAP® CO₂ module series GMM221 sensor built into the growth chamber. The CO₂ sensors had an accuracy of ±1.5% of its range, plus 2% of the actual reading. Therefore, a reading of 380 ppmv would have an associated
error of ±37.6 ppmv, and 180 ppmv ±33.6 ppmv. The CO₂ sensor had an operating range of 0-2000 ppmv, a temperature range of -20°C to +60°C, a pressure range of 700-1300 hPa, and a relative humidity range of 0-100%. The CO₂ sensors were periodically tested against calibration gases for accuracy during the experiment.

2.4.2 CO₂ Control and Distribution System (CCDS)

The objective of the experiment was to create a LGM CO₂ (treatment-180 ppmv) and modern day atmospheric CO₂ (control-380 ppmv) concentration within the CEUs. To achieve this, a self-regulating CO₂ control and distribution system (CCDS) (figure 2.5) was constructed to work with the Snijders CEU software. This system was designed to ensure that reliable and reproducible specific CO₂ concentrations were maintained throughout the duration of the experiment. To avoid any unnecessary blocking effects within the experiment, the CCDS was designed so that CO₂ concentrations could be periodically rotated between the CEUs. This allowed mesocosms to be moved between CEUs whilst maintaining the appropriate CO₂ treatment. Figure 2.5 shows a schematic of the required equipment and configuration needed to create the two different CO₂ concentrations. Fundamental to the set up was the CMC 28 1 purge gas generator (labelled P.G. in figure 2.5).

The CMC purge gas generator uses pressure swing adsorption (PSA) technology to remove CO₂ from compressed air. This type of generator is typically used in fourier transform infrared spectroscopy (FTIR) analysis to improve the resolution of the instrument by purging the analysis chamber with CO₂-free (<1 ppmv) and dry (<0.01 ppmv) air. The
filters also remove dust and oil from the gas stream. PSA essentially relies on the fact that under pressure, gases are adsorbed or attracted to different surface types. When the bed
(molecular sieve) reaches the end of its capacity to adsorb, it regenerates by reducing the pressure which releases the CO₂ and H₂O ready for another cycle of production. Two adsorbent vessels allow near-continuous production of the CO₂-free air. The main reason for using PSA technology is that it removes the need for CO₂-free air cylinders. To output the required 28 l at 2-3 bar pressure, the purge gas generator is provided with between 5.5-8 bar of compressed air from oil-free compressors. Both compressors used in the system were attached to drier units that filtered and dried the air.

2.4.3 Creating Experimental CO₂ Atmospheres

To create a LGM [CO₂] atm (180 ppmv), 26 l of CO₂-free air was passed directly into the treatment CEU every minute. At this rate, purging takes ~67 minutes for all the air to be replaced once in the CEU. However, because they are not fully sealed units, it takes longer to reduce the concentration to the required set point. The actual length of time it takes to reach the set point depends on the starting concentration within the CEU, the number of mesocosms and their photosynthetic rate (influenced by temperature and light settings), and the number of ventilation and drainage vents which are open on the unit. Increasing the temperature and light intensity within the CEU increases photosynthesis and the drawdown of CO₂ out of the atmosphere by the mesocosms. Therefore, the warmer and brighter the cabinet, the faster the set point is reached. An example of the time taken to reach the LGM set point concentration can be seen in figure 2.6.

It was necessary to perform this experiment in 'open' growth chambers to prevent an increase in atmospheric pressure in the CEUs when purging with CO₂-free air. An unavoidable consequence of open chambers is that it increases the work load on the
compressors and purge gas generator in the CCDS when trying to achieve the CO₂ set points. A continual supply of CO₂-free air channelled straight into a CEU if left unchecked, would eventually create an atmosphere of <50 ppmv. When the concentration falls below a set limit, the CEU software activates a solenoid inlet valve in the growth chamber. Connected to this inlet valve is the 'top-up' section of the CCDS. The top-up gas introduced into the treatment CEU is a mixture of laboratory air (~2 l/min) and pure CO₂ cylinder air (~5 ml/min). This enriched CO₂ air tops up the concentration within the unit until the set point is reached, after which, the CEU software then closes the solenoid valve. This results in a [CO₂] which oscillates around the set point as demonstrated in figure 2.7.

Figure 2.6 Time taken to reduce the treatment CO₂ concentration to set point. Concentration was manually recorded every minute using the internal CO₂ sensor. At 53 mins the CEU was opened and the mesocosms watered. The CCDS takes 91 minutes to reduce the concentration back to the 180 ppmv set point. Cabinet temperature was 15°C; light level 250 μm m² s⁻¹; 24 mesocosms present.
Figure 2.7 Regulated atmospheric [C\textsubscript{O\textsubscript{2}}] in the treatment CEU. Data recorded (06/03/09) during daylight hours. Section A, B and C represent different top-up mixtures. A, 1.3 L/min lab air mixed with 17 ml/min concentrated CO\textsubscript{2}. B, 1.3 l/min:10 ml/min. C, 2 l/min:5 ml/min. Cabinet temperature was 15°C; light level 250 \mu m m\textsuperscript{-2} s\textsuperscript{-1}; 24 mesocosms present. Concentration manually recorded every minute by noting down the internal CO\textsubscript{2} sensor value.

The same mechanics and principals used to produce the LGM [C\textsubscript{O\textsubscript{2}}] were used to create the modern day [C\textsubscript{O\textsubscript{2}}] control. As the CEUs were located within working laboratories, unaltered CO\textsubscript{2} concentrations were higher (∼450 ppmv) compared to ambient modern day values. To achieve a modern day [C\textsubscript{O\textsubscript{2}}], the control CEU was purged with 7 l/min of laboratory air mixed with 2 l/min of CO\textsubscript{2}-free air. When the set point was achieved, an enriched mixture of pure CO\textsubscript{2} cylinder air (10 ml/min) and laboratory (500 ml/min) air was added. An example of the CO\textsubscript{2} concentrations for both the control and treatment units over an extended period of time is shown in Chapter 3, figure 3.1. The average concentration was 406±23 (S.E.) ppmv and 196±28 ppmv in the control and treatment over year 1 and 2.
respectively. The CO$_2$ values achieved were slightly higher than aimed for, however the important 200 ppmv experimental difference was created and maintained.

2.4.4 CCDS Modifications

The CCDS system was improved many times over the course of the experiment to get closer to the set points (180 & 380 ppmv). The most significant change was to switch from ambient air to concentrated CO$_2$ for topping up in both the control and treatment CEUs. This was introduced at the end of year 1 in response to the challenges faced in creating the LGM [CO$_2$]$_{arm}$. The original CCDS system (design not shown) worked well in the colder winter months, using ambient laboratory air to top up the treatment CEU when the concentration fell below 180 ppmv. During this period, plant productivity would have been at its lowest, which would have had minimal influence on the CEUs [CO$_2$]. As the year progressed and temperatures increased, it is hypothesised that the plants within the mesocosms began to assimilate more CO$_2$ during daylight hours. This increased productivity combined with the CO$_2$-free air purging the CEU, creating a situation where using laboratory air was insufficient to bring the concentration back to the set point. To alleviate this problem, the amount of CO$_2$-free air delivered into the CEU was reduced. This solved the original CCDS systems inability to top up the treatment CEU, however a drawback to this action was an increase in the time taken to reduce the elevated night time [CO$_2$] down to the set point value during daylight hours. The [CO$_2$] was always near to the set point by the midday recording time, however figure 2.8 clearly shows that as daytime temperature increased during the season, it became increasingly more difficult to control the [CO$_2$]. The CCDS was altered to use a combination of pure CO$_2$ mixed with laboratory air for both treatment and control top-up functions at the end of year 1. This allowed for
an increase in the amount of CO₂-free air delivered into the unit, which reduced the time outside the set point and increased the overall CO₂ control of both the treatment and control CEUs.

![Graph](https://via.placeholder.com/150)

Figure 2.8 Daytime temperature overlaid by a smoothed averaged treatment [CO₂] during two growing years. Major modifications to the CCDS were carried out between 386-400 days into the experiment. CO₂ concentrations were manually recorded from the internal CO₂ sensor at midday during the daylight section of a diurnal cycle.

2.4.5 Experimental Environmental Variables

Two experimental growing years were created in both control and treatment CEUs over 727 days. The environmental variables which were replicated are shown in table 2.1.
Monthly average maximum and minimum temperatures from 1970-2000 for England and Wales were used to represent day and night time temperatures respectively (Met Office, 2006). Daylight length was based on longitude and latitude estimates for England and Wales. Light intensity was set to 250 µm m$^{-2}$ s$^{-1}$, which is a commonly used value in controlled environment studies (Blodau & Moore, 2003, Blodau et al., 2004). Relative humidity was set to 70% during daylight hours to recreate a moist but not saturated environment. Humidity was dropped to 60% during the night cycle to alleviate the pressure on the condenser and humidification unit within the CEUs.

Table 2.1 Monthly environmental variables used in the long term experiment. The * symbol indicates the actual data which was used in the experiment.

<table>
<thead>
<tr>
<th>Month</th>
<th>Day Temperature (°C)</th>
<th>Night Temperature (°C)</th>
<th>Daylight time (mins)</th>
</tr>
</thead>
<tbody>
<tr>
<td>January</td>
<td>6.6</td>
<td>1.2</td>
<td>493.2</td>
</tr>
<tr>
<td>February *</td>
<td>6.8</td>
<td>1.1</td>
<td>574.2</td>
</tr>
<tr>
<td>March *</td>
<td>9.0</td>
<td>2.4</td>
<td>691.8</td>
</tr>
<tr>
<td>April *</td>
<td>11.4</td>
<td>3.5</td>
<td>811.2</td>
</tr>
<tr>
<td>May *</td>
<td>15.0</td>
<td>6.2</td>
<td>924.6</td>
</tr>
<tr>
<td>June *</td>
<td>17.5</td>
<td>8.9</td>
<td>982.8</td>
</tr>
<tr>
<td>July *</td>
<td>19.9</td>
<td>11.2</td>
<td>967.2</td>
</tr>
<tr>
<td>August *</td>
<td>19.7</td>
<td>11.0</td>
<td>854.4</td>
</tr>
<tr>
<td>September *</td>
<td>16.9</td>
<td>9.1</td>
<td>742.2</td>
</tr>
<tr>
<td>October *</td>
<td>13.2</td>
<td>6.6</td>
<td>622.8</td>
</tr>
<tr>
<td>November</td>
<td>9.4</td>
<td>3.6</td>
<td>509.4</td>
</tr>
<tr>
<td>December</td>
<td>7.4</td>
<td>2.1</td>
<td>450.0</td>
</tr>
</tbody>
</table>

A graphical representation of the change in environmental values over a 32 (night-day-night) hour period in the CEUs is illustrated in figure 2.9a. Parameters were altered to create a day and night period where the light intensity, temperature, and humidity were changed accordingly. A 2 hour transition period was created where environmental variables were slowly changed between day and night settings. This simulation was
Figure 2.9 A typical 32 hour period within the CEUs. Graph A shows a graphical representation of how the environmental variables of light, relative humidity and temperature change diurnally. Graph B shows an example of the diurnal temperature change recorded using soil temperature probes and data logger (IceSpy QL) equipment. The temperature set points were associated with those of May (table 2.1). The 4 large temperature deviations are associated with defrost programs. Data was recorded every 2 minutes. Recorded temperatures were lower than May set points because the temperature probes were placed in a different location to the CEU temperature sensor.
intended to create a dawn and dusk period, and used to avoid a sharp contrast in environmental variables in the CEUs. Figure 2.9b shows an example of this pattern, where temperature was programmed to be low during the night, high during the day time, and slowly changed between the two different set points. During the experiment, water-tables within the mesocosms were maintained to within 2-3 cm of the surface vegetation using distilled water. Watering was performed on average once a week during cold periods and ~3 times a week during peak summer.

2.4.6 Experiment Duration

Conducting an experiment where plant physiology and rhizosphere biogeochemistry are altered, potentially requires a long-term approach to ensure the experimental effect is fully filtered through complex ecosystems (Gauci et al., 2005). Guaranteeing at least a two year experiment was crucial to the experimental design if the true effects of the treatment were to be observed. A full breakdown of the months simulated in the experiment and the timescales that were involved can be seen in table 2.2. To facilitate a long-term experiment whilst operating under time restrictions, a normal two year (24 month) growing cycle was reduced by ~6 months. The autumn/winter months of November, December and January were replaced by a shorter time period using February settings (table 2.1, 2.2). The winter months were shortened because natural wetlands emit less CH4 during winter months compared to the summer months (Dise, 1992, Dise, 1993, Rinne et al., 2007). The reduction in CH4 flux is associated with the temperature regulation of archaea activity and redox status (Smemo & Yavitt, 2006). The further below the optimum CH4 production temperature, which is 38°C (Jerman et al., 2009), the more this inhibits methanogenesis. The winter period is therefore unlikely to show the full effects of any CO2 treatment when
the methanogen populations are operating under temperature limited conditions. It was this period of reduced methanogen activity that was shortened in both year 1 and 2. It was important that the winter period was not completely removed from the experiment because acetate has been shown to accumulate during this season (Hines et al., 2001), and would be an important methanogen resource when temperature limitations on biological activity are relaxed in spring.

Table 2.2. Experimental timescales and simulated monthly variables.

<table>
<thead>
<tr>
<th>Real time (dd/mm/yy)</th>
<th>Cabinet time (day number)</th>
<th>Cabinet month</th>
<th>Experimental segments</th>
</tr>
</thead>
<tbody>
<tr>
<td>28/11/06 - 06/02/07</td>
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<td>Continued treatment with limited measurements</td>
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2.5 CH₄ Measurements and Flux Calculations

2.5.1 CH₄ Sampling Techniques
The most common ways to measure CH$_4$ emissions from wetlands in the field include: micrometeorological eddy covariance techniques (Rinne et al., 2007), tuneable diode laser (TDL) (Hargreaves & Fowler, 1998) and traditional surface chambers (soil enclosures) (Bellisario et al., 1999). Eddy covariance and TDL methods have the advantage of providing flux averages for large areas when compared to chambers. Eddy covariance also has the added advantage of recording prevailing meteorological conductions at the same time as [CH$_4$]. Both eddy covariance and TDL methods do not affect temperature, radiation and wind speed; factors which can affect CH$_4$ emissions. However, their disadvantages include a lack of portability, expense and are labour intensive. The most common method for determining CH$_4$ emission from wetlands is to use chambers. Their widespread use is due to their portability, ability to make measurements over a wide range of wetland types and the relatively cheap cost to produce compared to purchasing eddy covariance equipment and TDLs. Chambers can also measure small fluxes that may be below the detection limits of micrometeorological instruments. The main disadvantage of using chambers is that they can alter diffusion gradients and methanogenic pathways by changing pressure, temperature and light intensity. Therefore, careful consideration is required when designing surface chambers and the sampling strategy used to measure CH$_4$ emissions.

Chamber design can be broadly categorised as either open or closed. Open (dynamic) chambers circulate the air over the wetland surface from an open inlet and expel the air via a different route. The change in concentration between the inlet and outlet forms the basis of the flux calculation. Closed (static) chambers do not circulate air from outside and are closed self-contained systems. Unlike dynamic chambers, static chambers require no hardware other than their enclosure, therefore making them the cheapest method to monitor emissions. Fluxes from both are calculated by taking a series of measurements
from the chamber and plotting the change in concentration against time (see section 2.5.6 for full details on flux calculations). Gas samples collected from both chamber methods can be analysed using a variety of instruments. The most common approach is to measure \([\text{CH}_4]\) from headspace samples by Gas Chromatograph Flame Ionising Detector (GC-FID), however a variety of alternative techniques now exist. Examples of more recent techniques include using gas chromatography mass spectroscopy (Beckmann & Lloyd, 2001) and FTIR spectroscopy (Esler et al., 2000) to measure \([\text{CH}_4]\).

The best option for recording \(\text{CH}_4\) flux in this experiment was to use custom designed chambers. It was important that the LGM CO\(_2\) treatment was adequately replicated using multiple mesocosms; this enabling an appropriate statistical analysis of the treatment effect. In this experiment, emissions were monitored using static chambers during year 1 with a GC-FID. In year 2, dynamic chambers and cavity ring down laser spectroscopy (CRDLS) were used to measure emissions. The principles and methods used for GC-FID and CRDLS are explained in sections 2.5.4 and 2.5.6.

2.5.2 Flux Chamber Design

The two methods used to determine \(\text{CH}_4\) flux during the experiment each required a specific chamber design (figure 2.10). Both chambers were designed to capture the diffusive \(\text{CH}_4\) flux without removing the peat mesocosms from the CEUs. The chambers were also designed to eliminate changes in temperature and pressure during sampling periods. The best way to avoid these changes was to include a proportionately sized and appropriately located vent tube (Hutchinson & Livingston, 2001). By allowing a small
controlled gaseous exchange with the atmosphere, the valve negates against both changes
unavoidable changes in pressure (or volume) caused by attaching the chamber, and rapid
fluctuations in air pressure induced by turbulence (Hutchinson & Mosier, 1981). The
location of this vent was carefully chosen because Conen and Smith (1998) found
when experimenting in a natural environment, vented chambers produced higher fluxes
when compared to completely sealed chambers. Depressurisation of the chamber caused
by wind blowing over the vent (venturi effect) was thought to be the cause of this
observation. A decrease in pressure is known to cause an increase in CH₄ flux from
wetlands by increasing ebullition (Tokida et al., 2007). The position of the vent used in
this study was therefore carefully chosen to be away from the influence of turbulence. As
this experiment was performed in CEUs, horizontal wind turbulence was not an issue. Any
movement of air in the CEUs was generated by the mixing fans in the base of the growth
chambers, which created a vertical flow of air. Therefore, the logical location for the vent
was in the roof of the chambers, which is down wind of the turbulence. The needle vent
also played a part in controlling temperature changes in the chambers, however the most
effective temperature control mechanism was to keep sampling times to a minimum.
Sampling time was 40 minutes when collecting samples for GC-FID analysis. This
decreased dramatically to <5 minutes in year 2 when the CRDLS was used.

2.5.3 Sampling Methods

Gas samples were taken at the same point during the day cycle (midday) at every sample
point throughout the experiment. Sampling frequency was kept to a minimum (bi-monthly
on average) to maintain the LGM CO₂ treatment and control concentration for long periods
of time. Empty chambers showed neither a decrease in [CH₄], associated with adsorption
of CH₄ molecules onto the surface of chamber materials, or an increase in [CH₄] caused by
photo degradation of the plastics. The recorded changes in [CH₄] in the chambers over
time were therefore not a result of any artificial interference. In year 1, three 60 ml gas
samples (T0=0, T1=20 and T3=40 minutes) were taken from the static chambers to
calculate the mesocosm flux. Three samples were adequate to verify the flux because the
relationship between flux and time was determined as linear (figure 2.11a). The 60 ml
syringe samples were purged through 40 ml headspace autosampler vials using 2 needles.
Headspace vials were pre-purged with N₂ for 1 minute so that the [CH₄] within them was
Figure 2.11 Graph A shows examples of the linear relationship between mesocosm [CH₄] and time, samples collected every 10 minutes and analysed using GC-FID. The first bog measurement resides directly behind the fen first measurement. Graph B shows a real-time linear flux recorded from a fen mesocosm (cg21) using CRDLS. The x-axis in both graphs represents time since the chamber was placed on the mesocosms.

below the detection limit of the GC-FID. Additional tests also showed that vial septa were compromised after 10 needle punctures, therefore new septa were used after every analysis. In year 1, if the initial chamber measurement was considerably higher than the ambient concentration (~1.8 ppmv), e.g. >5 ppmv, this was used as an indicator of
ebullition and the flux measurement was then performed again. Figure 2.12 shows a photograph of the static chamber method being used to monitor CH₄ emissions in year 1.

![Multiple static chambers capturing CH₄ emissions from mesocosms in year 1](image)

Figure 2.12 Photograph showing multiple static chambers capturing CH₄ emissions from mesocosms in year 1 of the study.

In the second year, a CRDLS system (Los Gatos Research RMA-200 Fast Methane Analyser) was used to analyse CH₄ emissions. To analyse the flux a closed loop configuration was created between the static headspace and instrument (figure 2.13). The real time recording and display function of the instrument removed the need to extract gas samples for GC-FID analysis and also allowed immediate recognition of ebullition caused by disturbance. If ebullition was recorded, the chamber was instantly removed and the mesocosm re-sampled later that day.
2.5.4 Gas Chromatography

Gas Chromatography (GC) is an analytical separation technique where a mixture of volatile chemical constituents of a substance are vaporised, then resolved through migration of the constituents over an adsorbent or liquid carried by an inert gas. GC analysis can be used both qualitatively and quantitatively. When combining GC with a flame ionisation detector (FID), organic compounds can be detected and analysed. An FID
passes separated analytes mixed with hydrogen, nitrogen or helium over a small flame and polarising voltage (160 V). This ionises the sample and produces an increase in current which is displayed as a 'peak'. An FID produces a linear response to a range of organic compounds including CH$_4$. The end product is a chromatogram which illustrates the data output from a GC showing retention time versus response.

![Graph showing linear relationship between CH$_4$ concentration and peak area.](image)

**Figure 2.14** An example of a 6 point CH$_4$ calibration (1, 10, 100, 250, 500, 1020 ppmv) performed on the GC-FID (07/09/07). Points represent the average of 4 replicates and error bars of ±2 standard deviations.

During this experiment a Tekmar 7000 auto sampler and Cambridge Ai GC94 with FID detector was used to quantify the CH$_4$ concentration in headspace vials. The Porapak Q packed column temperature was set to 40°C, the injector to 51°C and detector to 320°C. Nitrogen was used as a carrier gas. An initial exploratory investigation of CH$_4$ concentration versus FID response confirmed the expected linear relationship (data not shown). Once this linear range was established, it was only necessary to carry out a full calibration every month (figure 2.14), when the column was changed or when any major repairs were carried out. CH$_4$ standards with an accuracy of ±2% were used to calibrate
the GC over the expected sample concentration range. Standards were plotted against peak area with typical regression coefficients >0.99. A one point calibration using a mid-range standard was used for the majority of sample runs. If the area response for the standard fell outside ±3 standard deviations then a full calibration was repeated. The GC-FID set-up and method used for this experiment had a reproducibility of ± 0.2 (S.D) ppmv.

2.5.5 Cavity Ring Down Laser Spectroscopy

A Los Gatos Research RMA-200 Fast Methane Analyser (FMA) was used to measure CH₄ concentration in year 2. The FMA uses a cavity-enhanced absorption-spectroscopy technique that uses a diode laser operating in the near-infrared. This system utilises an optical cavity as an absorption cell that uses mirrors to effectively trap the laser photons, so that they make thousands of passes on average before leaving the cell. This results in an optical path length that is several thousands of meters and provides an accurate measure of light absorption as it passes through the optical cavity. Path length depends on optical losses in the cavity and is determined by switching the laser off and measuring the time necessary for light to leave the cavity (typically tens of microseconds). The wavelength of the laser is tuned to CH₄ and the measured absorption spectra is recorded. A direct quantitative measurement of mixing ratio is determined by combining the measured gas temperature, pressure in the cell and effective path length. After factory set-up the system can operate without any external calibration, however the instrument was checked everytime against a known CH₄ standard prior to it being used. The instrument always returned the correct [CH₄] standard value to within ±100 ppbv, which was well within the stated error of the calibration gas (±10% on a 10 ppmv CH₄ standard).
2.5.6 CH₄ Flux calculations

The linear change in static chamber [CH₄] over time was converted to an appropriate measure of ecosystem flux, for example mg m⁻² day⁻¹. The conversion process used in this study is a common method used in terrestrial ecosystem trace gas flux conversions (Alm et al., 2007). A linear regression slope calculation was used to characterise the relationship between time and [CH₄]. The derived r² coefficient was then used to determine a sufficient linear relationship, where a value of >0.8 was used as an indicator of an undisturbed natural flux. During the experiment, r² coefficient values were generally >0.9. The derived slope function was then multiplied by the chamber volume and divided by the surface area of the core (mesocosm diameter) to express the flux in terms of µl m⁻² sec⁻¹. Values were then converted from micro litres into moles using a rearranged Ideal Gas equation (equation 2.1):

\[ n = \frac{PV}{RT} \]  

(Equation 2.1)

where n is the number of moles of analytical gas, P is atmospheric pressure (in atmospheres), V is the volume of analyte, R is the ideal gas constant and T represents temperature in Kelvin. For the experiment, internal volume (V) was calculated by multiplying the volume of the chamber by the fraction of the analyte per unit volume of gas. The volume of the static chamber was calculated from the internal diameter and the internal height. Temperature (T) is ideally measured within the headspace, however a CEU internal measure was used instead. This is an accurate substitution because static chambers were not kept on long enough to create a greenhouse effect that would warm the soil and alter CH₄ production. Pressure (P) was assumed to be normal (1 atm). After
calculating the number of moles, the molecular weight of CH\(_4\) was then used to convert values into grams and then multiplied by a unit of time. During subsequent Chapters, fluxes will be expressed in terms of mg m\(^{-2}\) day\(^{-1}\). Only using a single measurement to represent a daily flux has its limitations. Fluxes are known to exhibit diurnal variation, however without automated chambers, it was impossible to employ a sampling regime to fully capture diurnal variation over timescales associated with this project. In recognition of the diurnal variation in CH\(_4\) emissions from wetlands, a short-term experiment was conducted during the main experiment to fully characterise the variation (Chapter four).

2.5.7 Dissolved Pore Water CH\(_4\)

1 ml of pore water was removed from the mesocosms and analysed by GC-FID for dissolved CH\(_4\) content bi-monthly during year 2. Pore water samplers were permanently fixed into the mesocosms from the beginning of the experiment and remained there until the end (figure 2.15). The same samplers were also used to extract water for dissolved carbon analysis (see section 2.6). Pore water samplers were constructed from 1 ml Plaskipak syringes with holes drilled into them. The end of the syringe was blocked with silicone sealant and packed with glass wool. Rhizon/pore water samplers were installed into the peat mesocosms 10 cm below the surface of the vegetation. 10 cm below the surface was specifically chosen because this is a highly productive and active area, which is frequently used in studies of this nature (Freeman et al., 2004a). Samples were collected by applying a prolonged suction pressure with a syringe. The presence of glass wool in the samplers provided a sufficient barrier to maintain the integrity of the rhizosphere whilst removing water. Samples were analysed in headspace vials that had been pre-purged with
N₂ within ~2 days. Before analysis, the headspace vials were shaken on a horizontal shaker for 5 minutes and vibrated within the GC auto sampler for 1 minute.

![Diagram of pore water sampler design](image)

Figure 2.15 Pore water sampler design used for dissolved CH₄ and dissolved carbon analysis. Holes were drilled into the 1 ml syringe at 0.3, 0.5, 0.6, 0.7, 0.8 and 0.9 ml. This ensured the water extracted was representative of the horizon and not extracted from the side of the mesocosm.

### 2.6 Dissolved Pore Water Carbon

#### 2.6.1 Introduction

Gaseous carbon emissions are not the only mechanism by which carbon is lost from natural wetlands (Clair et al., 2000). The fluvial flux of carbon from wetlands is a significant
pathway that also needs quantifying. Fluvial carbon is lost in the form of DOC, POC, DIC and dissolved CO$_2$ (Worrall et al., 2005). During the experiment, pore water samples were collected at a minimum of monthly intervals for dissolved carbon analysis. A Shimadzu Total Organic Carbon VCSN (TOC) analyser combined with a Shimadzu ASI-V auto sampler was used to measure both the DIC and DOC fractions within samples. The TOC analyser oxidises organic matter to CO$_2$ and utilises a nondispersive infrared (NDIR) detector to measure the CO$_2$ that is produced. The quantity of CO$_2$ produced is directly proportional to the amount of organic and inorganic material present in the sample.

2.6.2 Dissolved Inorganic Carbon (DIC)

DIC is derived from carbonate sources such as weathering of the underlying strata (Worrall et al., 2003). DIC therefore comprises of HCO$_3^-$, CO$_2^-$, H$_2$CO$_3$ ions, or exists as dissolved free CO$_2$ (Hope et al., 1994). Biological processes such as photosynthesis, respiration and decomposition can influence the degree of free CO$_2$ in water samples, altering the concentration of inorganically derived HCO$_3^-$ ions (Strumm & Morgan, 1981). Quantifying the concentration of DIC in water samples simply requires the addition of a controlled amount of acid. By acidifying the sample with 2 M HCl to a pH between 2 and 3, all carbonates are converted to CO$_2$ by the reactions shown in equation 2.2 and 2.3. CO$_2$ and dissolved CO$_2$ in the sample are volatilised by sparging CO$_2$ free gas through the sample.

\[ \text{Me}_2\text{CO}_3 + 2\text{HCl} \rightarrow \text{CO}_2 + 2\text{MeCl} + \text{H}_2\text{O} \]  
(Equation 2.2)

\[ \text{MeHCO}_3 + \text{HCl} \rightarrow \text{CO}_2 + \text{MeCl} + \text{H}_2\text{O} \]  
(Equation 2.3)
2.6.3 Dissolved Organic Carbon (DOC)

DOC is composed primarily of two categories of substance: non-humic and humic substances (Hope et al., 1994). Non-humics include low molecular weight compounds such as carbohydrates and proteins, whereas humic substances are generally heavier and form most of the organic matter in waters (Wetzel, 1992). The TOC analyser can determine DOC in three ways. Option 1 performs separate IC and total carbon (TC) analysis and subtracts the difference. When performing a TC analysis, the TOC analyser injects the entire sample into a combustion tube situated in a 680°C furnace. The sample oxidises over a platinum catalyst and the CO₂ created is measured by the nondispersive infrared (NDIR) detector. Option 2 determines DOC by using a purgeable organic carbon and non-purgeable organic carbon (NPOC) analysis. When performing an NPOC analysis samples are acidified to pH 2-3 and then sparged with CO₂-free gas to eliminate the IC component. The remaining sample is injected into the combustion tube (680°C) complete with a platinum catalyst. A purgeable organic carbon analysis directs the sparge gas containing the volatilised CO₂ and volatile components of the sample to a lithium hydroxide-filled CO₂ absorber to eliminate the CO₂. The remaining gas is then directed into the combustion tube to be oxidised to CO₂. The third option is to perform a NPOC analysis without a purgeable organic analysis.

2.6.4 Nondispersive Infrared (NDIR) Detector

All forms of analysis on the Schimadzu TOC instrument use a NDIR detector to measure [CO₂]. Before sample gases arrive at the detector they pass through a dehumidifier, membrane filter and halogen filter. The NDIR detector is equipped with an infrared light
source, an IR sensor and a filter that blocks all light except the 4.26 μm wavelength that the CO₂ molecule absorbs. The IR light passes through the sensor chamber and makes contact with the CO₂ molecules that have diffused through the walls of the chamber. The intensity of the 4.26 μm light that reaches the detector is inversely proportional to the [CO₂]. To correct for background fluctuations, the detector continuously alternates taking a sample measurement followed by a reference measurement. The sample then passes through a soda lime CO₂ absorber before being expelled. The CO₂ measured is converted to an analogue signal and interpreted by the TOC-Control V software.

2.6.5 Experiment Sampling Method

Pore water samples were collected bi-monthly on average during the experiment. 10 ml of pore water was removed 10 cm below the surface vegetation using 1ml syringes inserted into the mesocosms (figure 2.15). The use of customised syringes is a technique which is common in wetland fluvial carbon analysis (Freeman et al., 2004a). See section 2.5.7 for a full description of how water was removed from the mesocosms. After extraction, pore water samples were filtered through 0.45µm syringe tip filters and stored at -20°C before analysis. Due to the nature of this experiment, POC could not be collected for analysis. To maintain the anaerobic conditions in the peat, mesocosms were designed to be stand alone and ‘closed’ units. Therefore, there was no through flow of water available to collect for POC analysis. Removing the glass wool barrier in the pore water samplers may have allowed POC to be collected, however the samplers would have blocked very easily and needed replacing regularly.
Extracted samples were not analysed immediately, therefore an appropriate preservation method for DOC and DIC was introduced. Preserving DOC is particularly important because non-humic substances are easily utilised and degraded by microorganisms (Chen & Wangersky, 1996). There is no widely accepted opinion regarding the best preservation method for DOC, however preservation options fall into two distinct categories, physical and chemical (Kaplan, 1994, Dafner & Wangersky, 2002, Sliwka-Kaszynska et al., 2003). Physical passive preservation techniques include freezing samples to -20°C or -70°C with no alteration of the medium required. Freezing is particularly effective at preserving low molecular weight compounds (Karlsson et al., 1999). Using chemical preservation by acidifying to pH 2 (HCl, H$_2$SO$_4$, HNO$_3$) prevents precipitation, flocculation, complexing of some sample components and also inhibits growth and biological activity of microorganisms (Sliwka-Kaszynska et al., 2003). Adding compounds that inhibit biological activity, such as chloroform, formaldehyde and thymol, is also an option (Ogawa et al., 1981), however, this may also add unwanted organic compounds to the solution.

The inorganic fraction of water does not require the same preservation techniques as used for DOC. Molecular bonded carbonate ions created by mineral weathering or dissolution of carbonate minerals in sediment rock, remain stable in solution when nothing is added to the water medium. A small study was performed to verify this and showed that over 96 hours, pore water samples left at room temperature showed no change in IC concentration (results not shown). One important consideration for DIC analysis was that water samples collected under sub-ambient CO$_2$ conditions may have a lower CO$_2$ partial pressure compared to the atmosphere. Delaying the inevitable equilibration by freezing the samples immediately and storing them in containers with no or limited headspace is therefore advantageous. Published literature and my own small scale experiment suggest the best
option for preserving both DOC and DIC, is to freeze the sample with no acid addition. When samples were collected they were placed in a -20 °C freezer within 5 minutes.

2.6.6 Instrument Method

To determine the DOC in pore water samples, an NPOC analysis was chosen. A TC-IC method was not used because it can result in a large error when combining the results of each analysis. The IC detection accuracy on the TOC instrument decreases with increasing IC concentration, therefore this is an avoidable limitation when other DOC methods exist. An NPOC only analysis was preferred to a combined purgable organic carbon and NPOC analysis, because pore water volatile fatty acids were analysed using a different method (solid phase extraction and GC mass spectroscopy). However, a purgable organic carbon analysis also has its limitations. Removing volatile organic carbon during the sparge process depends on the actual organic compound, the gas/liquid contact with the sparge gas, and the ambient temperature during sparging. Organic compounds that are highly soluble in water (such as methanol or ethanol) are not easily volatilized by sparging, whereas organic compounds with low solubility in water (such as methylene chloride) are easily expelled. The Shimadzu ASI-V auto sampler provided the option of externally adding acid to samples in the carousel to reduce run times. This option was not used because the addition of acid caused a 20% decrease in DIC concentration when compared to DIC samples analysed without this option.

Samples were typically analysed against two 8 point calibrations for both IC and NPOC analysis. Two calibration curves were required to cover the range of values produced by the samples. The IC method contained calibration curves covering 0-10 and 0-100 mg/l,
and the NPOC method 0-50 and 0-250 mg/l. The TOC instrument software automatically selected (on a measurement-by-measurement basis) the calibration curve with a concentration range that was greater than, or closest, to the measured value. The calibration correlation coefficient was never below 0.99 $r^2$. Figure 2.16 shows an example of a NPOC calibration curve used in the experiment. Full calibrations were performed every month or when any changes were made to the instrument. Calibration standard checks were run at the beginning, after every 8 samples and at the end of every sample run to check the validity of the current calibration. Blank deionised water samples were also processed at the beginning of every run as controls.

![Figure 2.16 An example of a NPOC calibration curve (0-250 mg/l) used in the experiment.](image)

2.7 Statistical Analysis and Experimental Design

The experiment was designed to be a fixed factor study where the $[\text{CO}_2]_{\text{tan}}$ treatment was fixed to the LGM level. Including two ecosystem types into the experimental design (bog
and fen) made the study more representative of wetlands and the experimental treatment more random. The ecosystems chosen represented the full nutrient gradient of wetland habitats, making them a good indicator of the range of CH₄ emissions from wetlands in natural environments. Including more than one ecosystem allowed for the experimental findings to be cautiously extended to all wetland types. The experiment was performed using two CEUs to investigate a between-cabinet treatment effect (CO₂ concentration) and within treatment (time influenced by seasonal changes in temperature and day length) influence on mesocosm CH₄ emissions. The experiment was designed based on a split-plot experimental set-up. Split-plot designs differ from ordinary ANOVA designed experiments because they assume some correlation among treatment levels within a block (CEU). Rather than the experiment manipulating at the ‘pot’ level within a single chamber, the experimental unit becomes the whole growth chamber. When performing experiments such as this, it is common place to test for between-chamber differences subjected to the same treatment level (Saarnio & Silvola, 1999). Therefore, an experiment such as the one undertaken here would ideally have a between-chamber factor represented by two levels. To truly replicate the effects of the treatment, it is suggested that the minimum number of growth chambers in this case would be four (Potvin, 2001). Due to financial limitations, four CEUs were not available and therefore no between-chamber analysis could be performed during this experiment. The main disadvantages of only using two CEUs is that it does not enable the partition of random deviation caused by the treatment factor, the experimental error and the undesirable environmental (block) effect. A strategy of alternating mesocosms between CEUs every month was used to reduce the outlined issues.

The statistical test most frequently used during this experiment was a General Linear Repeated Measures Model (ANOVA repeated measures). This test procedure provides
analysis of variance when the same measurement is made several times on each subject or case. With a repeated measures design it is possible to test the null hypothesis for both between-subject and within-subject factors. A repeated measures test is subject to the same basic assumptions as other parametric tests, e.g. normality, however an ANOVA repeated measures test also requires that variances and the covariances of the set scores are equal. The advantage of this type of analytical method is that time can be included in the model as a variable. By including time in the model, the interaction of treatment and time can be addressed. A significant result was classified as being below the 5% level (p<0.05) level of uncertainty.
3.1 Introduction

Ice core records show that $[\text{CH}_4]_{\text{atm}}$ over the last 800,000 years has varied from lows of ~350 ppbv during Pleistocene glacial maxima, to highs of ~800 ppbv during interglacials (Loulergue et al., 2008). The reason behind this natural variation is not fully understood, but variations in both wetland CH$_4$ emissions (Chappellaz et al., 1993a, Chappellaz et al., 1997) and the strength of the tropospheric sink (reaction with the OH radical) (Valdes et al., 2005, Kaplan et al., 2006) are thought to be major contributing factors. During the LGM, the combination of colder global temperatures (Guilderson et al., 1994, Jahn et al., 2005, Affek et al., 2008) and the presence of ice sheets across northern boreal latitudes (Abe-Ouchi et al., 2007) and the $[\text{CO}_2]_{\text{atm}}$ (Petit et al., 1999, Luthi et al., 2008) are thought to have limited the global wetland area. More recently however, it has been suggested that wetland area may not have varied substantially between the LGM and present day due to the creation of new wetlands on exposed continental shelves created by lower global sea levels during the LGM (Kaplan et al., 2006). Should wetland area have remained constant,
this would suggest the low \([\text{CH}_4]_{\text{atm}}\) at the LGM was the result of either an elevated atmospheric OH radical sink, caused by a global reduction in BVOC (e.g. isoprene, monoterpenes) fluxes from forests (Adams et al., 2001), or relatively low wetland methane emissions per unit area. Modern wetland \(\text{CH}_4\) emissions are known to be sensitive to higher than current \([\text{CO}_2]_{\text{atm}}\) (Dacey et al., 1994), critically however, wetland productivity and the response of \(\text{CH}_4\) emissions to LGM conditions has not been fully quantified. Wetland \(\text{CH}_4\) flux responses to modern controlling variables are well characterised (Bellisario et al., 1999), yet wetland \(\text{CO}_2\) starvation experienced at the LGM has received little attention.

Wetlands are known to be controlled by temperature, water table position, \([\text{CO}_2]_{\text{atm}}\), plant composition and productivity (Dacey et al., 1994, Macdonald et al., 1998, Blodau & Moore, 2003, Strom et al., 2005). Wetland plant composition is particularly important as areas with contrasting plant species can produce contrasting \(\text{CH}_4\) fluxes. For example, Strom et al., (2005) found the stimulation of methanogenesis through root exudation of substrate and the subsequent \(\text{CH}_4\) flux, was dependent upon the species of vascular plant. Plant productivity also plays a significant role in determining \(\text{CH}_4\) flux (Whiting & Chanton, 1993). A positive linear relationship exists between NEE and \(\text{CH}_4\) flux in wetlands (Whiting & Chanton, 1993, Waddington et al., 1996, Joabsson & Christensen, 2001), therefore it is important to understand the species composition of wetlands to fully understand the \(\text{CH}_4\) flux.

Current approaches to explaining the low \([\text{CH}_4]_{\text{atm}}\) at the LGM and glacial-interglacial \(\text{CH}_4\) differences, have focused on either ‘bottom-up’ or ‘top-down’ modelling. Bottom-up (process-based) models attempt to represent the processes leading to \(\text{CH}_4\) emissions in a
mechanistic manner (Cao et al., 1996; Potter, 1997; Walter et al., 1996; Zhuang et al., 2004), whilst reconstructing palaeovegetation in conjunction with atmospheric chemistry and circulation models (e.g. Valdes et al., 2005). Top-down (inverse) models infer the magnitude of wetland CH\(_4\) emissions by constraining atmospheric chemistry models with recorded ice core CH\(_4\) concentrations (Crutzen & Bruhl, 1993, Chappellaz et al., 1997, Brook et al., 2000, Dallenbach et al., 2000). To constrain the LGM wetland CH\(_4\) source, process-based models could be improved by including any ecophysiological responses of wetland ecosystems to a LGM [CO\(_2\)]\(_{atm}\). Current models are parametrised using values and CH\(_4\) emission controlling variables empirically established under a modern day atmosphere, which are therefore unlikely to be the best analogue of wetland ecosystems experiencing CO\(_2\) starvation at the LGM.

Studies have shown that [CO\(_2\)]\(_{atm}\) is an important variable that determines CH\(_4\) emissions from wetland ecosystems (Dacey et al., 1994). Simulated future elevated atmospheric CO\(_2\) concentrations, have been shown to produce larger CH\(_4\) fluxes from wetlands experiencing both in-situ CO\(_2\) enrichment using small scale free-air CO\(_2\) enrichment (mini-FACE) techniques (Saarnio et al., 2000) and in more manipulated mesocosm studies (Hutchin et al., 1995, Megonigal & Schlesinger, 1997, Saarnio & Silvola, 1999, Kang et al., 2001, Ellis et al., 2009). It is thought that the increase in CH\(_4\) emissions result from an increase in photosynthetive allocation from wetland plants to the rhizosphere, with plant root exudates known to be an important substrate for CH\(_4\) production (Kim & Kang, 2008). These results demonstrate that at, and above modern ambient CO\(_2\) concentrations, CO\(_2\) is a key controller of CH\(_4\) flux from wetlands, however no direct measurements of the effect of LGM [CO\(_2\)]\(_{atm}\) on wetland CH\(_4\) flux has been made.
Given that the LGM is characterised by exceptionally low CO₂ concentrations of ~180 ppmv (Petit et al., 1999) or approximately half of modern [CO₂]ₐir, it is hypothesised that CO₂ starvation during that time would have an important limiting effect on CH₄ flux in a way that contrasts with those observed in CO₂ enrichment studies, i.e. a decrease in flux would be observed. To test this central hypothesis, a two year controlled environment experiment was designed to investigate how LGM [CO₂]ₐir influences CH₄ flux from two contrasting natural temperate wetland ecosystems. The two wetland ecosystems were a nutrient poor bog (Migneint) and a nutrient rich fen (Cors Goch). At the end of the experiment the influence of LGM [CO₂]ₐir on net primary production was also investigated.

3.2 Methods

3.2.1 Experimental Design

Peat mesocosms (110 x 400 mm) were collected in autumn 2006 from a base-rich minerotrophic fen (Cors Goch) on the Isle of Anglesey, Wales, UK (SH 504 817) and from a base-poor ombrotrophic bog (Migneint) located in the Snowdonia National Park, Wales, UK (SH 816 440–central point). A total of 16 bog and 16 fen mesocosms were collected with intact surface vegetation. Mesocosm containers were constructed from opaque PVC pipe segments and sealed base caps that maintained the anaerobic condition of the core. Mesocosms were randomly assigned to one of two Snijders Microclima MC1750E CEUs, where 2 temperate growing seasons were recreated. One of these groups was the
designated control and the other was the designated treatment group. Light intensity was set to 250 µm m⁻² s⁻¹ and relative humidity to 70% during daylight hours (60% when lights were off at night). Monthly temperatures were based on local 30 year averages from 1970-2000 (Met Office, 2006) and daylight hours were estimated based on the longitude and latitude of the local area. The water-table was maintained to within 2-3 cm of the surface of the mesocosms by frequent (between 1-3 times per week) applications of distilled water.

3.2.2 Modification of [CO₂]ₘₐₜ

An auto-regulating CO₂ system was designed to maintain [CO₂] within the treatment CEU at LGM concentrations (i.e. ~180 ppmv) and modern day [CO₂] in the control CEU (i.e. ~380 ppmv). The CO₂ regulating system included a purge gas generator (CMC Ltd) that generated zero [CO₂] air by using PSA technology to remove CO₂ from compressed air. The purge gas generator creates a near-continuous source of CO₂-free (<1 ppmv) dry (<0.01 ppmv) air by switching between two adsorbent vessels (molecular sieves). Over 2 years the control [CO₂] averaged 406 ± 23 (S.E.) and the treatment 196 ± 28 ppmv (figure 3.1). Mesocosms (and their associated CO₂ exposure) were rotated between the two cabinets to minimise any possible block effects within the cabinets. CH₄ emissions were measured within the CEUs using static and dynamic chambers. Chambers were constructed from clear perspex pipe (110 x 500 mm) and a three-way valve sample port. Pressure changes were prevented by allowing a small needle hole (0.8 mm) through a resealing membrane (Suba Seal). Full details of the method used to create the LGM [CO₂]ₘₐₜ within the CEUs and the design of the chambers can be found in 2.2.4 and 2.2.5 respectively.
Figure 3.1 Manually recorded CO₂ concentrations during the experiment using the internal Vaisala CARBOCAP® CO₂ sensor. The experiment started on day 95, represented by the dashed line. Pre-day 95, both control and treatment mesocosms were maintained in the same CEU at the same ambient [CO₂]. Control = 406 ± 23 (S.E); treatment = 196 ± 28 ppmv over the 2 year experiment.

3.2.3 CH₄ Flux Measurements

Gas samples were taken at the same point during the day cycle (midday) at each sample point throughout the experiment. Sampling frequency was kept to a minimum (bi-monthly on average) to 1) allow characterisation of seasonal CH₄ flux responses to the treatment and 2) to minimise intrusion on LGM CO₂ treatment as gas sampling involved opening cabinet doors and temporarily (hours) elevating CO₂ concentrations. Chamber [CH₄] was determined by GC using a Poropack Q column and FID (Ai Cambridge GC94), and CRDLS (Los Gatos Research RMA-200 Fast Methane Analyser). CH₄ fluxes were calculated from the linear increase in gas concentration in the chamber with time, using a
linear regression equation (Christensen et al., 1995). Total emitted CH$_4$ was calculated using equation 3.1 (Melling et al., 2005), where $R_i$ is the mean gas flux (mg m$^{-2}$ day$^{-1}$) of two sampling points, $D_i$ is the number of days in the sampling interval and $n$ is the number of sampling times.

\[
\text{Total emitted CH}_4 = \sum_{i=1}^{n} R_i D_i
\]

(Equation 3.1)

3.2.4 Net Ecosystem Exchange (NEE)

NEE is the net carbon gain (or loss) by ecosystems and is defined as the measured difference between gross primary production (GPP) and respiration. GPP is the rate at which an ecosystem captures and stores a given amount of chemical energy as biomass over a given length of time. A PP Systems CIRAS-2 portable photosynthesis system was used to determine NEE on the wetland mesocosms after they had been exposed to a LGM [CO$_2$] for more than 1000 days. The analyser uses 4 non-dispersive infrared measurements to accurately measure both CO$_2$ and H$_2$O. The CIRAS-2 has internal air sampling pumps with mass flow controllers that pump air through the cells at ~100 ml/minute. The analyser measures absolute concentration of a reference gas sample and calculates the difference in concentration to a second sample.

To measure NEE, the CIRAS-2 instrument was configured to operate using a closed system chamber. The same dynamic chamber used to determine CH$_4$ flux in the second year of the experiment was adapted to monitor the change in [CO$_2$]$_{atm}$. A fan secured to the inside of the chamber ensured air was mixed evenly during sampling. NEE was
measured on 2 separate occasions (1129 and 1131 days into the experiment), at midday during the light section of the diurnal cycle. On each occasion NEE from all bog and fen mesocosms was recorded over four 1 minute time periods. The temperature was 15°C, relative humidity 70% and the light flux was 250 μm m⁻² s⁻¹ during the measurements. Total ecosystem respiration was measured during the night/dark section of the diurnal cycle on the following days after NEE was measured (1130 and 1132 days into the experiment). For this measurement, the chamber was covered in tinfoil to completely eradicate any background laboratory lighting. Temperature and humidity were maintained at daylight levels. Gross photosynthesis was calculated based on the difference between NEE and dark respiration. The equipment and methods used to determine NEE, dark respiration and gross photosynthesis in this experiment are commonly used in wetland studies (Joabsson & Christensen, 2001, Christensen et al., 2003a).

3.2.5 Statistics

A general linear model (ANOVA repeated measures) was used to analyse for within-subject (time) and between treatment difference during the experiment. The same test was also used to test for an interaction between time and treatment effect. Where the assumptions for this test (normality, equal variances and sphericity) were not satisfied, transformations and corrections were applied accordingly. Data that continued to fail to meet the criteria were analysed using a non-parametric Kruskal-Wallis one-way analysis of variance and Friedman’s repeated measures test. When using non-parametric alternatives, the interaction of within-subject (time) and between-subject (treatment) could not be analysed. Independent t-tests were used when analysing statistical differences between total, cumulative CH₄ emitted from controls and treatments.
3.3 Results and Discussion

3.3.1 Introduction

All the underlying data presented in this Chapter can be found on a compact disc at the rear of the thesis.

The LGM $[\text{CO}_2]_{am}$ suppressed the total emitted $\text{CH}_4$ after the 2 year experiment by 19% ($P > 0.05$) and 29% ($p<0.05$) in the bog and fen treatment mesocosms compared to their controls, respectively. During this time, $\text{CH}_4$ emissions (control and treatment values) were of a similar magnitude (figure 3.2) to other mesocosm studies from the same locations (Hutchin et al., 1995, Kang et al., 2001) and fit comfortably within the broad range of measured $\text{CH}_4$ fluxes from northern latitude wetlands (Dise, 1993, Silvola et al., 2003). NH wetland fluxes typically range from 0-200 mg m$^{-2}$ day$^{-1}$, however larger values of up to 1000 mg m$^{-2}$ day$^{-1}$ have been measured (Dise, 1993).

3.3.2 Pre-Treatment Fluxes

Prior to $\text{CO}_2$ treatment initiation (0-95 days) all bog and fen mesocosms were maintained in winter conditions and in the same $\text{CO}_2$ environment of 426 ± 4.5 (S.E.) ppmv (figure 3.1). During this period, no differences between bog and fen average $\text{CH}_4$ flux or between total $\text{CH}_4$ emitted from each treatment group was observed ($P > 0.05$) (table 3.1). Both bog and fen control groups emitted more $\text{CH}_4$ (21 and 18% respectively) compared to their
Figure 3.2 Average CH$_4$ fluxes measured over 2 years (~650 days) from (A) bog and (B) fen mesocosms. The control category represents ambient [CO$_2$]$_{am}$ and the treatment the simulated LGM [CO$_2$]$_{am}$. Each point shows the mean flux of ~8 peat mesocosms. Error bars represent ±1 standard error of the mean. The shaded area represents the temperature during the day light simulation.
Table 3.1 Comparison of control and treatment CH$_4$ flux data from bog and fen mesocosms during the 2 year experiment.

| Period           | Site | Treatment      | Mean (mg m$^{-2}$ day$^{-1}$ ± S.E.) | Difference between control and treatment means (%) | Within-subject effects (time) | Within-subject interaction (time * treatment) | Between subject effect (treatment) | Total CH$_4$ emitted (g m$^{-2}$ ± S.E.) | Difference between control and treatment total emitted CH$_4$ (%) | Between subject difference (total emitted data) *
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<tr>
<td>Year 1 (pre-CO$_2$ treatment)</td>
<td>Bog</td>
<td>Control Treatment</td>
<td>12.0 ± 3.51</td>
<td>-21</td>
<td>F(2) = 2.52, p&gt;0.05</td>
<td>F(2) = 0.96, p&gt;0.05</td>
<td>F(1) = 0.56, p&gt;0.05</td>
<td>0.78 ± 0.18</td>
<td>-33</td>
<td>T(14) = 1.14, p&gt;0.05</td>
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<td>Control Treatment</td>
<td>9.53 ± 3.12</td>
<td>-18</td>
<td>F(2) = 4.88, p&lt;0.05</td>
<td>F(2) = 0.32, p&gt;0.05</td>
<td>F(1) = 0.21, p&gt;0.05</td>
<td>1.39 ± 0.51</td>
<td>-25</td>
<td>T(14) = 0.60, p&gt;0.05</td>
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<td>Fen</td>
<td>Control Treatment</td>
<td>24.0 ± 7.07</td>
<td>-20</td>
<td>F(4.62) = 4.84, p&lt;0.01</td>
<td>F(4.62) = 2.03, p&gt;0.05</td>
<td>F(1) = 0.005, p&gt;0.05</td>
<td>4.64 ± 0.89</td>
<td>-26</td>
<td>T(14) = 1.44, p&gt;0.05</td>
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<td>Control Treatment</td>
<td>25.3 ± 3.10</td>
<td>-17</td>
<td>F(4.84) = 1.86, p&gt;0.05</td>
<td>F(4.84) = 0.72, p&gt;0.05</td>
<td>F(1) = 0.74, p&gt;0.05</td>
<td>7.14 ± 1.38</td>
<td>-26</td>
<td>T(14) = 1.44, p&gt;0.05</td>
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<td>Year 2</td>
<td>Bog</td>
<td>Control Treatment</td>
<td>17.6 ± 2.52</td>
<td>-13</td>
<td>F(5.48) = 4.67, p&lt;0.01</td>
<td>F(5.48) = 0.86, p&gt;0.05</td>
<td>F(1) = 4.68, p&gt;0.05</td>
<td>5.30 ± 1.70</td>
<td>-15</td>
<td>T(9.07) = 1.81, p&gt;0.05</td>
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<td></td>
<td>Fen</td>
<td>Control Treatment</td>
<td>26.3 ± 3.02</td>
<td>-31</td>
<td>X$^2$ (14) = 37.5, p&lt;0.01</td>
<td>N/A</td>
<td>H(1) = 13.2, p&lt;0.01</td>
<td>7.61 ± 2.47</td>
<td>-32</td>
<td>T(14) = 3.67, p&lt;0.01</td>
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<tr>
<td>Year 1 + 2</td>
<td>Bog</td>
<td>Control Treatment</td>
<td>20.0 ± 1.84</td>
<td>-17</td>
<td>X$^2$ (27) = 77.8, p&lt;0.01</td>
<td>N/A</td>
<td>H(1) = 2.60, p&gt;0.05</td>
<td>9.94 ± 2.04</td>
<td>-19</td>
<td>T(14) = 0.91, p&gt;0.05</td>
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<td>Fen</td>
<td>Control Treatment</td>
<td>28.1 ± 2.42</td>
<td>-25</td>
<td>X$^2$ (27) = 78.0, p&lt;0.01</td>
<td>N/A</td>
<td>H(1) = 16.2, p&lt;0.01</td>
<td>14.74 ± 2.93</td>
<td>-29</td>
<td>T(14) = 2.87, p&lt;0.05</td>
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*Plant corrected data. Mesocosm fluxes divided by the number of vascular plants present.
treatment groups before the experiment began. The pre-treatment period showed that average fen CH$_4$ fluxes were $\sim$51% larger than bog fluxes. Trophic status plays a significant role in the magnitude of wetland CH$_4$ fluxes, with nutrient rich wetlands known to emit more CH$_4$ than nutrient poor wetlands (Juottonen et al., 2005, Hornibrook & Bowes, 2007). The experimental mesocosms were, therefore, emitting CH$_4$ at rates consistent with the ecosystems from which they were extracted.

3.3.3 Year 1 CH$_4$ flux

After CO$_2$ treatment initiation (day 95), a pronounced decrease in CH$_4$ flux was measured from the bog and fen treatment groups, when compared to their equivalent controls (figure 3.2). This decrease is most evident in the bog experimental group, where during the first month of CO$_2$ manipulation, the bog control group averaged 47 mg (CH$_4$) m$^{-2}$ day$^{-1}$ compared to only 19 mg (CH$_4$) m$^{-2}$ day$^{-1}$ in the treatment, a difference of $\sim$60%. Bog and fen control group CH$_4$ emissions peaked immediately after the CO$_2$ treatment began in year 1, whereas bog and fen treatment CH$_4$ fluxes peaked later in the year during the simulated month of August.

Explaining the early year 1 CH$_4$ peak in the control groups is challenging. Wetland CH$_4$ fluxes are known to respond exponentially in laboratory environments to temperature increases (Daulat & Clymo, 1998, Gauci et al., 2004). In the field, the highest wetland CH$_4$ flux values are measured during the summer months of July and August in the NH (Dise, 1993). Summer exhibits the highest wetland CH$_4$ fluxes because temperature becomes less of a limiting factor on carbon decomposition, plant productivity and methanogenesis compared to colder seasons. One possible explanation for this peak is that
by shortening the spring to accommodate a long-term 2 year growing experiment, this caused an early collapse in CH₄ emissions from the control mesocosms. Increasing the temperature quickly could have stimulated biological activity and caused the rapid consumption of organic compounds that accumulate over winter by methanogens (Hines et al., 2001, Duddleston et al., 2002). In addition, the plants within the mesocosms would have also started to export more labile carbon into the rhizosphere as temperature restrictions on productivity were removed. Treatment mesocosms would have experienced the same change in temperature, however plant productivity in the mesocosms may have been suppressed in the LGM [CO₂] atm compared to the control. This would have potentially limited the release of newer root exudates into the rhizosphere and restricted the amount of substrate available for methanogens (Whiting & Chanton, 1993) when compared to the controls.

Treatment mesocosm fluxes in year 1 peaked during the August simulation (figure 3.2). These peaks coincided with the second highest maximum daily temperature and day length in the year. The peaks also coincided with a period in the experiment where the CCDS struggled to accurately maintain the treatment [CO₂] set point (as detailed in chapter 2.4.4). This problem was rectified by redesigning the CCDS (~380 days into the experiment), thus improving the accuracy, efficiency and reproducibility of the LGM [CO₂] atm treatment in the CEUs in year 2 (figure 3.1). During this time, it was the only period in the experiment that three consecutive (bog and fen) treatment flux measurements were higher compared to their equivalent control values. This suggests that plant productivity plays a significant role in determining CH₄ flux by increasing or decreasing the quantity of root exudates according to changes in [CO₂] atm. It also suggests that this ‘knock-on’ effect may only take a matter of days to begin altering CH₄ emissions from wetlands. This theory is supported
by the rapid divergence in control and treatment flux values measured at the onset of the experiment.

During the first year, there was no significant difference in average CH$_4$ flux between the LGM [CO$_2$]$_{ahn}$ treated mesocosms relative to the modern day control mesocosms (P > 0.05). The bog control group averaged $23.2 \pm 2.66$ mg (CH$_4$) m$^{-2}$ day$^{-1}$, whereas the treatment mesocosms averaged $18.6 \pm 2.21$ mg (CH$_4$) m$^{-2}$ day$^{-1}$. The fen control average CH$_4$ flux was $30.5 \pm 3.97$ mg (CH$_4$) m$^{-2}$ day$^{-1}$ and the fen treatment average flux was $25.3 \pm 3.10$ mg (CH$_4$) m$^{-2}$ day$^{-1}$. These values represent a difference of 20 and 17% between fen and bog experimental groups respectively. At the end of the season, there was also no statistical difference between total CH$_4$ emitted between bog and fen experimental groups (P > 0.05). The bog control group emitted a total of $4.64 \pm 0.89$ g (CH$_4$) m$^{-2}$, whereas treatment mesocosms emitted $3.55 \pm 0.50$ g (CH$_4$) m$^{-2}$, a difference of 23%. The fen control group emitted $7.14 \pm 1.38$ g m$^{-2}$, which was 26% more than the treatment group ($5.28 \pm 1.06$ g m$^{-2}$).

The difference in CH$_4$ emissions between the control and treatment groups in year 1 was similar to that measured during the pre-treatment period. The difference between bog control and treatment total CH$_4$ emitted values actually showed that the difference had decreased slightly from 33 to 23%. A direct comparison between the pre and post treatment periods can only be carried out with a degree of caution. The pre-treatment period contained only three sampling points during the winter and spring of year 1. Therefore comparing the differences measured between the experimental groups pre-CO$_2$ treatment to post-CO$_2$ treatment initiation periods, where mesocosms had experienced a greater range of environmental variables and produced a larger range of CH$_4$ emissions, is
not an appropriate comparison. During the first year, only the bog mesocosms showed a within-subject effect where fluxes were auto-correlated with time (P < 0.01). This indicates that the bog flux values in both groups were changing over time, but in a similar way so that there was no interference with the between-subject effect (time x treatment interaction) (P > 0.05).

3.3.4 Year 2 CH₄ flux

In year 2 bog control and treatment flux values were of similar magnitude to one another and frequently overlapped (figure 3.2). This pattern remained the same until during the late summer/autumn period, where treatment fluxes were consistently lower (~68%) when compared to the control. Both bog control and treatment flux peaked at 32.5 mg (CH₄) m⁻² day⁻¹ in year 2, however the bog treatment peak flux lagged behind the control by ~1 month. The average bog control flux was 17.6 ± 2.52 mg (CH₄) m⁻² day⁻¹, whereas the treatment average flux was 13% lower at 15.3 ± 2.84 mg (CH₄) m⁻² day⁻¹. A repeated measures analysis of this data showed that fluxes changed over time (P < 0.01) during year 2, yet there was no interaction with the between-subject treatment effect. The between-subject effect was on the boundary of significance (p = 0.05). Bog control mesocosms emitted 5.30 ± 1.70 g (CH₄) m⁻² by the end of the season. This was not significantly different (P > 0.05) to the 4.51 ± 2.50 g (CH₄) m⁻² emitted by the bog treatment mesocosms.

Fen control mesocosms emitted a total of 7.61 ± 2.47 g (CH₄) m⁻² in year 2, which was 32% more (P < 0.01) when compared to the treatment mesocosms total of 5.18 ± 2.04 g (CH₄) m⁻². During the year, control and treatment fen CH₄ emissions shared a similar
pattern in the majority of simulated months, except for the summer months of June, July and August (figure 3.2). Fen control and treatment mesocosms emitted CH$_4$ at similar rates (37.6 and 35.0 mg (CH$_4$) m$^{-2}$ day$^{-1}$ respectively) for the first measurement in June, however after this point the two groups clearly diverged, where the gap in CH$_4$ flux grew over the following ~3 months. Over this period, fen control fluxes were on average 38% higher than fen treatment fluxes. This divergence reached a maximum in August, where the fen control emitted 54.0 ± 18.6 and treatment group 27.7 ± 12.4 mg (CH$_4$) m$^{-2}$ day$^{-1}$. This was the largest difference measured (~49%) between the two groups in year 2. The average year 2 fen control flux was 26.3 ± 3.02 mg (CH$_4$) m$^{-2}$ day$^{-1}$, whereas the average treatment flux was measured at 18.1 ± 2.44 mg (CH$_4$) m$^{-2}$ day$^{-1}$. This is a statistically significant difference ($P < 0.01$) of 31%, which is larger than the difference measured in the pre-treatment period (18%) and the difference exhibited in year 1 (17%).

Both fen control and treatment flux data changed over the duration of year 2 ($P < 0.01$), however because the data set failed the assumptions required for an ANOVA repeated measures analysis, the interaction of time and between-subject treatment could not be examined using this approach. Based on observing no interaction in either year 1 flux data or in bog flux data from year 1 and 2, it is unlikely that there would have been any interaction; however the possibility of an interaction cannot be completely ruled out.

3.3.5 LGM [CO$_2$]$_{am}$ Influence on CH$_4$ Emission Over 2 Years

Combining both the years results together to analyse the end of experiment (year 1+2) total CH$_4$ flux, showed that the LGM [CO$_2$]$_{am}$ treatment had significantly suppressed ($P < 0.05$)
the CH₄ flux from the fen by an average of 29% (figure 3.3). In contrast, although the bog treatment CH₄ flux was 19% lower than the control, this proved to be insignificant (P > 0.05). The same pattern was observed when the mean CH₄ flux values were analysed. The fen treatment average (21.2 ± 1.94 g (CH₄) m⁻²) CH₄ flux was suppressed when compared to the modern day control (28.1 ± 2.42 g (CH₄) m⁻²) (P < 0.01), whereas the bog data showed no statistical difference (P > 0.05) between control (20.0 ± 1.84 g (CH₄) m⁻²) and treatment (16.7 ± 1.88 g (CH₄) m⁻²). Both bog and fen flux data sets failed to meet the
assumption for an ANOVA repeated measures analysis. A non-parametric Friedman's test showed that both bog and fen CH₄ fluxes changed over the duration of the experiment (P < 0.01). On completion of the experiment, total CH₄ emitted from each of the experimental groups followed this pattern, bog treatment<bog control<fen treatment<fen control (figure 3.3). Total CH₄ emitted from the fen control group (14.74 g (CH₄) m⁻²) was statistically different to the fen treatment (10.46 g (CH₄) m⁻²) and to both bog control (9.94 g (CH₄) m⁻²) and treatment (8.06 g (CH₄) m⁻²) totals (P < 0.05).

In this experiment, applying an LGM [CO₂] atm starvation treatment to wetland mesocosms suppressed CH₄ emissions in nutrient-rich fen peat mesocosms. The cause of this suppression is likely to have been a decrease in primary productivity which would have limited root exudates from plants (Whiting & Chanton, 1993). Decreasing the [CO₂] atm may have limited photosynthesis (Tissue et al., 1995) and reduced the carbon allocation to the rhizosphere (Dippery et al., 1995). The effect of this would be to limit the supply of carbon for methanogenesis. During the 2 year experiment, dissolved carbon in pore water was also measured (Chapter 6). The findings from this analysis could help to confirm this hypothesis.

At the end of the experiment the fen LGM [CO₂] atm treatment had emitted 29% less CH₄ than the modern day [CO₂] atm control. The suppression effect was larger in year 2 compared to the previous year, therefore it is possible that in subsequent years, the LGM [CO₂] atm would have further decreased the CH₄ flux. In contrast to the fen mesocosms, the nutrient-poor bog mesocosms showed no significant CH₄ flux response to the LGM [CO₂] atm treatment. Modern nutrient-rich wetlands produce more CH₄ compared to nutrient deficient wetlands (Hornibrook & Bowes, 2007). The experiment showed that this pattern remains the same under CO₂ starvation conditions. CH₄ emissions were
significantly suppressed from the mesotrophic fen where the ecosystem was operating under CO₂ limitation rather than nutrient limitation. In contrast, CH₄ emissions from the ombrotrophic bog were potentially regulated by the availability of nutrients, a limitation that could potentially render CH₄ fluxes from such ecosystems insensitive to reductions in [CO₂]ₐₚ. The assumption that glacial maxima reduces the productivity of wetland ecosystems and CH₄ emissions is supported by CO₂ fertilisation studies which report increases in DOC and plant biomass at elevated CO₂ concentrations (Kang et al., 2001, Freeman et al., 2004a). It is assumed the opposite is taking place in the fen LGM treatment mesocosms which reduced the plant productivity and the supply of fresh labile carbon substrate to methanogens.

The results from this experiment suggest that if wetland extent did not change considerably since the LGM (Kaplan et al., 2006), then models could be significantly over-estimating wetland CH₄ emissions both during the LGM and in the immediate post-glacial period leading into the Holocene. For example, Smith et al., (2004) identify that post-glacial peatland expansion in the West Siberian Lowland (WSL) was dominated by relatively nutrient-rich fen ecosystems which, under modern [CO₂]ₐₚ, peatlands in these latitudes are known to be highly CH₄ emitting (Bubier et al., 1995). The results from this study suggest that the CH₄ contribution of the WSL fens during the early Holocene (Smith et al., 2004) is likely to have been overstated as fen ecosystems exposed to sub-ambient [CO₂]ₐₚ representative of this time, produce less CH₄ compared to those of the modern day. The findings in this study therefore suggest that the increase in [CH₄]ₐₚ associated with the Pleistocene-Holocene transition might be more likely due to the creation of highly CH₄ emitting thermokarst lakes during deglaciation (Walter et al., 2007), rather than increases in fen dominated global wetland areas (Smith et al., 2004, MacDonald et al., 2006).
3.3.6 CH₄ Flux After 1000+ Days of LGM [CO₂] atm Treatment

After year 2 had finished, the LGM [CO₂] treatment was continued for more than ~420 days with environmental parameters maintained at a constant climate simulation of the month of May (see Chapter 2, table 2.1 for settings). During this time the [CO₂] atm in the treatment did not exceed 200 ppmv during daylight hours in the cycle. Two final CH₄ fluxes were measured on days 1116 and 1123 into the experiment. The bog control mesocosms averaged 6.61 ± 4.66 mg (CH₄) m⁻² day⁻¹, whereas the treatment mesocosm averaged 4.77 ± 4.42 mg (CH₄) m⁻² day⁻¹ (P > 0.05). Fen treatment mesocosms averaged 0.46 ± 0.33 mg (CH₄) m⁻² day⁻¹, which was 97% less than the control mesocosm average of 13.7 ± 5.38 mg (CH₄) m⁻² day⁻¹ (P < 0.01). Such a large difference between the fen control and treatment CH₄ flux is unlikely to have been exclusively driven by the LGM CO₂ treatment. Between the end of year 2 and the final flux measurements, *Juncus subnodulosus* had proliferated in the fen treatment mesocosms. Vascular plants can account for up to 90-97% of measured CH₄ flux from wetlands (Waddington *et al.*, 1996, Kelker & Chanton, 1997, King *et al.*, 1998, Frenzel & Karofeld, 2000) due to their internal structure and ability to export labile carbon into the rhizosphere (Strom *et al.*, 2005). Up to 56 *Juncus* shoots were counted in the treated fen mesocosms (average = 26 per mesocosm) before the CH₄ flux was measured. Such a large density in small mesocosms (110 x 400 mm) could therefore have aerated the core with O₂ and reduced the CH₄ flux.

3.3.7 The Influence of Plants on CH₄ flux

Removing the influence of vascular plants is important when analysing flux data (Saarnio & Silvola, 1999) as CH₄ emissions are strongly associated with wetland plant composition.
(Schimel, 1995, Strom et al., 2005). In particular, accounting for differences in vascular plants is important because they provide a path of least resistance for CH4 to diffuse through (Thomas et al., 1996, Lloyd et al., 1998, Christensen et al., 2003b). Uneven distributions between bog and fen experimental groups were measured during year 1 and 2, which could have potentially disguised the effect of CO2 starvation (table 3.2). To solve this problem, mesocosm fluxes were divided by the number of recorded plant shoots for total emitted CH4 analysis and vascular plant numbers were used as a covariate in ANOVA repeated measures analyses.

Table 3.2 The number of vascular plants recorded at three periods during the experiment.

<table>
<thead>
<tr>
<th>Experimental Group</th>
<th>Year 1 (summer)</th>
<th>Year 2 (summer)</th>
<th>1000 + days of LGM [CO2]am exposure</th>
<th>Approximate increase from 1 to 1000+ days (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bog Control</td>
<td>4.63 ± 2.53</td>
<td>0</td>
<td>13.3 ± 7.13</td>
<td>187</td>
</tr>
<tr>
<td>Bog Treatment</td>
<td>1.13 ± 0.52</td>
<td>2.00 ± 2.00</td>
<td>3.00 ± 2.00</td>
<td>165</td>
</tr>
<tr>
<td>Fen Control</td>
<td>3.65 ± 1.60</td>
<td>3.38 ± 2.03</td>
<td>7.75 ± 3.05</td>
<td>112</td>
</tr>
<tr>
<td>Fen Treatment</td>
<td>5.5 ± 1.91</td>
<td>12.5 ± 4.09</td>
<td>25.5 ± 8.31</td>
<td>364</td>
</tr>
</tbody>
</table>

In all experimental groups regardless of treatment, the average number of vascular plants per mesocosm increased by the end of the experiment (table 3.2). This was mainly caused by an increase in Juncus effuses and subnodulosus abundance in both the bog and fen ecosystems. The largest increase was measured in the fen treatment mesocosms, where Juncus subnodulosus began to dominate entire mesocosms by the end of the experiment. Studies which have exposed wetlands to elevated [CO2]am have found that this can cause increased above-ground biomass (Dacey et al., 1994, Fenner et al., 2007) and below-
ground biomass (Dacey et al., 1994, Marsh et al., 2005) in both vascular and bryophyte vegetation types. However, many studies have also found no statistical differences in biomass after long-term elevated CO₂ exposure (Megonigal & Schlesinger, 1997, Berendse et al., 2001, Hoosbeek et al., 2001, Milla et al., 2006). The exact influence of elevated [CO₂]_{atm} on biomass is therefore unclear, however, determining how biomass and plant photosynthesis respond to CO₂ starvation is extremely important as both are strongly correlated with CH₄ emissions from wetlands (Vann & Megonigal, 2003).

The large increase in Juncus in the fen treatment when exposed to CO₂ starvation conditions was an unexpected result. Where atmospheric CO₂ is severely limited, C₃ (of which Juncus spp are examples) plants show a reduction in root mass, lower growth rates and lower specific leaf mass (Dippery et al., 1995). The increase in Juncus could have been independent of the treatment, or perhaps due to an ‘edge’ effect in the mesocosm. If the LGM [CO₂]_{atm} had actually provided the Juncus with a competitive advantage over the other vegetation, this would have implications for carbon sequestration. Juncus could be accessing CO₂ released from decomposition which is channelled through its aerenchyma tissues and subsidising photosynthesis (Li & Jones, 1995). If CO₂ starvation conditions give vascular plant species an advantage, this could mean that LGM or early Holocene wetlands would be potentially dominated by plants which are more easily decomposable compared to modern day wetlands that include recalcitrant species such as Sphagnum. Moreover, any increases in vascular plants would have implications for CH₄ transport (Joabsson et al., 1999).
3.3.8 Influence of Temperature on CH₄ Flux

CH₄ flux under laboratory controlled conditions responds exponentially to a linear increase in temperature from wetlands (Daulat & Clymo, 1998). The influence of LGM [CO₂] atm on this relationship was investigated by plotting flux data from year 1 and 2 against the corresponding temperature when it was measured. As day length (hours of light) also varied over both years, the results from this analysis can only be used as an indication of CH₄ flux response to temperature. The direct influence of LGM [CO₂] atm on the temperature response of CH₄ emissions was studied in more detail in a separate investigation (Chapter 5).

In year 1 there was no clear relationship between CH₄ flux and temperature, however in year 2, CH₄ fluxes from all experimental group showed a linear response to temperature (figure 3.4). Bog control and treatment shared a similar flux response across the temperature range, whereas increasing temperature caused a divergence in the temperature response of CH₄ between fen control and treatment. This finding is similar to the results of the temperature response experiment that was performed during the spring of year 2 (Chapter 5). One reason for this ecosystem dependence in the treatment response could be due to different dominant CH₄ production pathways. Bog methanogens are predominantly CO₂/H₂ utilisers, whereas nutrient-rich fens are inhabited by a greater presence of obligate acetotrophs (Galand et al., 2005, Juottonen et al., 2005). This potentially makes acetotrophic methanogenesis in fens more susceptible to changes in plant derived substrates which are influenced by both temperature and atmospheric [CO₂] atm.
3.3.9 NEE Exchange

At the end of the experiment the LGM CO₂ treatment groups both produced more negative NEE values when compared to their controls (table 3.3). Whilst there was no statistically significant difference between the bog experimental groups (P > 0.05), there was a clear pattern that bog treatment mesocosms that shared similar vegetation to controls had contrasting NEE values. The fen treatment NEE rate was significantly different (P < 0.05) from the NEE measured for the control. The reason for this was that the treatment group had more *Juncus* in their mesocosms compared to the control (table 3.2), resulting in a higher photosynthesis rate. Therefore, a fair comparison of fen control and treatment NEE values cannot be made. NEE and CH₄ emissions are closely correlated (Whiting & Chanton, 1993, Joabsson & Christensen, 2001), however there was no correlation in this study between the two.
Table 3.3 Average NEE, respiration and gross photosynthesis from bog and fen mesocosms.

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Average NEE (mg CO₂ m⁻² h⁻¹ ± 1 S.E.)</th>
<th>Average respiration (mg CO₂ m⁻² h⁻¹ ± 1 S.E.)</th>
<th>Average gross photosynthesis (mg CO₂ m⁻² h⁻¹ ± 1 S.E.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bog control</td>
<td>22.7 ± 21.5</td>
<td>111.7 ± 13.7</td>
<td>-89.1 ± 30.1</td>
</tr>
<tr>
<td>Bog Treatment</td>
<td>-81.6 ± 67.9</td>
<td>96.1 ± 58.3</td>
<td>-177.7 ± 125.3</td>
</tr>
<tr>
<td>Fen Control</td>
<td>-32.9 ± 24.9</td>
<td>223.8 ± 63.2</td>
<td>-256.6 ± 69.9</td>
</tr>
<tr>
<td>Fen Treatment</td>
<td>-213.0 ± 68.8</td>
<td>306.4 ± 87</td>
<td>-519.4 ± 149.2</td>
</tr>
</tbody>
</table>

3.5 Conclusion

Estimating the contribution of wetlands to [CH₄]ₐm during the LGM remains extremely difficult. This study demonstrates that the [CO₂]ₐm present at the LGM significantly limits CH₄ emissions from more nutrient-rich wetlands by a minimum of 29% while having no effect on nutrient-poor bogs. This suggests that there is considerable uncertainty in the way that late Quaternary glacial wetland CH₄ emissions are represented in models. The results from this experiment show that the trophic status of natural wetlands is a key determinant in their response to atmospheric CO₂ concentrations during the LGM and Holocene. Nutrient availability should therefore be an important consideration when attempting to estimate the contribution of wetlands to ancient CH₄ budgets. Current estimates of wetland CH₄ emissions during the PIH and the LGM suggest there was little difference between these two time points in terms of global extent or emissions (Valdes et al., 2005, Kaplan et al., 2006). Results gained from this study therefore suggest that the source strength of late-glacial and early Holocene wetlands are currently over-estimated because fen ecosystems are a far smaller CH₄ source under low [CO₂]ₐm than they are today.
4.1 Introduction

Wetlands are an important component of the global carbon cycle because they account for 16-33% of the world’s soil carbon store (Gorham, 1991, Maltby & Immirzi, 1991, Bridgham et al., 2006) and emit between 100-231 Tg of CH$_4$ per year (Hein et al., 1997, Houweling et al., 2000, Wuebbles & Hayhoe, 2002, Fletcher et al., 2004, Wang et al., 2004, Chen & Prinn, 2006). CH$_4$ is a powerful greenhouse gas with a radiative forcing value of 0.48 W m$^{-2}$ (Ramaswamy et al., 2001). Wetlands are the single largest contributor of CH$_4$ to the atmosphere (Lelieveld et al., 1998), therefore appreciating how this flux varies both spatially and temporally can inform future climate prediction models and also contribute to the understanding of why variations in [CH$_4$]$_{am}$ exist between glacial and interglacial periods (Loulouergue et al., 2008).

Over diurnal timescales the temporal change in CH$_4$ flux from wetlands is likely to be caused by localised changes in: temperature (Macdonald et al., 1998), water-table position (Moore & Dalva, 1993b), light intensity levels (Kaki et al., 2001), plant transpiration rates
(Chanton et al., 1997), plant biomass and gas transport mechanisms (Van der Nat et al., 1998, Kaki et al., 2001), and the amount of root exudates released into the rhizosphere by plants (Panikov et al., 2007). Temperature variations influence CH$_4$ production rates by altering carbon mineralisation and substrate supply, and the rate of methanogenesis (van Hulzen et al., 1999, Hoj et al., 2008). The water-table position defines the aerobic layer and hence the oxidising capacity of wetlands. Lowering the water-table enlarges the aerobic zone which increases the CO$_2$ flux and decreases the CH$_4$ flux (Blodau & Moore, 2003). Plant mediated pathways are important over diurnal timescales as they introduce oxygen into the soil to support respiration (Armstrong et al., 1991) and allow CH$_4$ produced in anoxic zones to bypass methanotrophes in oxygenated surface layers (Bellisario et al., 1999). This same pathway can also stimulate below ground oxidation of CH$_4$ in areas localised to the roots (Watson et al., 1997). The stomata/transpiration pathway is sensitive to changes in environmental variables such as light, temperature and water vapour pressure (Yang et al., 2005), therefore a strong diurnal pattern of CH$_4$ released through wetland plant stomata is frequently measured (Morrissey et al., 1993, Knapp & Yavitt, 1995, Garnet et al., 2005). See Chapter 1.4.6 for more details on the importance of plants in affecting wetland CH$_4$ fluxes.

Understanding the relationships between controlling flux variables both spatially and temporally is important when trying to predict or model wetland CH$_4$ emission. In this Chapter, the results of a short experiment investigating the diurnal pattern of wetland CH$_4$ flux will be presented. The diurnal pattern of wetland CH$_4$ emissions has been shown to be inconsistent, with peak CH$_4$ flux having been measured both during the day (Wang & Han, 2005, Panikov et al., 2007) and also during the night (Yavitt et al., 1993, Moore et al., 1994, Mikkela et al., 1995). The diurnal study reported here was performed as part of a long-term experiment where the effects of a glacial maximum [CO$_2$]$_{atm}$ on wetland CH$_4$
emissions were measured (Chapter 3). The diurnal analysis was carried out on ombrotrophic and minerotrophic mesocosms that had received >300 days of exposure to CO₂ starvation conditions.

4.2 Methods

4.2.1 Experimental Design

Diurnal variations in CH₄ flux were measured from contrasting nutrient status wetland mesocosms which were maintained in CEUs. Fluxes were measured from ombrotrophic bog (n=16) and minerotrophic fen (n=16) mesocosms (110 x 400 mm) split between two CEUs. The CEUs created a modern day [CO₂]ₐtm (~380 ppmv) and LGM [CO₂]ₐtm (~180 ppmv) as part of a long-term experiment (Chapter 3). More details of the sample sites and CEU set-up can be found in Chapter 2. CH₄ flux was measured at 6 points over a period of 32 hours (dark-light-dark cycle) during the simulated month of May. This period was 440 days into the two year experiment and 340 days after the CO₂ treatment had begun. CH₄ flux was measured 4 times during two night periods and twice during a day simulation. During the night, the temperature was set to 6°C and relative humidity to 60%. During the day, the temperature was set to 15°C, humidity to 70% and light intensity to 250 µm m⁻² s⁻¹. A 2 hr dawn and dusk program was used to gradually change the settings from day into night and visa versa. During this transition no measurements were made. Temperatures applied to represent May were based on local 30 year averages (1970-2000) (Met Office., 2006) and the day length based on longitude and latitude values for the local area. The control CEU was programmed to start before the treatment CEU (2 hours), so that this lag
period could be used to measure CH₄ emission at the same time point in the diurnal cycle in both CEUs.

4.2.2 CH₄ Fluxes from Mesocosms

CH₄ was measured using CRDLS and static chambers in a closed loop configuration. For full details of the theory behind the analytical instrument, the design of the static chambers and the sampling protocol, see Chapter 2.5.

4.2.3 Experimental [CO₂]

During this short experiment, frequently opening the CEUs to measure CH₄ fluxes interrupted the CCDS used to maintain the appropriate concentration in the units, i.e. 380 ppmv (control) and 180 ppmv (treatment). This resulted in CO₂ concentrations in the control and treatment unit averaging 565 (±20 S.E.) and 345 (±25) ppmv during the night simulation, respectively. The night CO₂ concentrations uninterrupted would normally be ~450 and ~220 ppmv. During the day simulation, [CO₂] averaged 504 (±12) ppmv in the control and 205 (±3) ppmv in the treatment. CO₂ concentrations would be <420 and <200 ppmv in control and treatment respectively, in closed and uninterrupted CEUs with the same day time environmental settings used in this experiment.
4.2.4 Statistical Analysis

Independent t-tests were used to test for significant differences between day and night CH$_4$ flux in both bog and fen data. Data was common log or square root transformed to meet the assumptions of this test. Mann-Whitney U non-parametric analysis was used when transformed data failed to meet the parametric criteria. A general linear repeated measures model was used to determine significant differences between control and treatment groups over the diurnal cycle, and between bryophytes and vascular plants. To correct for violations of sphericity in bog and fen analysis, a more conservative Greenhouse-Geisser correction was used. The fen data needed a square root whereas the bog required a common log transformation to meet the requirements of normality and equal variances before analysis. Bog and fen were analysed by repeated measures both with and without vascular plant numbers as a covariate.

4.3 Results

4.3.1 Bog Diurnal CH$_4$ flux

The largest control CH$_4$ flux was measured during the night at 56.8 mg m$^{-2}$ day$^{-1}$, and the smallest flux measured during the day at 19.1 mg m$^{-2}$ day$^{-1}$ (figure 4.1a). Dividing all the control measurements between the two categories of day and night (figure 4.2a), shows that CH$_4$ emission was on average 46% lower during the day (19.1 mg m$^{-2}$ day$^{-1}$) compared to at night (35.1 mg m$^{-2}$ day$^{-1}$) (P > 0.05). The treatment mesocosms showed a less distinct
Figure 4.1 CH₄ flux measured at 6 time points over 32 hours from the (A) bog and (B) fen mesocosms. Error bars represent ±1 standard error of the mean.
diurnal pattern in CH$_4$ flux compared to the controls (figure 4.1a), with similar mean CH$_4$ flux values of 8.18 and 9.24 mg m$^{-2}$ day$^{-1}$ during the day and night respectively (P > 0.05) (figure 4.2a). The largest measured treatment flux was however still recorded during the night (13.1 mg m$^{-2}$ day$^{-1}$) (figure 4.1a). Over the total 32 hour period, the control CH$_4$ flux averaged 29.8 mg m$^{-2}$ day$^{-1}$, whereas the treatment averaged 8.89 mg m$^{-2}$ day$^{-1}$, a difference of 70% (P < 0.05). Further investigation into this result showed a significant difference between the control and treatment CH$_4$ flux during both the day and night (P < 0.05), where treatment emission was 57% lower during the day and 72% lower during the night when compared to the control (figure 4.2a). Vascular plant abundance in the bog mesocosms was low, therefore there was no need to perform any corrections for differences in plant density between groups, or include them as a covariate in statistical analysis.

Figure 4.2 Control (modern day [CO$_2$]) and treatment (LGM [CO$_2$]) CH$_4$ fluxes measured during the day and night from (A) bog and (B) fen mesocosms. Error bars represent ±1 standard error of the mean. Bars with different letters are significantly different.
4.3.2 Fen Diurnal CH₄ Flux

The fen mesocosms produced a diurnal CH₄ pattern which was similar to the bog control, mesocosms (figure 4.1a), however the difference between night and day flux (averaging 35%) was not statistically significant (figure 4.2b) (P > 0.05). The largest day control flux was measured at 32.2 mg m⁻² day⁻¹, compared to 55.1 mg m⁻² day⁻¹ measured at night. Treatment mesocosms showed a less distinct diurnal pattern compared to the control, with flux values remaining fairly constant over the experiment (figure 4.1b). The largest treatment flux value was measured at night at 25.6 mg m⁻² day⁻¹ (figure 4.1b), but there was no statistical difference (P > 0.05) when comparing the average values for night (21.7 mg m⁻² day⁻¹) and day (20.1 mg m⁻² day⁻¹) (figure 4.2b).

Over the total 32 hour period, control CH₄ flux averaged 34.8 mg m⁻² day⁻¹ whereas the treatment mesocosms averaged 21.1 mg m⁻² day⁻¹, a difference of 39% (P > 0.05). There was no difference between control (25.7 mg m⁻² day⁻¹) and treatment (20.1 mg m⁻² day⁻¹) daylight flux (P > 0.05), however during the night, control mesocosms on average emitted 39.3 mg m⁻² day⁻¹ compared to 21.7 mg m⁻² day⁻¹ from treatment mesocosms. This was a statistically significant difference of 45% (P < 0.05). Fen mesocosm had uneven distributions of vascular plants (*Juncas subnodulosus; Carex lepidocarpa; Carex hirta*) between treatments, however when this was included as a covariate, this did not change the outcome of the statistical analysis.
4.3.3 Bog Verses Fen Comparison

There was no statistically significant difference between control flux values ($P > 0.05$) despite control fen mesocosms emitting 26% more CH$_4$ during the day and 11% more at night compared to control bog mesocosms (figure 4.3a). The fen and bog treatment mesocosm fluxes were significantly different during both day and night ($P < 0.05$) (figure 4.3b). The treatment fen mesocosms emitted 60% more CH$_4$ than the bog treatment group during the day and 57% more during the night.

![Graph showing comparison between bog and fen CH$_4$ flux during the day and night.](image)

Figure 4.3 Comparison between bog and fen CH$_4$ flux during the day and night in the (A) control and (B) treatment experimental groups. Error bars represent ±1 standard error of the mean.

4.3.4 The Influence of Plants on Diurnal CH$_4$ Flux

During the experiment there were 5 bog mesocosms with a mixture of vascular plants and bryophytes growing in them. The other bog mesocosms were exclusively dominated by
bryophytes, such as Hypnaceous mosses (e.g. *Hypnum cupressiforme*) and *Sphagnum*. Only one bog mesocosm in both the control and treatment experimental groups had a large density of vascular plants (16 x *Juncus effuses*). The densely populated mesocosm with *Juncus effusus* showed a strong diurnal response to daylight and temperature changes during the experiment (figure 4.4), where all 4 night fluxes were lower than the 2 day measurements. The largest flux value (32.6 mg m\(^{-2}\) day\(^{-1}\)) was measured early in the morning after the 2 hour dawn simulation where temperature, light and humidity were gradually changed from night into day settings. The smallest value (23.0 mg m\(^{-2}\) day\(^{-1}\)) was measured in the second night period just before the start of another 2 hour transition period into full day time conditions. The average percentage difference between day and night was 21%.

![Graph showing diurnal CH\(_4\) flux from a bog mesocosm with *Juncus effuses* plants growing (n=16). This was the only bog mesocosm with *Juncus spp.* growing and was part of the treatment group. Dotted line represents CEU temperature.](image)
The fen mesocosms had more vascular plant species growing in them compared to the bog mesocosms. *Juncus subnodulosus* and *effuses* was the dominant vascular species and *Campylium stellatum, Sphagnum tenellum* and *recurvum* the dominant bryophyte species. The greater presence of vascular plants allowed for a comparison between the diurnal CH4 response of bryophyte dominated mesocosms compared to mesocosms containing a mixture of both vascular plants and bryophytes (figure 4.5). The CH4 emissions from mesocosms containing vascular plants did not exhibit the variation observed from bryophyte only mesocosms between day and night conditions. In the control mesocosms (figure 4.5a), the vascular plant CH4 flux exhibited limited variation over the duration of the experiment. The peak emission value was measured during the day at 40.5 mg m\(^{-2}\) day\(^{-1}\) and the smallest during the night at 34.9 mg m\(^{-2}\) day\(^{-1}\). Mesocosms containing only bryophytes showed a contrasting response to the diurnal cycle, with both the largest and smallest fluxes measured during night periods (67.1 and 9.1 mg m\(^{-2}\) day\(^{-1}\) respectively). The average 'bryophyte mesocosm' CH4 flux was 33.3 mg m\(^{-2}\) day\(^{-1}\), whereas the average 'vascular plant' CH4 flux was 20% lower at 26.5 mg m\(^{-2}\) day\(^{-1}\) (figure 4.6) (P > 0.05).

The fen treatment group (figure 4.5b) 'bryophyte only' mesocosms emitted 16% more CH4 during the night when compared to the day. Like the bryophyte mesocosms in the control CEU, the night period produced both the largest (38.6 mg m\(^{-2}\) day\(^{-1}\)) and smallest flux value (19.9 mg m\(^{-2}\) day\(^{-1}\)). The treatment vascular plant flux shares a similar pattern to the control vascular plant flux (figure 4.6). The flux does not exhibit large variation over the diurnal cycle, however the peak emission is measured during the day (11.8 mg m\(^{-2}\) day\(^{-1}\)) and the lowest emission measured during the night (14.6 mg m\(^{-2}\) day\(^{-1}\)). The average treatment CH4 emission over the experiment from the vascular plants was 56% lower than the bryophytes (figure 4.6). Despite the large difference between the two time periods, this
Figure 4.5 Comparison of diurnal CH$_4$ emission from fen mesocosms with a mixture of vascular plants (v.plants) and bryophytes, and mesocosms with only bryophytes growing (bryophytes). A = Fen control, B = Fen treatment. Error bars represent ± 1 standard error of the mean.
was not a statistically significant difference (P > 0.05). A comparison of CH$_4$ emissions between control and treatment mesocosms containing similar plant assemblages (figure 4.6), showed that mesocosms containing vascular plants in the LGM atmospheric [CO$_2$], emitted 51% less CH$_4$ than mesocosms containing vascular plants in the control (P < 0.05). There was no statistical difference between control and treatment bryophyte CH$_4$ fluxes.

4.5 Discussion

A strong diurnal CH$_4$ pattern existed at both ambient [CO$_2$]$_{atm}$ and LGM [CO$_2$]$_{atm}$ in the bog and fen mesocosms (figures 4.1 and 4.2). CH$_4$ fluxes were generally higher during the night and lower during the day. This finding is consistent with a number of diurnal wetland studies which show higher emission rates during the night in both temperate and boreal wetlands (Yavitt et al., 1993, Moore et al., 1994, Mikkela et al., 1995, Ding et al.,
2004). The finding of higher CH$_4$ emissions at night suggests that the rate of CH$_4$
production is not the major controlling variable behind the wetland diurnal CH$_4$ pattern in
this study. If CH$_4$ production alone was responsible for changes in the diurnal pattern,
warmer temperatures associated with the day would result in higher CH$_4$ emissions
(Dunfield $et$ $al.$, 1993, Macdonald $et$ $al.$, 1998) compared to cooler night periods.
Moreover, with plant mediated pathways increasing during the day because of light
induced stomatal opening (Yang $et$ $al.$, 2005, Shimazaki $et$ $al.$, 2007), a significant day
process would be required to counter-act increased CH$_4$ production and transportation.

One possible mechanism suggested by Ding $et$ $al.$, (2004) to explain larger CH$_4$ fluxes at
night, is enhanced oxidation of CH$_4$ and reduced CH$_4$ production caused by an increased
oxygen (O$_2$) content in the soil during the day. The increase in oxygen content in the soil
would originate from plant photosynthesis or via stomatal openings. Oremland and Taylor
(1977) reported that the oxygen concentration in plants could be higher than in the
atmosphere during the day, therefore day time plant photosynthesis could release oxygen
into the rhizosphere. The opening of stomata during the day would also increase oxygen
supply into the rhizosphere and contribute to CH$_4$ oxidation in the roots of wetland plants
(Calhoun & King, 1997). The oxygen content in wetland rhizospheres has been shown to
increase after sunrise in other studies (Thomas $et$ $al.$, 1996, Ding $et$ $al.$, 2004, Albanito $et$
$al.$, 2009), which implies that the diurnal CH$_4$ pattern could be driven more by irradiance
and the effect this has on rhizosphere oxidation, rather than temperature (King, 1990, Kaki
$et$ $al.$, 2001).

Not all wetland diurnal studies report the largest CH$_4$ fluxes at night (Wang & Han, 2005,
Panikov $et$ $al.$, 2007). An alternate theory was put forward by Panikov $et$ $al.$, (2007) to
explain diurnal wetland CH₄ emissions that peak during the day and decrease during the night. Panikov et al., (2007) hypothesised that the daylight induced photosynthetic CO₂ uptake causes the transport of photosynthate to roots. This leads to enhanced root respiration and root exudation, which results in microbial oxidation of exudated compounds to CO₂. The rhizosphere subsequently becomes O₂ depleted, which causes the expansion of an anaerobic zone around plant roots where exudates and CO₂ are converted to CH₄. Panikov et al., (2007) suggest that the combination of O₂ diffusion and the termination of photosynthesis restricting root exudation causes the night time decrease in CH₄ flux.

A change in the rate of release of root exudates between night and day could explain the contrasting diurnal variation in CH₄ flux pattern of vascular plants and bryophytes that was observed in this study. Mesocosms containing vascular plants all produced the largest CH₄ flux during the day (figures 4.4 and 4.5). Light induced increases in plant root exudates to the rhizosphere could increase the supply of methanogen substrates and cause an increase in CH₄ production. This increase in CH₄ production, combined with the opening of the gaseous exchange pathway (stomata) through the plants, could explain the higher CH₄ emission during the day in vascular dominated mesocosms. This hypothesis is consistent with previous findings, for example, Morrissey et al., (1993) found that dark enclosures reduced CH₄ emission by an average of 25% from Carex dominated wetlands, this coinciding with a decrease in stomatal conductance in response to the dark. Waddington et al., (1996) reported a similar finding with Carex rostrata, where a build-up of CH₄ in the stem was measured during darkness. Bryophyte mesocosms exhibited a contrasting diurnal CH₄ pattern to vascular plant mesocosms in this study. The largest CH₄ flux from bryophyte dominated mesocosms was measured during the night. Bryophytes deliver photosynthate to the rhizosphere more slowly than vascular plants (Thomas et al., 1988),
therefore a time delay could exist from peak photosynthetic CO₂ uptake to enhanced root respiration and root exudation. Although the duration of this delay is unknown in this bog, this could explain why in this experiment, bryophyte mesocosms had larger CH₄ emissions during the night compared to the day.

The LGM [CO₂]ₘₐₜ treatment in this experiment significantly reduced the CH₄ flux from the bog mesocosms (figure 4.1 and 4.2). The cause of this suppression is likely to have been a decrease in plant primary productivity which would have limited the export of plant root exudates into the rhizosphere (Whiting & Chanton, 1993). Decreasing the [CO₂]ₘₐₜ would limit photosynthesis (Tissue et al., 1995) and reduce the carbon allocation to the rhizosphere (Dippery et al., 1995). This would place a limit on the supply of carbon for methanogenesis and reduce CH₄ production. There was only a significant difference between the fen control and treatment CH₄ flux during the night in this experiment (figure 4.1 and 4.2). This could be explained by the greater presence of vascular plants in the fen mesocosms compared to the bog. Vascular plants deliver photosynthate to roots in a matter of hours (King & Reeburgh, 2002, King et al., 2002, Strom et al., 2003, Dilkes et al., 2004), therefore any changes in plant physiology which affects plant derived root exudates, will quickly impact on wetland CH₄ emissions. It is possible that a higher than normal sampling resolution increased the [CO₂]ₘₐₜ above that normally experienced in the experiment and stimulated the production of root exudates and elevated the CH₄ emissions from fen treatment mesocosms containing vascular plants. The bryophyte dominated mesocosms are unlikely to have responded to any short-term elevation in [CO₂]ₘₐₜ in terms of root exudate production (Thomas et al., 1988), therefore CH₄ emissions from these mesocosms could still reflect the plant processes under CO₂ starvation. However, there was only a statistically significant difference between the fen control and treatment mesocosms containing a mixture of bryophytes and vascular plants (figure 4.6). This
implies that reduced CH₄ emissions from CO₂ starved wetlands is mainly driven by a reduction in root exudates from vascular plants.

4.6 Conclusion

The diurnal CH₄ flux measured in this experiment showed a pattern of higher CH₄ emissions during the night compared to the day. This could have been caused by increased oxidation in the rhizosphere driven by plant photosynthesis during the day (Ding et al., 2004). Equally plausible is a time delay from peak photosynthetic CO₂ uptake, to root exudation in the rhizosphere from bryophyte plants (Thomas et al., 1988). The LGM CO₂ treatment reduced the bog diurnal CH₄ emission by 70% (P < 0.05) and the fen diurnal CH₄ emission by 39% (P > 0.05). The results from this experiment suggest that a decrease in vascular plant primary productivity and the subsequent limitation this would place on plant root exudates, is the main reason for reduced CH₄ emission from wetlands subjected to LGM atmospheric [CO₂].
Influence of Simulated Glacial Carbon Dioxide Concentrations on the Temperature Response of Wetland CH₄ Emissions

5.1 Introduction

CH₄ is a powerful long-lived GHG that is second only to water vapour and CO₂ in terms of radiative forcing (Ramaswamy et al., 2001, Shindell et al., 2009). Understanding the natural ~50% decline in [CH₄]ₓₒₓ during glacial maxima (Loulergue et al., 2008) requires accurate characterisation of CH₄ sources and sinks in the past. Earth system modelling provides a useful approach to explaining this natural variation however, due to the complexities of recreating and experimenting under simulated glacial environmental conditions, process-based and empirically derived models which are currently used to estimate glacial biogenic CH₄ emissions (e.g. Cao et al., 1996, Christensen et al., 1996), rely on terrestrial ecosystem relationships established under modern day environmental conditions. Predictions of the CH₄ cycle during Pleistocene glacial maxima would therefore be more representative, if they were to include relationships established from wetland experiments that were established in glacial environmental conditions.
Pleistocene and Holocene variations in atmospheric CO2 and CH4 concentration are strongly associated with past temperature [Petit et al., 1999], however, the effect of glacial [CO2]_{\text{air}} on the temperature response of wetland CH4 emissions is currently unknown. Temperature variations influence CH4 production rates by altering carbon mineralisation, substrate supply and the rate of methanogenesis [Hoj et al., 2008; van Hulzen et al., 1999]. Long term temperature variations may also cause an increase in methanogen population numbers and diversity in anaerobic environments [Hoj et al., 2008]. In situ wetland CH4 emissions are highest during the summer [Dise, 1993] and in controlled laboratory studies they exhibit an exponential increase in CH4 flux when exposed to linear increases in temperature (0 to 30°C) [Daulat and Clymo, 1998; Thomas et al., 1996]. The influence of temperature on wetland CH4 flux is often incorporated into process-based models [e.g. Cao et al., 1996] in terms of the Q10 factor, which describes the change in reaction rate with an increase of 10°C in temperature. Temperature coefficients (Q10) show a large range of 1 to 35 for methanogenesis in wetland soils [Segers, 1998; Whalen, 2005]. The wide range in values most likely reflects the temperature sensitivity of microbial processes that precede methanogenesis, as these processes limit the temperature response of methanogens [Bergman et al., 1998]. Although the Q10 range for CH4 emissions is well characterised, there are no data currently available for wetlands experiencing glacial maxima CO2 starvation. Current models [e.g. Valdes et al., 2005] use Q10 values that are appropriate for the modern day when predicting glacial maxima wetland behaviour which are unlikely to fully represent the effect of global CO2 starvation on wetland CH4 emissions.

CH4 emitted from wetlands is mainly derived from recently fixed carbon (Chanton et al., 1995, Bellisario et al., 1999), therefore substrate availability and supply should be considered a major factor in influencing CH4 emission rate (Segers, 1998). An increase in methanogen substrate (organic acids) supply, either from increased fermentation or
increased plant root exudates (Whiting & Chanton, 1993, Strom et al., 2003) due to temperature increases, could also contribute to the observed relationship between wetland CH$_4$ emissions and temperature. For example, Christensen et al., (2003a) found that the combined influence of temperature and microbial substrate availability accounted for close to 100% of the seasonal variation in CH$_4$ emissions from high latitude wetland sites.

Given that CO$_2$ starvation has been shown to reduce photosynthesis and productivity [Sage and Kubien, 2007; Tissue et al., 1995] and also affect growth and biomass allocation in plants [Dippery et al., 1995] all of which may affect substrate supply to methanogens and vascular transport emission pathways in C$_3$ species, this Chapter tests the hypothesis that where nutrient limitation is absent, glacial [CO$_2$]$_{atm}$ would reduce the temperature response of CH$_4$ emissions from wetlands. This experiment was performed in controlled environment units (CEUs) using minerotrophic and ombrotrophic wetland mesocosms that had been exposed to glacial maxima [CO$_2$]$_{atm}$ (180-200 ppm) for over 1 year (Chapter 3).

5.2 Methods

5.2.1 Site Description and Field Sampling

Wetland mesocosms were collected in autumn 2006 from peatlands of contrasting nutrient status in the UK, for a long-term glacial maximum CO$_2$ starvation experiment. The temperature response study was performed during the second year (400+ days into the study) of this experiment. A total of thirty two 110 x 400 mm peat mesocosms, complete with intact surface vegetation were collected from a minerotrophic fen in Anglesey, Wales.
(Cors Goch-SH 504 817) and an ombrotrophic bog in Snowdonia, Wales (Migneint-SH 816 440). Both wetland sites have been previously used to provide mesocosms for wetland biogeochemistry experiments (Hutchin et al., 1995, Kang et al., 2001, Freeman et al., 2004a). Cors Goch is a base rich alkaline fen that overlies carboniferous limestone. Mesocosm samples were taken from areas containing Sphagnum papillosum, S. plumulosum, Juncus subnodulosus and Carex spp. Migneint is a base poor ombrotrophic blanket bog that only receives nutrients from rainwater. Cores were collected from representative sites containing the species Juncas effusus, Sphagnum papillosum and S. magellanicum. Mesocosms were created by inserting sections of PVC pipe (110 mm x 400 mm) into representative locations at both bog and fen sites. Each mesocosm was excavated and the base sealed in the field with a PVC end cap to maintain the anaerobic condition of the core. Samples were promptly transported to the laboratory where they were placed into CEUs.

5.2.2 Experimental Design

16 bog and 16 fen mesocosms were split between two Snijders Microclima MC1750E CEUs. One set of mesocosms (8 bog and 8 fen) were treated to simulated glacial maximum [CO₂]ₚₑₖ with a target concentration of ~180 ppmv. Control mesocosms were maintained at modern day [CO₂]ₚₑₖ. CO₂ concentrations in both were maintained using a purge gas generator (CMC Ltd). During this experiment, average CO₂ concentrations were 395 ± 21 (S.D.) ppmv and 199 ± 28 ppmv in the control and treatment CEUs respectively. Temperature and lighting levels throughout the experiment reflected seasonal changes recorded at meteorological stations local to the site collection points.
Mesocosms within the control and treatment CEUs received 12 hrs of light at 250 µm m\(^{-2}\) s\(^{-1}\) and 12 hrs of complete darkness on each day of the temperature response study. Relative humidity was set to a constant 70% and the water-table fixed to within 2-3 cm of the surface with distilled water. Mesocosms in both CEUs were maintained at 5°C for 24 hours to equilibrate to the change in temperature after which CH\(_4\) fluxes were measured. Temperatures were then elevated to 10 then 15, 20 and 25°C for 24 hours at each temperature with fluxes measured at each temperature as for 5°C. A further monitoring period consisted of 24 hours at each of these temperatures but in reverse order to account for any possible lag effects from the temperature treatments. CH\(_4\) emissions were measured using CRDLS to provide real time CH\(_4\) measurements. CH\(_4\) emissions were measured using static closed chambers that were constructed from clear perspex pipe (11 cm x 50 cm). A mixing fan secured to the inside of the chambers ensured an evenly mixed chamber atmosphere. Pressure changes were prevented by allowing a small needle hole (0.8 mm) through a resealing membrane (see Chapter 2.5 for full details on chamber design). Sampling time accounted for <1 hour out of the 48 hours at each temperature point. The temperature coefficient (Q\(_{10}\)) for a given 10°C temperature range was calculated using the linear increase between two temperature points in the experiment, as shown in equation 5.1:

\[
Q_{10} = \left( \frac{R_2}{R_1} \right)^{\frac{10(T_2 - T_1)}{T_2 - T_1}} \\
\text{(Equation 5.1)}
\]

5.3 Results

CH\(_4\) emissions increased exponentially between 5 and 25°C in both the bog and fen ecosystems, (figure 5.1 and table 5.1) a result which is consistent with other controlled
environment studies (Daulat & Clymo, 1998, Macdonald et al., 1998, Gauci et al., 2004) and field observations (Christensen et al., 2003a). Bog and fen CH$_4$ emissions exhibited a contrasting response to the glacial maximum CO$_2$ treatment when compared to their equivalent controls (figure 5.1a). Bog control and treatment emissions did not differ significantly in their response over the entire temperature range. Comparing treatment and control regression curves plotted through the bog data shows that the glacial maximum [CO$_2$]$_{atm}$ treatment made little difference to the temperature response of CH$_4$ emissions (figure 5.1). In contrast, fen mesocosms demonstrated a pronounced treatment effect on temperature CH$_4$ flux response (figure 5.1b). Comparing regression equations fitted to both fen groups shows a clear separation in CH$_4$ response above 10°C. This result suggests that below 10°C a reduction in [CO$_2$]$_{atm}$ to glacial maximum levels, has a limited effect on CH$_4$ emissions in fens, whereas above 10°C, CO$_2$ starvation affects temperature response.
Temperature response experiments often express results in terms of Q_{10} values, which is the rate of biological change as a consequence of increasing the temperature by 10°C. Q_{10} values were calculated for 3 different temperature ranges (5-15, 10-20 and 15-25°C), with Mann-Whitney U tests used to determine any statistical significant differences between the control and treatments (figure 5.2). The average Q_{10} value for the control and treatment in the bog mesocosms was 3.9 and 3.6 respectively, but the differences were not statistically significant (P > 0.05). There was also no difference between bog control and treatment (P > 0.05) Q_{10} values in any of the three temperature ranges. The fen produced average Q_{10} values of 3.3 and 1.8 in the control and treatment respectively, the difference being statistically significant (P < 0.05). Considering the different temperature ranges, fen mesocosms showed ~50% decrease in Q_{10} over 5-15°C and 10-20°C (P < 0.05), although there was no statistical difference between control and treatment Q_{10} values at 15-25°C (P > 0.05). The Q_{10} values (change in CH_{4} flux over a given 10°C range) reported in this study fit in the range of 1.5-35 previously summarised by Segers (1998) and Whalen (2005). This wide range in reported values could be due to the temporal and spatial differences in substrate availability and quality within wetland soils (Davidson & Janssens, 2006). The results from this experiment are towards the lower end of the reported Q_{10} range, which is in agreement with the majority of observations.

Table 5.1 Summary table showing CH_{4} flux vs. temperature regression equations, average CH_{4} emissions and average Q_{10} values in the experiment (S.E. = standard error).

<table>
<thead>
<tr>
<th>Wetland Type</th>
<th>Experimental Group</th>
<th>Regression Equation</th>
<th>R^2</th>
<th>Average CH_{4} emission (mg m^{-2} day^{-1} ±1 S.E.)</th>
<th>Average Q_{10} (±1 S.E.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bog</td>
<td>Control</td>
<td>y = 17.735e^{0.0653x}</td>
<td>0.98</td>
<td>29.6 ± 3.00</td>
<td>3.98 ± 0.92</td>
</tr>
<tr>
<td></td>
<td>Treatment</td>
<td>y = 20.532e^{0.0418x}</td>
<td>0.91</td>
<td>25.8 ± 4.76</td>
<td>3.63 ± 0.52</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>y = 9.3966e^{0.069x}</td>
<td>0.97</td>
<td>52.8 ± 5.54</td>
<td>3.31 ± 0.58</td>
</tr>
<tr>
<td></td>
<td>Treatment</td>
<td>y = 6.8752e^{0.0766x}</td>
<td>0.93</td>
<td>40.4 ± 4.52</td>
<td>1.77 ± 0.25</td>
</tr>
</tbody>
</table>
**Figure 5.2** A comparison of modern day (control) and glacial maximum (treatment) Q10 values over 3 different temperature ranges in (A) an ombrotrophic bog and (B) a minerotrophic fen. Error bars represent ±1 standard error of the mean. The * symbol is used to represent a significant (P < 0.05) difference between control and treatment values within the temperature range.

### 5.4 Discussion

The contrasting \(\text{CH}_4\) temperature response to LGM \([\text{CO}_2]_{\text{atm}}\) in the bog and fen, is likely to be caused by the two ecosystems responding differently to the same environmental variables (Weltzin *et al.*, 2000). One potential explanation for this contrasting result could be the different nutrient statuses of the bog and fen. In modern day atmospheres, ombrotrophic bogs have lower \(\text{CH}_4\) emissions compared to minerotrophic fens (Keller *et al.*, 2006, Hornibrook & Bowes, 2007), therefore it is possible that the inherent nutrient deficiency present in ombrogenous ecosystems, is exerting a stronger control over \(\text{CH}_4\) emissions when compared to the effects of \(\text{CO}_2\) starvation. This could imply that \(\text{CH}_4\) emissions from bogs may be nutrient, rather than \(\text{CO}_2\) limited, even under atmospheres containing ~180 ppmv \([\text{CO}_2]\). Minerotrophic fens however, do not have the same nutrient constraints as ombrotrophic bogs and so become limited by \(\text{CO}_2\) availability.
An alternative explanation for the contrasting results could be due to differences in species composition in the bog and the fen. The bog mesocosms were dominated by bryophyte species which are known to supplement photosynthesis with sub-surface CO$_2$ (Turetsky & Wieder, 1999). It is therefore possible that a reduction in the [CO$_2$]$_{\text{air}}$ would not reduce the photosynthetic behaviour of bryophyte plants in wetlands and therefore not alter their carbon allocation to the rhizosphere. As the bog mesocosms were dominated by bryophytes species, it is hypothesised that sub-surface CO$_2$ counteracted the LGM treatment, resulting in no change in the CH$_4$ temperature response in the bog (figure 5.1). In contrast, the fen mesocosm had a higher number of vascular plants growing in them which are known to rapidly change their photosynthetic behaviour in sub-ambient CO$_2$ conditions (due to the limitations of C$_3$ photosynthesis (Tissue et al., 1995)) and are likely (when temperature limitations are not applied) to reduce their output of root exudates in a matter of hours (King & Reeburgh, 2002, King et al., 2002), which would have an immediate impact on CH$_4$ emissions.

Differences in dominant methane production pathways between minerotrophic fens and ombrotrophic bogs, may also explain the contrasting CH$_4$ flux response to temperature among different wetland types under glacial maxima [CO$_2$]. CH$_4$ is produced in anaerobic environments by methanogenic archaea in two distinctive ways. Acetotrophic methanogens reduce acetate to CH$_4$ and CO$_2$, whereas hydrogenotrophic methanogens reduce CO$_2$ in the presence of H$_2$ and produce CH$_4$ and water as by-products (Chapelle, 2001). Fens and bogs exhibit contrasting dominant CH$_4$ production pathways with bog methanogens being predominant CO$_2$/H$_2$ utilisers and the more nutrient rich fens inhabited by a greater presence of obligate acetotrophs (Galand et al., 2005, Juottonen et al., 2005). Differences in abiotic and biotic factors between fens and bogs such as pH and plant community composition could further explain contrasting dominant CH$_4$ production
pathways. For example, ombrotrophic bogs exhibit lower degradation rates than fens (Aerts et al., 1999) which are likely to be caused by lower pH, nutrient and microbial decomposition (Belyea, 1996). CH₄ emissions from fens are therefore more susceptible to changes in plant root exudates due to the dominance of acetotrophic methanogenesis (particularly in the surface layers), and therefore more likely to show a response to changing CO₂ levels in the atmosphere.

Different CH₄ production pathways could therefore contribute to the reason why there was a contrasting effect of reduced atmospheric [CO₂] on CH₄ flux temperature response in the bog and fen. Bog CH₄ emissions are unlikely to respond to short-term CO₂ starvation induced reductions in plant productivity and root exudates, because the main CH₄ pathway is hydrogentrophic methanogenesis from old recalcitrant peat and not recent labile carbon. Fen CH₄ emissions are however more dependent on freshly provided labile carbon (Strom et al., 2003). This potentially makes acetotrophic methanogenesis in fens more susceptible to changes in plant derived, and hence, atmospheric [CO₂] controlled substrate supply. It cannot, however rule out the possibility that longer-term changes in bog peat quality would result from low [CO₂] atmospheres which may, at a later stage, affect methanogenesis and the response of CH₄ emissions to temperature.

In the fen there was a difference in the treatment effect observed between <10°C and >10°C. This could have been caused by cool temperatures constraining biological activity in methanogen communities (Hoj et al., 2008) under both CO₂ starvation and control conditions, this causing a reduction in the overall rate of decomposition (Davidson & Janssens, 2006). In contrast, increasing the temperature beyond 10°C removes this constraint, at which point the CO₂ starved fen mesocosms become substrate rather than
temperature limited. It is therefore possible that during the summer, CH$_4$ emissions from the CO$_2$ starved fen switched from being temperature to substrate limited. In addition to a potential nutrient status control on CH$_4$ flux under CO$_2$ starvation, therefore, the response of wetland CH$_4$ emissions to glacial [CO$_2$]$_{atm}$ could also be moderated by changes in latitudinal temperature gradients. The results in this Chapter suggest that the largest suppression in CH$_4$ flux at the LGM compared to modern day may have been in the warmest parts of the world, i.e. in the low latitudes, with a diminishing effect at higher latitudes.

The suppression of CH$_4$ temperature response in the fen was examined using the Cao et al. (1996) equation for CH$_4$ production in wetlands (equation 5.2). The equation predicts CH$_4$ production based on the fraction of the dissolved carbon pool (SOMD), temperature (f(TEM)), water table position (f(WTP)) and a fixed factor of 0.47 (proportion of the decomposed organic carbon transformed to CH$_4$ under optimal conditions of temperature and soil water status for methanogens). The additional equations used to determine f(TEM) can be found in Cao et al., (1996).

\[
\text{CH}_4 \text{ production} = 0.47 \times \text{SOMD} \times f(\text{WTP}) \times f(\text{TEM}) \quad \text{(Equation 5.2)}
\]

Equation 5.1 was able to reproduce the CH$_4$ temperature response exhibited by the fen control group using a $Q_{10}$ value of 2 and SOMD value of 670 mg C m$^{-2}$ d$^{-1}$. The fen treatment pattern could only be achieved by reducing the $Q_{10}$ value to 1.5 and reducing the dissolved carbon pool by 50%. Water-table (F(WTP)) fluctuations were not factored into either analysis as this was maintained at the surface of the mesocosms during the experiment. This exercise shows that current predictions of wetland CH$_4$ flux at the LGM
that use this equation (e.g. Valdes et al., 2005), are unlikely to be accurately predicting fen
CH\textsubscript{4} flux, which could lead to an overestimation of the LGM global wetland CH\textsubscript{4} flux.

5.5 Conclusion

In summary, fen ecosystems experiencing glacial maxima [CO\textsubscript{2}]\textsubscript{atm} may have had CH\textsubscript{4} Q\textsubscript{10}
values that are only half those of modern day values (figure 5.2b) which suggests a smaller
global CH\textsubscript{4} source than was previously thought during the LGM. Current models could be
overestimating wetland CH\textsubscript{4} emissions at the LGM and earlier glacial maximum periods in
Earth's history. The experiment detailed in this chapter showed that low atmospheric CO\textsubscript{2}
concentrations during the LGM could have limited wetland CH\textsubscript{4} emission responses to
temperature, possibly by limiting plant root exudates and substrate supply to methanogens.
Furthermore, our results indicate that the largest LGM [CO\textsubscript{2}]\textsubscript{atm} induced suppressions in
CH\textsubscript{4} flux occurred when temperature limitation on carbon mineralisation was at its lowest
(i.e. during the warm summer months at temperatures >10°C). These findings need to be
incorporated into further glacial maximum simulations of the wetland CH\textsubscript{4} source in order
to accurately asses the influence of wetlands on glacial to interglacial variations in
atmospheric [CH\textsubscript{4}].
CHAPTER SIX

Wetland Rhizosphere Responses to Glacial Carbon Dioxide Starvation

6.1 Introduction

With unprecedented (800 ka) increases in $[\text{CO}_2]_{\text{am}}$ since the industrial revolution, researchers have been eager to understand the effect this may have on natural ecosystems (e.g. Dacey et al., 1994, Ainsworth & Long, 2005). Wetlands have received a significant amount of this attention in the last 20-30 years because, due to slow decomposition rates, they have accumulated a large pool of organic carbon and currently hold 390-455 Pg (1 Pg $= 10^{15}$ g) of terrestrial carbon, or approximately one-third of the global carbon stock (Gorham, 1991, Jenkinson et al., 1991). Wetland biogeochemistry has been shown to be particularly sensitive to future elevated atmospheric CO$_2$ levels, with numerous studies having reported increased CH$_4$ emissions, DOC flux and changes in species composition as a result of experimentally increased $[\text{CO}_2]_{\text{am}}$ (Hutchin et al., 1995, Freeman et al., 2004a, Fenner et al., 2007). There are however, no investigations into the effects of the exceptionally low CO$_2$ concentrations of the LGM on wetland ecosystem carbon cycling processes and CH$_4$ emissions beyond those presented in this thesis. Chapters 3-5 have demonstrated the impact that a sub-ambient [CO$_2$] of ~180 ppmv present at the LGM can
have on reducing wetland CH$_4$ flux. This chapter will address the impact of LGM CO$_2$ starvation has on belowground processes which are of fundamental importance to carbon cycling and the CH$_4$ emissions reported and discussed in those chapters. This chapter presents the effects of a LGM [CO$_2$] on DM, dissolved carbon and acetate concentrations in wetland pore waters.

Wetlands are largely anaerobic environments in which gaseous products (CH$_4$, CO$_2$) are released during the degradation of readily available organic matter (Wuebbles & Hayhoe, 2002, Whalen, 2005, Lai, 2009). In wetland soils two metabolic pathways of methane production exist: acetoclastic, in which acetate is an immediate precursor of CH$_4$, and autotrophic (hydrogen-dependent), in which CH$_4$ is produced from H$_2$ and CO$_2$ (Chapelle, 2001). In acidic wetlands the hydrogen pathway of methanogenesis is the more important of the two (Chasar et al., 2000a, Duddleston et al., 2002, Strom et al., 2005), however the strength of the pathway is strongly determined by vegetation classes (Hines et al., 2008). The influence of changing CO$_2$ levels on methanogenic pathways has not been thoroughly explored, therefore by measuring acetate over the course of the experiment, this will yield insights into the suppression of CH$_4$ emissions.

The CH$_4$ formed in wetland soils is transported to the atmosphere via diffusion, ebullition and plant mediated transport (Chanton, 2005, Tokida et al., 2005), yet not all CH$_4$ is immediately released to the atmosphere and can dissolve into solution in the rhizosphere. DM varies both temporally and spatially in wetlands (Dise, 1993, Benstead & Lloyd, 1996) and responds to changing [CO$_2$]$_{am}$ (Marsh et al., 2005, Keller et al., 2009), therefore it is likely to be a good indicator of the effects of CO$_2$ starvation on CH$_4$ production in the rhizosphere. Carbon lost in the dissolved form is estimated to account for ~10% of total
carbon released from peatland catchments (Worrall et al., 2003) and has also been shown
to increase in elevated CO₂ studies (Fenner et al., 2007). DOC is generated by the
decomposition of dead plant material and release of exudates from the roots of plants,
which is an important supply of methanogenic substrates (Chanton et al., 1995, Hutsch et
al., 2002).

6.2. Methods

6.2.1 Experimental Design

16 bog and 16 fen mesocosms (110 x 400 mm) were split between two Snijders
Microclima MC1750E Controlled Environment Units over 2 years. One unit was used to
recreate the LGM [CO₂] (treatment) and the other to maintain a modern day [CO₂]
(control). Over 2 years the control [CO₂] averaged 406 ± 23 (S.E.) and the treatment
averaged 196 ± 28 ppmv. Full details of the experimental approach are included in
Chapter 2 and in the methods section of Chapter 3 (3.2.1).

6.2.2 Dissolved CH₄

Pore water samplers were constructed from 1 ml Plastipak syringes (Chapter 2.5.7) and
permanently fixed 10 cm below the surface vegetation in each mesocosm at the beginning
of the experiment. From these samplers, 1 ml of unfiltered pore water was collected bi-
monthly from the bog and fen mesocosms during the second year of the experiment. Pore
water [DM] was determined on a Cambridge Ai Gas Chromatography (GC) 94 equipped
with a flame ionising detector and Tekmar 7000 auto sampler. A headspace technique was used to measure DM, using 1 ml of pore water in N₂ purged 20 ml vials. The original dissolved concentration was reconstructed using the headspace concentrations, the volume of headspace and water phase, and Henry’s Law. Henry’s Law states that the equilibrium value of the mole fraction of gas dissolved in a liquid is directly proportional to the partial pressure of the gas above the liquid surface. Using this method, the total gas concentration (TC) in the original water sample is calculated by determining the gas concentration of the headspace by GC analysis. This is subsequently converted to the partial pressure of the gas, which can then be used to calculate the aqueous gas concentration that is partitioned into the gas phase (C_G) and the remaining concentration in the aqueous phase (C_W). The total gas concentration in the aqueous phase is then:

\[ TC = C_G + C_W \]  
(Equation 6.1)

The method for calculating the [DM] involves several steps to determine C_G and C_W. The parameters that inform those steps include the molecular weight of CH₄, the headspace volume, the sample vial volume, temperature of the sample (assumed to be 25°C) and atmospheric pressure (assumed to be 1 atm). Calculating DM using this approach is common in belowground wetland/peatland experiments (e.g. Blodau et al., 2007).

6.2.3 Dissolved Carbon

Pore water samples for dissolved carbon analysis were collected bi-monthly on average and analysed for DIC and DOC content using a Shimadzu Total Organic Carbon (TOC)
VCSN analyser, combined with a Shimadzu ASI-V auto sampler. Samples were collected using the same custom built samplers used for DM analysis (Chapter 2.5.7). A total of 20 ml (10 ml of 0.45 µm filtered pore water + 10 ml deionised water) was required to determine DIC and DOC from the same sample. When samples were collected from mesocosms they were placed in a -20°C freezer within 5 minutes to preserve the concentrations of dissolved carbon before analysis (typically within 1 week). A full detailed description of the method used to determine DIC and DOC, and the principles behind the instrument can be found in Chapter 2.6.

6.2.4 Carboxylic Acids (Volatile Fatty Acids)

A Supelco Visiprep™ 24 solid phase extraction manifold and Biotage Isolute Env+ 200 mg/3 ml SPE tubes were used to extract the carboxylic acids of acetic, ethanoic, propanoic, butanoic, pentanoic, hexanoic, heptanoic and octanoic acid (C2-C8) from pore water samples. Glassware was cleaned using Decon-Glass, distilled water and methanol (CH₃OH), this included: beakers, pipettes and auto-sampler vials. Extractions were performed away from standards. Isolute Env+ columns were conditioned using 4 ml of 0.01M HCl. Pre-extraction, samples were acidified to ~pH 2 with HCl and 1.862 µg of 2-methylpentanoic acid was added as an internal standard. 2 ml of sample was placed through the tubes, followed by 4 ml of 0.01 HCl and 2 ml of methanol to elute the compounds of interest. Following each addition an air flush was performed. After the extraction, anhydrous sodium sulfate was added to the vials to remove any residual water. A procedural blank of deionised water was processed with every batch of samples. Response factors for the compounds of interest were calculated from six point calibration curves.
Gas chromatography-mass spectroscopy (GC-MS) analysis was carried out using an Agilent Technologies 6890 gas chromatograph coupled to a 5973 mass spectrometer. Separation was performed on a Phenomenex FFAP column (30 m length, 0.25 mm internal diameter and 0.25 mm film thickness) with a He carrier gas at a constant column flow rate of 1.1 ml min$^{-1}$. The GC oven temperature was held for 1 minute at 50°C and then ramped to 200°C at a rate of 10°C a minute and then held for 2 minutes. The injection was at 190°C with a 10:1 split and 1 μl injected. The MS was run in full scan and for quantitation in selective ion monitoring (m/z 43,45,60,73,74,87) with a dwell time of 50 ms for each ion.

6.2.5 Statistics

Data sets were analysed for treatment effects using repeated measures analysis of variance (ANOVA) where the assumptions of normality, equal variance and sphericity were satisfied. Non-parametric Kruskal-Wallis and Friedman's tests were used when transforming the data failed to comply with the necessary assumptions. Independent t-test and Mann-Whitney U tests were used to analyse for between treatment and ecosystem differences when data was segregated into calendar seasons. The statistical package used was SPSS Statistics, version 18.

6.3 Results

6.3.1 Summary
The results of all ground water variables measured during the experiment are summarised in table 6.1. Raw data is provided on a compact disc at the rear of the thesis.

Table 6.1 Average (n=8) DOC (mg l⁻¹), DIC (mg l⁻¹), pH (median), conductivity (µS cm⁻¹), acetate (µg l⁻¹) and dissolved CH₄ (mg l⁻¹) values measured in year 1 and 2. Error is ± 1 standard error of the mean in all cases except for pH, where 95% confidence limits are used.

<table>
<thead>
<tr>
<th>Period</th>
<th>Variable</th>
<th>Bog Control</th>
<th>Bog Treatment</th>
<th>Fen Control</th>
<th>Fen Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Year 1</td>
<td>DOC</td>
<td>67.7 ± 5.6</td>
<td>62.1 ± 3.9</td>
<td>82.1 ± 6.6</td>
<td>101.6 ± 9.3</td>
</tr>
<tr>
<td></td>
<td>DIC</td>
<td>34.1 ± 3.2</td>
<td>19.9 ± 3.2</td>
<td>23.7 ± 2.1</td>
<td>14.4 ± 1.5</td>
</tr>
<tr>
<td></td>
<td>pH</td>
<td>5.96 ± 0.16</td>
<td>5.11 ± 0.26</td>
<td>6.24 ± 0.28</td>
<td>5.77 ± 0.22</td>
</tr>
<tr>
<td></td>
<td>Conductivity</td>
<td>442 ± 23</td>
<td>305 ± 28</td>
<td>429 ± 21</td>
<td>407 ± 24</td>
</tr>
<tr>
<td></td>
<td>Acetate</td>
<td>8.9 ± 2.89</td>
<td>6.3 ± 1.97</td>
<td>2.5 ± 0.75</td>
<td>5.8 ± 2.22</td>
</tr>
<tr>
<td>Year 2</td>
<td>DOC</td>
<td>51.2 ± 3.4</td>
<td>44.7 ± 2.5</td>
<td>56.5 ± 3.1</td>
<td>49.5 ± 3.0</td>
</tr>
<tr>
<td></td>
<td>DIC</td>
<td>27.2 ± 1.7</td>
<td>15.7 ± 1.1</td>
<td>25.7 ± 1.2</td>
<td>13.1 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>pH</td>
<td>5.94 ± 0.17</td>
<td>5.56 ± 0.23</td>
<td>6.30 ± 0.19</td>
<td>5.79 ± 0.19</td>
</tr>
<tr>
<td></td>
<td>Conductivity</td>
<td>434 ± 23</td>
<td>386 ± 33</td>
<td>495 ± 30</td>
<td>500 ± 39</td>
</tr>
<tr>
<td></td>
<td>Acetate</td>
<td>3.4 ± 1.13</td>
<td>1.9 ± 0.21</td>
<td>1.4 ± 0.06</td>
<td>2.8 ± 1.01</td>
</tr>
<tr>
<td></td>
<td>Dissolved CH₄</td>
<td>2.55 ± 0.18</td>
<td>2.93 ± 0.22</td>
<td>1.93 ± 0.18</td>
<td>0.99 ± 0.13</td>
</tr>
</tbody>
</table>

6.3.2 Dissolved CH₄

Average pore water concentrations of DM in the bog ranged from 0.98 mg l⁻¹ measured towards the beginning of the year, to 4.15 mg l⁻¹ measured at the end of the year (figure 6.1a). Both the control and treatment bog mesocosms produced a fluctuating pattern of increasing [DM] as the year progressed (figure 6.1a). A notable decrease in both bog control and treatment [DM] was measured during the peak of summer, however this was only a temporary drop, as concentrations continued to increase going into autumn and winter (>550 days into experiment). The bog LGM treatment exhibited both the lowest
Figure 6.1 Pore water dissolved CH₄ concentrations measured in the (A) bog and (B) fen in the second season. Measurements were taken at 10 cm below the vegetation surface. Error bars represent ±1 standard error of the mean.

and highest concentration of DM when compared to the control group, however, out of the 16 measurements performed during the year, average control and treatment bog DM
concentrations were always within ± 1 standard error of the mean of each other (figure 6.1a). The fen mesocosms generally displayed lower concentrations of DM compared to the bog, where concentrations ranged from 0.30 to 3.03 mg l\(^{-1}\) during the year (figure 6.1b). The seasonal pattern of DM in the fen contrasts with that observed in the bog. The control and treatment fen groups both showed a summer time peak in DM. This pattern is more evident in the fen control group, where the lowest concentration was measured in the first winter period (0.59 mg l\(^{-1}\)) and the highest concentration measured during the summer (2.80 mg l\(^{-1}\)). On five occasions during the year, average [DM] measured in the fen treatment was lower than in the controls.

The average bog control [DM] in the 2\(^{nd}\) year was 5.23 mg l\(^{-1}\), which was 15% lower than the average bog treatment figure of 6.02 mg l\(^{-1}\). Non-parametric analysis of the year showed that both sets of data were correlated with time (P < 0.05), with no significant difference between the two groups (P > 0.05) (Appendix A). The fen control group averaged 3.96 mg l\(^{-1}\) over the year, which was 48% higher than the LGM treatment figure of 2.04 mg l\(^{-1}\) (P < 0.01). Both fen groups showed a correlation with time (P < 0.01) during the experiment. DM data collected during the 2\(^{nd}\) year was pooled into the four main seasons for analysis (figure 6.2). This exercise showed that in line with the Kruskal–Wallis analysis of results over the entire year, the bog control and treatment DM concentrations were not significantly different in any of the four seasons in the second year of the experiment (P > 0.05). In contrast, the fen control [DM] was significantly different from the fen treatment in the spring, summer and autumn of the year (P < 0.05). Grouping the data into seasons also showed that fen treatment mesocosms had significantly lower amounts of DM in their pore waters in every season compared to the other 3 experimental groups (figure 6.2).
6.3.3 Dissolved Carbon

6.3.3.1 Dissolved Inorganic Carbon

In year 1 the average [DIC] measured in the bog control group ranged from 10.8 to 44.1 mg l⁻¹ and from 7.9 to 24.6 mg l⁻¹ in the treatment group (figure 6.3a). Bog treatment values were consistently between 26 and 58% lower than the control throughout the first
year, with an average difference of 42% based on average concentration values of 34.1 and 19.9 mg l$^{-1}$ in the control and treatment respectively ($P < 0.01$). Data from both bog groups
showed a strong correlation with time during the same period (P < 0.01) (Appendix B). There was a seasonal pattern in [DIC] exhibited by both bog control and treatment mesocosms, where [DIC] was generally higher during the warmer summer months compared to the colder months of winter and early spring. Fen mesocosms shared a similar pattern of [DIC] over the first year and the difference exhibited between the experimental groups compared to the bog. Fen control [DIC] averaged 23.7 mg l\(^{-1}\), compared to 14.4 mg l\(^{-1}\) in the fen treatment, a suppression of 39% (P < 0.01). Fen treatment [DIC] shared a similar pattern to the control mesocosms, with concentrations lower during the colder months compared to summer values. [DIC] in both the control and treatment were significantly correlated with time (P < 0.01), with the interaction of time and treatment effect also testing as highly significant (P < 0.01).

In year 2 the pattern of [DIC] differs from the previous season in both bog and fen ecosystems, yet the suppression effect of the LGM [CO\(_2\)] remained the same in the bog and is enhanced in the fen by 10%. Bog control [DIC] ranged from 9.07 to 51.9 mg l\(^{-1}\) and bog treatment [DIC] ranged form 6.20 to 30.6 mg l\(^{-1}\) over the year (figure 6.3a). Bog treatment values were between 21 and 58% lower than the control group throughout the 2\(^{nd}\) year except for the first measurement in winter that showed a reversal in the prevailing trend (+19%). The average difference between bog control and treatment values was 42% based on the average concentration values of 27.2 and 15.7 mg l\(^{-1}\) in the control and treatment respectively (P < 0.01). Bog DIC values were correlated with time during the 2\(^{nd}\) year (P < 0.05), however there was no interaction with time and the treatment effect (P > 0.05). Fen control DIC values ranged from 17.3 to 39.5 mg l\(^{-1}\) and from 7.1 to 21.8 mg l\(^{-1}\) in the treatment (figure 6.3b). All measurements of fen treatment [DIC] were suppressed below control values (between 12 and 64%) with an average suppression of -49% (P < 0.01), based on the average [DIC] values of 25.7 mg l\(^{-1}\) and 13.1 mg l\(^{-1}\) in the control and
treatment respectively. Fen DIC data was correlated with time (P < 0.01), yet there was no 
time-treatment interaction (P > 0.05). The overall pattern of [DIC] during the second year was erratic with numerous peaks and troughs in both the bog and fen data set. One distinctive event consistent across all the datasets was a decrease in [DIC] concentration in the simulated months of July and August followed by a rebound in concentration by October (~600 days into experiment).

The DIC statistical test results are conducive to the theory that year 1 was a correctional period in the experiment. A significant result for the interaction of time and treatment effect in year 1 implies that bog and fen experimental groups were changing at different rates during the year. Both experimental groups responded to the change in seasonal environmental variables, therefore a correlation with time is to be expected, however the decrease in [CO2]am provides an additional feature exclusive to the LGM treatment groups that alters the [DIC]. In year 2, bog and fen [DIC] again interacted with time, however the influence of CO2 starvation on ecosystem processes may have reached a maximum as there was no evidence of an interaction between time and treatment.

6.3.3.2 Dissolved Organic Carbon

In year 1, the average [DOC] in the bog control mesocosms ranged from 49.8 to 104.3 mg l⁻¹ (figure 6.4a). Apart from an early peak in the bog control group in May (116 days into the experiment), bog treatment DOC values were evenly matched with control values during year 1 with a range of average values from 39.1 to 76.0 mg l⁻¹. Post-CO2 manipulation in year 1, bog control average [DOC] was 67.7 mg l⁻¹ and bog treatment was 62.1 mg l⁻¹, an 8% difference (P > 0.05) (Appendix B). A repeated measures ANOVA
Figure 6.4 Dissolved organic pore water concentrations measured from (A) bog and (B) fen mesocosms over 2 seasons. Each point represents an average of 8 replicates. Error bars show ±1 standard error of the mean. The dotted line shows when the LGM treatment was instigated. Control points represent the ambient [CO₂]ₐmb, and treatment points the simulated LGM [CO₂]ₐmb.

showed that the bog data set in this year was correlated with time (P < 0.05), and that time and the treatment effect had an interaction (P < 0.05). The general pattern of bog [DOC] in year 1 suggested that higher concentrations were associated with the warmer summer
months. This pattern was reproduced in the fen data set, however due to a small data set in this year and large standard errors with each measurement, no definitive relationship could be established with temperature. Average fen control [DOC] values ranged from 57.1 to 90.1 mg l⁻¹, which was lower than the average treatment range of 69.4 to 121.9 mg l⁻¹ for the same time period (figure 6.4b). Fen treatment DOC values were on average 24% higher than the fen control in year 1, however this was not a large enough difference to produce a significant statistical result (P > 0.05). Fen control and treatment DOC values were correlated with time during this period (P < 0.05), but there was no time-treatment interaction (P > 0.05).

In year 2, the average bog control [DOC] ranged from 33.6 to 81.5 mg l⁻¹ and the bog treatment average concentrations ranged from 32.7 to 70.9 mg l⁻¹ (figure 6.4b). This was a difference of 13%, which increased the difference between the two groups observed during the first year (8%), yet this was not large enough to produce a statistically significant result (P > 0.05). The pattern of [DOC] in the bog data set in year 2 showed no clear seasonal influence, however the largest concentration was still measured in summer (June) and the lowest in winter. Like the first year, control and treatment bog DOC values were correlated with time (P < 0.05), but there was no time-treatment interaction (P > 0.05). The pattern of [DOC] in the fen closely resembled that witnessed in the bog. The highest fen concentrations were measured in summer (June) and the lowest in winter, with no overall seasonal pattern evident. The average concentration in the fen control was 56.5 mg l⁻¹ and in the fen treatment it was 49.5 mg l⁻¹ (P > 0.05). The fen year 2 data set showed a time interaction (P < 0.05), but no time-treatment interaction (P > 0.05). The [DOC] concentration was generally lower in year 2 compared to year 1 in all bog and fen experimental groups.
Over the 2 year experiment there was no influence of the LGM \([\text{CO}_2]_{\text{tom}}\) on \([\text{DOC}]\). Unlike with DIC, there was no immediate suppression effect, however the correctional period to a new steady state may be longer for DOC than for DIC. In the bog, the difference between control and treatment average \([\text{DOC}]\) was 8% in year 1, which increased slightly to 13% in year 2. The same pattern is observed in the fen ecosystem, where the percentage difference changed from +24% in year 1 to -12% in year 2. If the experiment was continued, it is likely that the difference would continue to increase as there was a time-treatment interaction in year 2, which suggests that the control and treatment groups behaved differently to each during the season. An example of the growing trend of increasing difference between the fen control and treatment groups can be seen in figure 6.5. By taking the proportional difference in \([\text{DOC}]\) measured between control and treatment before the LGM treatment began, allows for a best estimate of background (non-treatment) variability and allows for an estimate of the relative extent to which \([\text{DOC}]\) was lowered over the course of the experiment. This analysis was performed on both bog and fen ecosystems, however only the relationship between fen control and treatment showed a consistently negative pattern (figure 6.5). The value of \(\Delta\text{DOC}\) (percentage change in \([\text{DOC}]\)) was calculated for every post-treatment flux and is defined as:

$$\Delta\text{DOC} = \left( \frac{y_1 \left( \frac{x_a}{y_b} \right) - x_1}{x_1} \right) \times 100$$

(Equation 6.2)

Where \(\Delta\text{DOC}\) is the percentage change in \([\text{DOC}]\) as a result of the treatment effect, \(x_1\) and \(y_1\) are the respective control and treatment DOC concentrations during the treatment
period, $x_a$ and $y_b$ are the respective mean control and treatment DOC concentrations prior to the onset of the treatment.

Figure 6.5 Percentage difference between treatment and control values for dissolved organic carbon in the fen. Negative values indicate a relative suppression treatment effect and the positive values indicate a relative stimulation in treatment dissolved carbon flux.

6.3.4 Acetate

The concentration of acetate was measured once during each calendar season in the experiment (figure 6.6). A series of blank (deionised water + internal standard) control samples were also analysed at the same time to account for background contamination. Blank samples averaged 1.1 µg ml$^{-1}$ for acetate, which when subtracted from mesocosms
concentrations, showed that only spring pore waters contained acetate at levels that were notably above background level. Bog [acetate] was highest during the first spring period in the experiment in both the control (31.3 µg ml\(^{-1}\)) and treatment (18.5 µg ml\(^{-1}\)) mesocosms. The subsequent seasons in the first and second year contained substantially less acetate in the bog pore waters. There was a second spike in acetate measured in the second spring in both bog control (6.7 µg ml\(^{-1}\)) and treatment (3.5 µg ml\(^{-1}\)) groups, however this was considerably lower when compared to year 1. Bog control [acetate]
averaged 8.9 µg ml\(^{-1}\) and treatment 6.3 µg ml\(^{-1}\) in season 1 (P > 0.05) (Appendix C, D). Values were lower in year 2, where the control group averaged 3.4 µg ml\(^{-1}\) and the treatment averaged 1.9 µg ml\(^{-1}\) (P > 0.05). Dividing the bog data into calendar seasons showed that there was only a significant difference between bog control and treatment during the autumn of year 1 (P < 0.05), where LGM treatment mesocosms had 100% more acetate in their pore waters. However, the concentrations during this time were both very low (control = 1.2 µg ml\(^{-1}\) and treatment = 2.4 µg ml\(^{-1}\)), therefore when compared to spring, autumn concentrations of acetate and differences between controls and treatment may be inconsequential.

Fen [acetate] in the control remained consistently low throughout the experiment with no notable peaks in any of the seasons. In contrast, the fen treatment exhibited a pattern of high concentrations in spring and considerably lower concentrations in the other seasons, a pattern that closely resembles that observed in the bog data. In the first spring, fen control measured 0.8 µg ml\(^{-1}\), which is lower than the background concentration, whereas the fen treatment [acetate] measured significantly higher at 18 µg ml\(^{-1}\) (P < 0.05) (Appendix C, D). This result is contrary to the relationship observed between bog control and treatment during the same period. Over the first year the fen control averaged 2.5 µg ml\(^{-1}\) and the fen treatment averaged 5.8 µg ml\(^{-1}\), a statistically significant difference of 132% (P < 0.05). The large difference measured during the first year is driven mainly by the large difference measured during the spring of that year. A second peak in [acetate] is measured in the second spring in the fen treatment, however like both the bog control and treatment [acetate] pattern, this is considerably lower compared to the previous spring. In year 2, the control mesocosms average [acetate] was 1.4 µg ml\(^{-1}\), whereas the treatment mesocosms averaged a higher 2.8 µg ml\(^{-1}\) (P > 0.05).
6.3.5 pH and Conductivity

pH and conductivity were measured in each season of the 2 year experiment. Average values can be seen in table 6.1 and Appendix E and F. There was no LGM [CO2]_{atm} effect on pH or conductivity in either the bog or fen that was beyond the error associated with the measurements, or that could not be accounted for by the pre-treatment differences. Therefore, this data will not be discussed in the following section.

6.4 Discussion

6.4.1 Dissolved CH₄

In year 2 of the experiment there was no difference in bog [DM] between control and treatment mesocosms (P > 0.05), yet there was a significant difference of 49% between fen control and treatment (P < 0.05) pore water [CH₄]. DM is a good indicator of CH₄ production rates in wetland soils and is therefore likely to be susceptible to change arising as a result of modification to the [CO₂]_{atm}. Previous studies that have exposed wetlands to elevated [CO₂]_{atm} have shown higher levels of DM compared to ambient controls, however not at the depth measured in this study (Keller et al., 2009). Marsh et al., (2005) reported results which showed mean [DM] was between 12-18% higher in elevated CO₂ treatment plots compared to ambient controls, however this was not significant (P > 0.05). In rice paddy soils, Cheng et al., (2005) measured an increase in DM at 10 cm below the surface, but the difference proved to be insignificant when tested (P > 0.05). When investigating the combined influence of nitrogen nutrition and elevated CO₂ on rice plant growth, Li et
al., (2004) found that at times DM showed a positive response to elevated CO$_2$ (P < 0.05) in plots subjected to a medium loading of nitrogen, phosphorus and potassium. In plots that had a large nutrient loading, DM was 25% higher than ambient controls, but large measurement variations resulted in an insignificant statistical result (P > 0.05). Results from other studies have overall been inconclusive regarding the effect changing the [CO$_2$]$_{amin}$ can have on DM in wetland pore waters. A consistent and prolonged significant difference (averaging 49%) was measured in the fen during this study, therefore this represents the first time that changing the [CO$_2$]$_{amin}$ has been reported as undoubtedly altering the [DM] in wetland pore waters.

The contrasting DM pattern of no response to the LGM [CO$_2$] in the bog and a ~50% decrease in the fen is challenging to explain. It is unlikely that the sampling resolution was unable to determine a treatment effect because DM shows little temporal deviation over diurnal timescales (Benstead & Lloyd, 1996). DM generally increases with depth (Romanowicz et al., 1995, Benstead & Lloyd, 1996, Alberto et al., 2000, Clymo & Bryant, 2008), therefore it is feasible that there may have been differences in [DM] in the bog and fen at depths not measured in this study. Contrasting statistical differences have been reported by other elevated CO$_2$ studies within profile measurements, for example, Keller et al., (2009) reported a significant result at -30 cm depth, but not at -10 or -75 cm. The major mechanism hypothesised to explain the increase in methanogenesis in wetlands exposed to elevated CO$_2$ is an increase in labile carbon availability as a consequence of increased plant productivity and biomass (as discussed in previous chapters). The opposite may be occurring as a result of CO$_2$ starvation in the fen and reducing the pore water concentration of CH$_4$. However if this was the only consideration, then the bog mesocosms would also have shown a similar reduction to the fen.
There are at least two reasons why [DM] does not appear to respond to the treatment in the bog. The first reason could be that the species composition in the bog treatment mesocosm group was not responsive to changes in \([\text{CO}_2]_{\text{atm}}\). Both the bog control and treatment groups had less vascular plants compared to the fen. Vascular plants are known to be associated with higher \(\text{CH}_4\) fluxes as a result of their ability to introduce root exudates into the rhizosphere and channel \(\text{CH}_4\) through aerenchyma tissues (Saarnio & Silvola, 1999, Strom et al., 2005). The bog mesocosms were dominated by bryophyte species during the 2\(^{nd}\) year, which are likely to have lower photosynthesis rates and export less labile carbon into the rhizosphere compared to the mesocosms containing greater abundances of vascular plants (Hines et al., 2008). Bryophyte species also have a tendency to use subsurface \(\text{CO}_2\) as a significant source of carbon for photosynthesis (Turetsky & Wieder, 1999), therefore any alteration to the \([\text{CO}_2]_{\text{atm}}\) may not be significant in altering plant physiology in the bog mesocosms. The second possible reason for the lack of a measurable treatment effect in the bog could be that bog mesocosms are nutrient limited and therefore unlikely to respond to a reduction in \([\text{CO}_2]_{\text{atm}}\) because biological processes are already severely limited.

6.4.2 Dissolved Inorganic Carbon

The LGM \([\text{CO}_2]_{\text{atm}}\) caused a suppression of DIC by 42\% in the bog (\(P < 0.05\)) and 49\% in the fen (\(P < 0.05\)) in the second year of the experiment. Since the very first measurement in year 1 (post-\(\text{CO}_2\) treatment), there was a clear difference between the control and treatment mesocosms [DIC] in both the bog and fen, therefore, this implies that the processes that are controlling [DIC] are extremely sensitive to changes in \([\text{CO}_2]_{\text{atm}}\). DIC is defined as:
\[ DIC = [CO_2^+] + [HCO_3^-] + [CO_3^{2-}] \]  
(Equation 6.3)

Where, \([CO_2^+] = [CO_{2aq}] + [H_2CO_3] \)  
(Equation 6.4)

The mesocosms used in this study were closed systems, therefore any changes to the concentration of DIC is directly related to a change in concentration of aqueous CO2. Reducing the atmospheric concentration of CO2 by 50% to LGM levels could have simply reduced the [CO2] dissolved in the pore water and subsequently decreased the [DIC], as stated by Henry’s Law. DIC concentrations are generally higher in wetland pore waters than in ambient air due to autotrophic and heterotrophic respiration (Keller et al., 2009), therefore a change in equilibrium state is unlikely to be the major control on the measured [DIC] in the treatment mesocosms.

Previous studies which have looked at the opposite effect of CO2 fertilisation have reported increases in [DIC] compared to the control groups (Kang et al., 2001, Marsh et al., 2005, Keller et al., 2009). Causes of this increase have been attributed to increases in respiration by soil microorganisms that were nourished by increased root biomass and higher root exudates from vegetation (Kang et al., 2001, Marsh et al., 2005). In other non-wetland studies a positive correlation has also been established between soil respiration and root biomass (Vose et al., 1995, Pregitzer et al., 2000). Root biomass (particularly in year 1) is unlikely to have decreased in the treatment or increased significantly in the control mesocosms to account for the observed changes. As the plants adapted to the LGM [CO2], it is possible that the plants decreased the quantity of root exudates entering the soil, thereby limiting respiratory substrates. In a greenhouse experiment, Wolf et al., (2007) demonstrated that elevated CO2 can increase O2 loss from roots of C3 marsh sedges (S.
Americanus) which could aid the decomposition of soil organic matter. If the opposite were to occur as a result of a reduction in $[\text{CO}_2]_{\text{air}}$, then this would limit respiration in the roots and the [DIC] in the rhizosphere. Ecosystem respiration rates were calculated at the end of year 2 (Chapter 3.3.9), which showed that there was no difference in respiration between the control and treatment experimental groups in either the bog or the fen. This whole-system approach may not have been focused or sensitive enough to account for the respiration changes in the roots alone.

6.4.3 Dissolved Organic Carbon

Over the 2 year experiment there was no statistical difference in [DOC] between control and treatment mesocosms in either the bog or fen ($P > 0.05$). It is hypothesised that the correctional period to a new LGM $[\text{CO}_2]$ altered steady-state, may take longer than the 2 years in this experiment. This is based on the observed widening trend in [DOC] over the course of the experiment between control and treatment mesocosms. DOC in wetland soils is derived from decomposing material and plant root exudates. The influence that changing the $[\text{CO}_2]_{\text{air}}$ has on plants metabolic behaviour and the export of carbon (root exudates) may be relatively quick, however altering the quantity and quality of decomposing plant material could take considerably longer for the full effects to filter through the system. Elevated CO$_2$ studies have reported increases in [DOC] which have been attributed to increased plant activity (e.g. root exudates), the effect of aerobic microbes metabolising complex organic carbon as a result of increased O$_2$ loss from roots, and a change in species composition (Kang et al., 2001, Kang et al., 2005, Fenner et al., 2007, Wolf et al., 2007, Kim & Kang, 2008, Keller et al., 2009). However, not all elevated CO$_2$ studies have induced higher DOC in wetland soils (Ellis et al., 2009).
Increasing the \([\text{CO}_2]_{\text{atm}}\) could potentially cause an increase in microbial biomass that could initiate a chain of biological consumption, which could lead to an increased decomposition rate of DOC and lower concentrations (Ellis et al., 2009). Based on the majority of elevated CO\(_2\) studies measuring an increase in \([\text{DOC}]\), it is possible that given a long enough time, that a LGM \([\text{CO}_2]\) would limit \([\text{DOC}]\) in wetland rhizospheres.

6.4.4 Acetate

The pore water concentration of acetate showed no difference between control and treatment during year 1 or 2 in the bog mesocosms (P > 0.05). There was more acetate in the fen treatment during year 1, largely due to a big difference between the experimental groups in spring (P < 0.05), and no difference in the second year (P > 0.05). The only notable period of acetate concentration in both the bog and fen was in spring. Acetate tends to accumulate in certain wetlands over the year (Duddleston et al., 2002). Measuring a peak in acetate in spring suggests that short chain acids are accumulating over the winter period when fermentation processes are limited by temperature. When this limitation was lifted in subsequent seasons, concentrations of acetate reduced dramatically. In natural wetland environments, the ultimate fate of acetate produced anaerobically is aerobic degradation to CO\(_2\) in either oxidised surfaces or near oxidising roots of vascular plants (Duddleston et al., 2002), in comparison, only a small fraction is utilised for methanogenesis.

In the experiment, bog mesocosms generally had higher concentrations of acetate compared to the fen mesocosms. Upland bogs and sites that are Sphagnum-dominated shown a predominance for the hydrogenotrophic CH\(_4\) production pathway which has been
shown by both isotope (Lansdown et al., 1992, Chanton et al., 1995, Chasar et al., 2000a) and microbiological (Duddleston et al., 2002, Horn et al., 2003) approaches. In wetlands such as these, acetate can be a terminal product of metabolism that accumulates over time (Hines et al., 2001). The lack of acetate use represents a decoupling between the terminal step in methanogenesis and primary and secondary fermentation processes that supply substrates to methanogenic bacteria (Hines et al., 2001, Hines et al., 2008). It is hypothesised that the lack of methanogenesis from acetate and C1 compounds in upland bogs could be due to a short growing season or aeration events (Hines et al., 2001). In this experiment, bog and fen mesocosms were subjected to the same growing year and high water-tables were maintained permanently close to the surface, therefore these hypotheses can be rejected. Other potential factors could include low pH conditions and nutrient limitation (Kiene & Hines, 1995). The lower concentrations of acetate in the fen could be explained by the ecosystem favouring acetoclastic methanogenesis (Galand et al., 2005). The low concentration of acetate measured during both spring periods in the fen control is challenging to explain. It may be that the sampling resolution missed the accumulated winter concentration or that, in comparison to the fen treatment, this is a highly productive system operating without severe CO₂ limitation, therefore acetate is frequently utilised when available.

6.5 Conclusion

The LGM [CO₂] had a clear impact on the [DIC] in both fen and bog systems. Both ecosystems showed a ~50% decrease in concentration from the immediate onset of the experiment. This could be due to a decrease in root respiration caused by a reduction in plant root exudates, or a reduction in O₂ release from the roots caused by CO₂ starvation on
plant productivity. The bog and fen showed a trend of widening [DOC] over the experiment between control and treatment values. The bog mesocosms showed a 5% and fen mesocosms a 36% reduction in [DOC] over the experiment, however this was not statistically significant. Bog and fen mesocosms exhibited a contrasting response to the LGM [CO₂] in their rhizosphere pore water DM concentrations. Fen mesocosms demonstrated a ~50% reduction in pore water concentrations, whereas the LGM treatment caused no effect in the bog mesocosms. This contrasting response could be explained by the dominance of bryophyte species in the bog that are unlikely to fully respond to CO₂ starvation as they use subsurface CO₂ as a significant source of carbon for photosynthesis. High acetate accumulation was witnessed in the bog and is indicative of a system which favours hydrogentrophic methanogenesis over the acetoclastic pathway. In contrast, lower concentrations of acetate in the fen are symptomatic of a wetland system that has a higher proportion of methanogenesis derived from acetoclastic reactions. Measuring a higher concentration of acetate in spring indicates that short chain acids are accumulating in the rhizosphere over the winter period when fermentation processes are potentially limited by temperature. Results gained from this rhizosphere investigation show that the influence of the LGM [CO₂] on belowground processes cannot be assumed to be uniform across all wetland types. The response of wetlands to CO₂ starvation is likely to be dependent on the nutrient status and species composition of the ecosystem.
CHAPTER SEVEN

Discussion

7.1 Introduction

The main aim of this study was to quantify the effect of the LGM [CO$_2$]$_{am}$ on CH$_4$ flux from wetlands. To achieve this aim, a uniquely constructed experiment was devised to accurately manipulate atmospheric CO$_2$ to a level associated with glacial maxima (180 ppmv), within CEUs sufficient to maintain representative wetland mesocosms over the long-term. Wetland mesocosms (110 x 400 mm) were collected from a UK bog and fen, and maintained in the CEUs for >3 years. During this period, mesocosms were split between a designated control (modern day [CO$_2$]$_{am}$) and treatment (LGM [CO$_2$]$_{am}$) group where they were maintained for two growing-seasons and exposed to a climate that was representative of the locations from which they were sampled. To address the principal research question, CH$_4$ emissions were measured using headspace chambers and two analytical techniques: GC-FID and CRDLS (Chapter 3). A deliberately low sampling resolution was employed during the study to accurately maintain the treatment [CO$_2$]$_{am}$ for long time-periods thus maximising the treatment exposure. In recognition of the diurnal variation in wetland CH$_4$ flux that the main experiment did not address, an investigation
into this was conducted during the second year (Chapter 4). Chapter 5 focused specifically on the effect of the LGM $[\text{CO}_2]_{\text{atm}}$ on the relationship between temperature and CH$_4$ emissions. This relationship is often expressed in terms of $Q_{10}$ values and is easily assimilated into wetland CH$_4$ models. This therefore represents a potentially straightforward way to factor in the effect of CO$_2$ starvation on one of the most important controlling mechanisms on CH$_4$ emissions from wetlands. Finally, to understand the findings that were presented in Chapters 3-5, the results from the long term monitoring of several below-ground variables (DM, DOC, DIC and acetic acid) were presented in Chapter 6. In this Chapter, the work is discussed and summarised, and recommendations for future work are made.

### 7.2 The Effect of LGM $[\text{CO}_2]_{\text{atm}}$ on Wetland CH$_4$ Flux

In Chapter 3 (2 year CH$_4$ flux monitoring) and Chapter 4 (diurnal CH$_4$ flux variations) the LGM $[\text{CO}_2]_{\text{atm}}$ had a suppressive effect on CH$_4$ emissions from the wetland mesocosms. The two contrasting sampling resolutions in each of the chapters showed a consistent trend of less CH$_4$ emitted from the fen treatment mesocosms than fen controls. During the second season of the experiment, the fen control and treatment average CH$_4$ fluxes differed by 25% and, over the entire year, this amounted to a 32% difference in total CH$_4$ emitted ($P < 0.05$) (figure 3.2 and table 3.1). This suppression pattern was also measured in the diurnal experiment. The fen treatment mesocosms emitted on average 39% ($P > 0.05$) less CH$_4$ than control mesocosms (figure 4.1b), with the largest suppression measured during the night (45% $P < 0.05$).
The effect of the LGM \([\text{CO}_2]_{\text{atm}}\) on the bog treatment \(\text{CH}_4\) flux was statistically indistinguishable from the modern day control over the 2 year experiment. However, when the sampling resolution was increased to measure the diurnal variation, bog treatment mesocosms emitted a significantly lower amount of \(\text{CH}_4\) (70%) (figure 4.1a). This contrasting response to the LGM \([\text{CO}_2]_{\text{atm}}\) by the bog uncovered in Chapter 3 and 4 is difficult to explain. The diurnal investigation was performed in the second year during the simulated month of May. In the main two-year experiment, both of the \(\text{CH}_4\) flux measurements carried out during that month showed that bog treatment \(\text{CH}_4\) emissions were on average 5% larger compared to controls. This contrasting result in Chapter 3 and 4 highlights the high temporal variability in \(\text{CH}_4\) flux which should be considered when examining short-term data in any longer-term wetland study.

To my knowledge, there are no other similar wetland LGM \([\text{CO}_2]_{\text{atm}}\) simulation studies for the results from the 2-year (Chapter 3) and diurnal (Chapter 4) experiment to be compared with. The results can, however, be compared with those from published studies that investigate the effect of elevated \([\text{CO}_2]_{\text{atm}}\) to see if the suppression effect detailed in this thesis is of equal magnitude to the stimulation effect. This comparison would have ideally plotted \(\text{CH}_4\) fluxes measured at different atmospheric CO2 concentrations by researchers, however, because there is no standard experimental design, inter-comparisons of reported values would have been misleading. Therefore, the effect of altering the \([\text{CO}_2]_{\text{atm}}\) is reported in terms of percentage difference from a modern day \([\text{CO}_2]\) control (figure 7.1). By plotting the percentage difference between the control and treatment groups from Chapters 3 and 4, and those from elevated CO2 studies (figure 7.1), a strong positive correlation \((r^2 = 0.60)\) was observed. The diagram clearly shows that a reduction or increase in the \([\text{CO}_2]_{\text{atm}}\) is likely to cause a linear change in \(\text{CH}_4\) flux proportional to the
change in $[\text{CO}_2]_{\text{atm}}$ over the range of 180 to 700 ppmv. The hypothesised mechanism for this relationship is summarised in section 7.4.

Figure 7.1 Scatter diagram showing the percentage difference between CH$_4$ emissions measured in modern day $[\text{CO}_2]$ atmospheres (X) and those measured in either a sub-ambient $[\text{CO}_2]_{\text{atm}}$ (results from this thesis at ~180 ppmv or elevated $[\text{CO}_2]_{\text{atm}}$ (~550 and 700 ppmv). Note: Not all of the percentage differences plotted in the diagram are statistically significant from their controls.
7.3 The Influence of Temperature and Species Composition on Wetland CH$_4$ Flux

Temperature is a key controller of decomposition and the rate of methanogenesis, which leads to higher CH$_4$ emissions from wetlands in the summer months (Dise, 1993, van Hulzen et al., 1999, Hoj et al., 2008) and far lower emissions during cool winters. In the 2-year experiment (Chapter 3) and the dedicated temperature control experiment (Chapter 5), CH$_4$ flux is shown to be directly influenced by temperature. The second season in the experiment showed a clear linear relationship between temperature and CH$_4$ flux in all experimental groups (figure 3.4). This linear relationship is unlikely to be exclusively caused by temperature, as day length also changed over the year and plants were at different growth stages, therefore this relationship represents the combined influence of the a number of seasonally affected variables on CH$_4$ flux. This relationship was not influenced in the bog by the LGM [CO$_2$]$_{am}$, which, when considering there was no overall difference between bog control and treatment CH$_4$ flux during the experiment, is not surprising. The fen did, however, demonstrate a treatment effect with seasonal change. As temperature and day-length increased in the second season, CH$_4$ emissions decreased from the fen treatment compared to the control. This lead to a larger difference in emissions during the summer compared to the winter.

The role of temperature and its relationship to CH$_4$ flux was explored in more detail in Chapter 5. Wetlands show an exponential increase in CH$_4$ emissions when temperature is increased and all other variables are maintained constant (Daulat & Clymo, 1998). This well documented pattern was demonstrated in Chapter 5 in every experimental group. There was however a contrasting LGM [CO$_2$]$_{am}$ response on the temperature dependency of bog and fen fluxes. In the bog, the relationship between temperature and CH$_4$ flux was
unaltered by the LGM [CO$_2$]$_{atm}$ (figure 5.1). In the fen, the relationship between temperature and flux was unchanged below \(\sim 10^\circ C\), whereas above this, the relationship was suppressed by the LGM [CO$_2$]$_{atm}$ leading to lower emissions from the fen at higher temperatures (figure 5.1) than in controls. This effect resulted in treatment $Q_{10}$ values which were approximately 50% lower than in controls. This pattern suggests that below 10°C CH$_4$ emissions from the fen were limited by temperature constraints on biological activity, whereas above 10°C, LGM [CO$_2$]$_{atm}$ limited substrate supply which restricted CH$_4$ production.

Despite the clear link between CH$_4$ flux and temperature, a higher atmospheric or soil temperature does not always lead to a higher CH$_4$ flux. In the diurnal study (Chapter 4) CH$_4$ fluxes were generally higher during the colder night period compared to during the warmer day period. This could have been caused by increased oxidation in the rhizosphere during the day (Ding et al., 2004) or a delay in the output of plant root exudates into the rhizosphere (Thomas et al., 1988). Measuring a higher flux in the night has been identified in other wetland studies (Yavitt et al., 1993, Moore et al., 1994, Mikkela et al., 1995) and highlights the potential error associated with up-scaling from single CH$_4$ flux measurements made during daylight hours (the approach employed in Chapter 3 and in many other published studies). In this experiment, the LGM [CO$_2$]$_{atm}$ caused a greater suppression of CH$_4$ flux at night in both the bog and fen mesocosms. This suggests that the CH$_4$ flux suppression outlined in Chapter 3 represents a conservative estimate. Had night sampling been implemented in conjunction with a day sampling strategy, a suppression effect may have also been measured in the bog over the 2 year experiment.
Wetland plants can act as conduits for CH$_4$ release (Morrissey et al., 1993, Bellisario et al., 1999) and export carbon into the ground in the form of root exudates (Saarnio et al., 2004). Carbon compounds released from plants provide the major source of carbon used in methanogenesis in wetlands (Chanton et al., 1995). The composition of wetland species therefore has a significant bearing on the CH$_4$ flux from wetlands (Strom et al., 2005). The bog mesocosms used in this study were dominated by Hypnum cupressiforme, Sphagnum papillosum and the occasional Juncus effusus shoot. This composition is not known for the prolific export of root exudates into the rhizosphere (Hines et al., 2008), which may help to explain why there was only no overall CO$_2$ starvation effect in this ecosystem (Chapters 3 and 6). Bryophyte species such as these deliver root exudates slowly into the soil, potentially creating a time delay from peak photosynthetic uptake, to peak CH$_4$ emissions at night (Chapter 4). The fen mesocosms had a larger proportion of vascular plants growing in them that consisted of Campylium stellatum, Juncus subnodulosus and Carex lepidocarpa. The greater presence of C$_3$ vascular plants could further help to explain why fen mesocosms demonstrated a CO$_2$ starvation effect. Vascular plants input more carbon into the soil compared to bryophytes (Hines et al., 2008). Therefore, limiting the photosynthetic rates due to a reduction in [CO$_2$]$_{atm}$ is likely to considerably alter the proportion of labile carbon in the soil and limit methanogenesis. In summary, Chapters 3-5 show that species composition can be as important as temperature when accounting for differences in wetland CH$_4$ flux.
7.4 Summary of, and Reasons for, the Contrasting Bog and Fen Response to CO₂ Starvation

Based on the results from Chapters 3-6, a summary of the main effects of CO₂ starvation on the bog and fen mesocosms is illustrated in figure 7.2. Overall, the bog ecosystem exhibited no response to CO₂ starvation, whereas the fen ecosystem showed considerable change.

7.4.1 Bog

At the end of the 2-year experiment the total amount of CH₄ emitted in the two bog experimental groups was statistically indistinguishable. There are two possible reasons for this: (1) nutrient limitation and (2) soil derived CO₂ supplemented photosynthesis. The low nutrient status of the bog would have limited plant growth and physiological processes from the onset of the experiment. It is therefore possible that wetland ecosystems which are nutrient deficient will not show a dramatic lowering in CH₄ flux in response to CO₂ starvation. The bog mesocosms in this study were mainly dominated by Sphagnum and Hypnaceous mosses. Bryophyte species such as these are unlikely to alter their physiological processes in a sub-ambient CO₂ atmosphere because they use belowground CO₂ to supplement photosynthesis (Turetsky & Wieder, 1999). This supplementing of limited atmospheric CO₂ will have countered the effects of CO₂ starvation and so may have maintained photosynthesis and the export of carbon into the rhizosphere at rates more similar to those found under ambient CO₂ atmospheres. It is likely that both sub-surface enrichment CO₂ within the sphagnum ‘canopy’ and nutrient limitation were both acting simultaneously to suppress CH₄ emissions. The results in Chapter 6 provide evidence for
Figure 7.2 Summary of the main effects of CO₂ starvation on bog and fen mesocosms during this experiment. This illustration represents a hybrid between measured parameters (indicated by the square symbol) and ideas based around published literature. The suppression figures for CH₄ flux were year 2 values.
this hypothesis. No change was observed in almost all bog variables measured during the 2-year experiment, with [DIC] being the only exception. DOC showed no reduction, furthermore, there were no changes in [acetate] or in [DM].

Acetate was found in higher concentrations in the bog than in the fen. This implies that hydrogentropic methanogenesis dominates in this system, which is in agreement with other studies that show upland bogs accumulate acetate over the winter (Duddleston et al., 2002). Hydrogentrophic methanogenesis in wetlands is generally associated with more acidic systems and found in deeper peat layers (Hornibrook et al., 1997, Chasar et al., 2000b). A wetland soil that favours hydrogentrophic methanogenesis is therefore indicative of a system that is deficient in labile carbon. Based on this assumption, any changes to the proportion of plant root exudates to the rhizosphere will have limited effect on CH4 emissions in bogs when acetotrophic methanogenesis is potentially negligible compared to hydrogentrophic methanogenesis.

7.4.2 Fen

The fen mesocosms exhibited a decline in CH4 emissions when exposed to the LGM [CO2]arm. The reason for this reaction is most probably due to a limitation in photosynthesis and NEE as a result of CO2 starvation, that resulted in a reduction in carbon allocation to the rhizosphere (Whiting & Chanton, 1993, Dippery et al., 1995, Tissue et al., 1995), thereby limiting substrate availability to methanogens. The opposite of this theory is used to describe the observation of increased CH4 emissions and DOC fluxes from wetlands exposed to elevated CO2 (Hutchin et al., 1995, Megonigal & Schlesinger, 1997, Freeman et al., 2004a). The fen mesocosms used in this study contained a larger quantity
of vascular plant species compared to the bog. This is likely to be a major reason why the fen mesocosms exhibited a suppression in CH₄ flux when maintained in the LGM [CO₂]₉₉. Ecosystems containing vascular plants export more labile carbon into the rhizosphere compared to areas dominated by bryophytes species (Saarnio et al., 2004, Hines et al., 2008). Therefore any changes to the physiology of vascular plants (particularly C₃) could result in considerable changes in the concentration of labile carbon compounds in wetland soils. When the majority of CH₄ from a wetland system originates from acetotrophic methanogenesis, as is the case in fens (Galand et al., 2005, Juottonen et al., 2005), it is likely that changing the export of root exudates from vascular plants through a suppression in photosynthetic rate, will ultimately lead to alterations in CH₄ emissions. This is because microbes rapidly metabolise root exudates into other substrates such as acetate (Saarnio et al., 2004), which is readily available for acetotrophic methanogenesis.

The belowground variables measured in Chapter 6 support the hypothesis that the proportion of carbon being allocated into the rhizosphere was reduced in the fen. There was a greater reduction in [DOC] measured in the fen compared to the bog during the 2-year experiment. The fen control and treatment mesocosms were statistically indistinguishable in terms of [DOC], however there was a clear trend of decreasing concentration in the treatment mesocosms (figure 6.4) that may have continued to decline in a longer experiment. A reduction of 50% in the [DM] measured in the second season of the experiment is also a clear indicator that methane production had been suppressed.

Examining the relationships between belowground variables and CH₄ flux provided further evidence of a fen ecosystem that was considerably altered by CO₂ starvation (figure 7.3).
Figure 7.3 The relationships between belowground variables (Chapter 6), CH₄ flux (Chapter 3) and the influence of CO₂ starvation measured in the fen. The relationship between [DOC] and CH₄ flux is shown in graph A, [DOC] and [DM] in graph B and [DM] and CH₄ flux in C.
A regression analysis showed a positive correlation between [DOC] and CH₄ flux, [DOC] and [DM], and CH₄ flux vs. [DM]. In every case, the LGM [CO₂] atm caused a suppression in the relationship, but still maintained the link between the variables. There was no direct relationship between [DOC] and [DM] in the fen treatment, however a clear pattern of suppression still existed. It is interesting to note that the largest differences between control and treatment regressions were at the highest measured flux and concentration values. As the highest fluxes and concentrations were generally associated with higher temperatures, this shows that the influence of the LGM [CO₂] is most prominent when temperature ceases to become a limiting factor, i.e. in the summer. Bog mesocosms showed no pattern between [DOC] and [DM], and CH₄ flux vs. [DM]. There was a positive correlation between CH₄ flux and [DM], but the bog experimental groups shared similar regressions.

### 7.5 Implications of Findings

The main mechanisms for glacial-interglacial atmospheric CH₄ differences are thought to be a change in wetland CH₄ emissions and global extent (Chappellaz et al., 1993a, Chappellaz et al., 1997), and the strength of the tropospheric sink (reaction with the OH radical) (Valdes et al., 2005). Determining the relative contributions of these controlling variables is extremely difficult. The contraction of global forests and lower global temperatures may have reduced global atmospheric emissions of BVOC (Adams et al., 2001, Petron et al., 2001, Cinege et al., 2009) and elevated the OH radical sink in the atmosphere, thereby lowering the atmospheric lifetime of CH₄ and the overall atmospheric concentration. However, there is some uncertainty as to what degree global BVOC emissions changed over glacial-interglacial timescales. Arneth et al., (2007) suggest only a 15% difference in isoprene and monoterpane emissions between the LGM and the PIH,
which would have created a more stable OH concentration during the late Pleistocene and Holocene, yet Kaplan et al., (2006) and Valdes et al., (2005) suggest that there was \(-65\%\)
less BVOC in the atmosphere at the LGM compared to the PIH. To add to this uncertainty, the results from this thesis suggest that the global wetland CH\(_4\) flux may have been lower than previously predicted at the LGM due to the effects of CO\(_2\) limitation on ecosystem behaviour, an effect which has not been recognised previously.

The results from this thesis question the assumption that global wetland CH\(_4\) emissions at the LGM would have been of similar magnitude to those predicted for the modern day (Kaplan et al., 2006). It has been shown that a [CO\(_2\)]\(_{am}\) similar to the LGM, reduces CH\(_4\) emissions in some wetland ecosystem types by as much as \(-30\%\) over 2 years. Furthermore, the largest LGM [CO\(_2\)]\(_{am}\) induced suppressions in CH\(_4\) flux were consistently measured when temperature limitation on carbon mineralisation was at its lowest (Chapters 3 and 5). This suggests that the dominant source of CH\(_4\) during the LGM (warm-temperate and tropical wetlands) (Chappellaz et al., 1993, Dallenbach et al., 2000) could be greatly overestimated. CO\(_2\) limitation on wetland CH\(_4\) emissions is currently unaccounted for when modelling ice age CH\(_4\) budgets. This therefore means that current LGM models are likely to overestimate the global contribution of wetlands to the [CH\(_4\)]\(_{am}\). Furthermore, it could mean that the fall in BVOC and increase in atmospheric sink were not as dramatic as suggested by Kaplan et al., (2006) and Valdes et al., (2005). It would be interesting to apply the experimental results from this study to address this issue.

The results from this thesis could have wider implications beyond the LGM. For example, ice core records show that after the low CH\(_4\) concentrations associated with the YD stadial, atmospheric CH\(_4\) concentrations peak around 700 ppbv in the early Holocene (11 to 8 ka)
before declining again between 8 to 6 ka (Brook et al., 2000). Recent isotope studies have been able to pin-point a low latitude wetland source as the most likely causes of this peak, rather than a destabilisation in marine clathrates (Schaefer et al., 2006, Sowers, 2006, Petrenko et al., 2009). During the early Holocene there was a rapid expansion in northern peatlands (MacDonald et al., 2006). Investigations have shown that there was a large growth in circumartic peatlands (e.g. around the West Siberian Lowlands) that began ~16.5 ka and expanded rapidly between 12 and 8 ka in conjunction with high summer insolation and increasing temperature (Smith et al., 2004, MacDonald et al., 2006). It has been hypothesised that many of these newly developed peatlands were warm and wet minerotrophic fens, often dominated by sedges. The assumption is that the CH$_4$ production rates of northern peatlands may have been considerably higher in the early Holocene than they are today, based on the fact that northern peatlands are composed of more ombrotrophic bogs today.

The results presented in the thesis challenge this hypothesis. The expansion of peatlands in the NH during the immediate post-glacial Pleistocene/early Holocene would have undoubtedly made a positive contribution to the [CH$_4$]$_{atm}$ at the time. What remains questionable however, is the assumption that NH peatland CH$_4$ production rates would have been higher compared to those of the modern day. This thesis has demonstrated that the amount of CH$_4$ emitted from wetland ecosystems is sensitive to the [CO$_2$]$_{atm}$. In particular, fen CH$_4$ emissions were shown to be susceptible to changes in the [CO$_2$]$_{atm}$ due to their vegetation composition and preference for acetotrophic methanogenesis. If early Holocene circumartic peatlands were dominated by fen ecosystems, then compared to modern day fens, their CH$_4$ emissions would have been suppressed by the low [CO$_2$]$_{atm}$ of the time (~260 ppmv). The contribution of CH$_4$ to the atmosphere from newly developed fens at the Pleistocene-Holocene transition could therefore be overstated. An alternative
explanation for the rise in atmospheric CH$_4$ at the time, could be an increase in CH$_4$
ebullition from newly formed thermokarst lakes in unglaciated regions in northern high
latitudes (particularly in Siberia) as the climate warmed (Walter et al., 2006, Walter et al.,
2007).

7.6 Recommendations for Future Work

Results from this thesis shed light on the response of peatland ecosystems and their
fluxes of CH$_4$ to low [CO$_2$]$_{atm}$ as experienced during the LGM. There does, however,
remain scope for further work as detailed below. These studies should include:

- A full global estimate of the LGM total wetland CH$_4$ flux informed by the results
  from this project. The first step would be to use an existing model for a direct
  comparison between a simulation with and without the impact of CO$_2$ starvation on
  wetland ecosystem processes e.g. Cao et al., 1996). However, to accurately
  estimate wetland CH$_4$ emissions in the past, models need to spatially distinguish
  between wetland ecosystems of different nutrient status as these will have
  contrasting responses to sub-ambient CO$_2$ concentrations.

- An investigation into the long term (>3 years) response of wetland ecosystems to
  CO$_2$ starvation. During this experiment, variables such as [DOC] were still
decreasing at the end of the second year in response to a reduction in CO$_2$ level.
Based on this declining trend, it is unlikely that the influence of the LGM [CO$_2$]$_{atm}$
had fully altered the CH$_4$ flux. Therefore an increase in the duration of the
experiment may show a greater suppression of CH$_4$ flux. A longer-term
investigation would also provide the opportunity to investigate whether the long-term carbon decomposition rate is altered by CO2 starvation.

- An assessment of the impact of the LGM [CO2]_atm on ebullition. The contribution of bubbles to the overall wetland CH4 flux could be as much as 50-64% (Tokida et al., 2007). During this project only the diffusive flux/plant mediated pathway was measured, therefore the suppression effect could have been different had all the wetland flux pathways been examined. Such a study could be extended to look at the effect of elevated CO2 on the same pathway.

- Building on the last point, investigating the combined effect of variations in [CO2]_atm and temperature on bubble formation and release should be investigated. It would be interesting for example, to recreate the freeze-thaw conditions of the arctic tundra to see if pulses in CH4 released when wetlands freeze are altered by CO2 (Mastepanov et al., 2008).

- A detailed assessment of the response in belowground rhizosphere variables to LGM [CO2]_atm should be performed. This may include vertical distributions of DIC, DOC and DM. These variables have been shown in elevated CO2 studies to vary in concentration with depth. In addition, measuring the vertical distribution of dissolved CO2 in pore waters would provide a better indication of soil respiration. A decrease in DOC input into the belowground environment could limit organic matter decomposition. It would therefore be advantageous to measure soil enzyme activities at various depths.
• The impact of LGM \([\text{CO}_2]_{\text{atm}}\) on methanogen communities should be investigated. A change in [DOC] and methanogen substrate quantity and quality in the rhizosphere, as a result of lowering the [CO\(_2\)]_{\text{atm}}\), could have an adverse effect on methanogen population size and may change the composition on the methanogen community. Understanding this change could help to explain the contrasting response of bog and fen ecosystems to the LGM [CO\(_2\)]_{\text{atm}}\).

• Investigate the combination of CO\(_2\) starvation and water-table manipulation on CH\(_4\) and CO\(_2\) emissions. During this experiment water-tables were maintained at a constantly high level. In the natural environment the water-table fluctuates on a regular basis. It would be interesting to measure whether the increase in CO\(_2\) flux associated with a water-table fall, is limited by the LGM [CO\(_2\)].

• A more detailed assessment of wetland plant response to CO\(_2\) starvation and CH\(_4\) emission should be carried out. An investigation into whether certain species/assemblages of species have a competitive advantage in a reduced CO\(_2\) atmosphere would be important to perform, because if the LGM [CO\(_2\)]_{\text{atm}}\) gives certain species an advantage, this could change the gas transport and decomposition properties of wetlands and ultimately the CH\(_4\) flux. During a study such as this, it would be informative to measure net ecosystem exchange and the long-term response of different plants to [CO\(_2\)]_{\text{atm}}\). It would also be important to analyse which plants are supplementing photosynthesis with belowground carbon through stable isotope techniques.
• An experiment focusing exclusively on the quantity and type of roots exudates from wetland plants. This may include a full characterisation of DOC to the molecular level. This could be achieved by isolating wetland plants, perhaps hydroponically or in artificial mesocosms, and focusing on root exudation over a year and how this was influenced by low CO₂ levels.

• More wetland ecosystems should be investigated for their response to the LGM [CO₂]ₐtm. An experiment may focus for instance on wetlands in coastal locations. During the LGM global seas levels fell and created exposed continental margins which were colonised by wetland ecosystems (Kaplan et al., 2006). Investigating this type of habitat for response to LGM [CO₂]ₐtm would more accurately represent some of the largest wetlands in the world at the time. Deep coring of such places would also show the change in C₃ and C₄ plant species which would indicate likely CH₄ emissions in the past. It would also be interesting to monitor the levels of sulphate reducing bacteria in coastal wetlands to investigate the role of alternative electron acceptors at sub-ambient [CO₂]ₐtm.

• An investigation in to the effects of Holocene [CO₂]ₐtm on anaerobic environments. The Holocene trend of decreasing atmospheric CO₂ and CH₄ concentrations mimics previous interglacial patterns until ~5000 yrs ago, where both rise uncharacteristically. Ruddiman et al., (2008) hypothesise that the anthropogenic influence of early human rice cultivation (potentially much earlier than previously thought) could explain this rise in [CH₄]ₐtm. This idea could be tested using wild and more modern day rice plants in the sub-ambient CO₂ levels of the time. Up-
scaling the results to predict a global contribution of rice farming during the Holocene should be the overall goal.

7.7 Summary and Conclusions

- 2 years of experimentally subjecting wetland mesocosms to the LGM \([\text{CO}_2]_{\text{atm}}\) suppressed the fen \(\text{CH}_4\) flux by an average of 29%. The fen showed a 26 and 32% suppression in the total amount of \(\text{CH}_4\) emitted during year 1 and 2 respectively. It is likely that if a third year had been simulated, the suppression of \(\text{CH}_4\) flux would have been larger. There was no statistically significant change in \(\text{CH}_4\) flux measured in the bog mesocosms.

- The cause of the suppression in the fen is likely to be a reduction in photosynthetically fixed carbon entering the rhizosphere (particularly from vascular plants), which limited acetotrophic methanogenesis.

- The most likely reason for no observed \(\text{CO}_2\) starvation effect on \(\text{CH}_4\) flux in the bog is that the dominant bryophyte vegetation supplemented photosynthesis with subsurface \(\text{CO}_2\), which counteracted the treatment effect. \(\text{CH}_4\) emissions would have also been subject to nutrient limitation on plant substrate supply.

- The same 2 year experiment showed that there was a seasonal effect on \(\text{CH}_4\) flux. The highest emissions were recorded during the summer and lowest during the winter, most likely reflecting changes in temperature controlled biological activity.
• In general, bog and fen mesocosms emitted more CH₄ during the night compared to the day. This could have reflected a lag between peak photosynthesis and root exudate release into the soil and subsequent methanogenic consumption.

• Mesocosms dominated by vascular plants emitted more CH₄ during the day. This is likely to have been caused by an increase in stomatal conductance.

• Linear increases in temperature produced exponential increases in CH₄ flux from the bog and fen mesocosms. The LGM treatment did not alter this relationship in the bog. In the fen, when temperature ceased to be a liming factor (>10°C), the higher the temperature the greater the CH₄ flux was suppressed. In the fen the LGM treatment caused a suppression of ~50% in Q₁₀ values.

• To recreate the relationship between temperature and CH₄ flux in the fen under LGM CO₂ conditions, a reduction in both the Q₁₀ value and a 50% reduction the amount of carbon available for decomposition was required when using the Cao et al. (1996) equation for CH₄ release from wetlands.

• The 2 year experiment caused a decrease in DIC of ~50% in both the fen and bog. This could have been due to a combination of lower O₂ and root exudates from plant roots.
The 2 year experiment caused no significant change in the [DOC] in either the fen or bog. The fen did however show a clear trend of reducing concentration over time.

There was no effect on D.M. concentrations in the bog, whereas the fen exhibited a ~50% reduction. This was a clear indication that the bog system that was not reacting to the LGM CO₂ treatment, whereas methanogenesis in the fen was being affected.

The fen mesocosms showed the largest suppression in CH₄ flux at the highest temperatures in the experiments. This suggests that largest suppression in CH₄ flux at the LGM would have happened in the warmest places, i.e. the tropics.

CO₂ limitation on wetland CH₄ emissions is not currently represented in models of ice age CH₄ budgets. This therefore means that current LGM models are likely to overestimate the global contribution of wetlands to the [CH₄] atm during the LGM.
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Appendices

Appendix A - Dissolved CH$_4$ concentrations measured during year 2 and statistical analysis

<table>
<thead>
<tr>
<th>Period</th>
<th>Site</th>
<th>Treatment</th>
<th>Mean (mg l$^{-1}$ ± 1 S.E.)</th>
<th>Difference between control and treatment means (%)</th>
<th>Within-subject effects (time)</th>
<th>Between subject effect (treatment)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Year 2</td>
<td>Bog</td>
<td>Control</td>
<td>2.55 ± 0.18</td>
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<td>$X^2(15) = 30.5$, p&lt;0.05</td>
<td>H (1) = 0.59, p&gt;0.05</td>
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<tr>
<td></td>
<td></td>
<td>Treatment</td>
<td>2.93 ± 0.22</td>
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<td>$X^2(15) = 29.8$, p&lt;0.05</td>
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<tr>
<td></td>
<td>Fen</td>
<td>Control</td>
<td>1.93 ± 0.18</td>
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<td>$X^2(15) = 45.7$, p&lt;0.01</td>
<td>H (1) = 27.0, p&lt;0.01</td>
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<tr>
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<td></td>
<td>Treatment</td>
<td>0.99 ± 0.13</td>
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<td>$X^2(15) = 40.4$, p&lt;0.01</td>
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<td>Variable</td>
<td>Period</td>
<td>Ecosystem</td>
<td>Treatment</td>
<td>Mean (mg L⁻¹ ± S.E.)</td>
<td>Difference between treatments (%)</td>
<td>Within-subject effects (time)</td>
</tr>
<tr>
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<tr>
<td>Dissolved Inorganic Carbon (DIC)</td>
<td>Year 1 (post-CO₂ treatment)</td>
<td>Bog</td>
<td>Ambient [CO₂]</td>
<td>34.1 ± 3.2</td>
<td>-42</td>
<td>$X^2(4) = 14$, p&lt;0.01</td>
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<td>LGM [CO₂]</td>
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<td></td>
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<tr>
<td></td>
<td></td>
<td>Fen</td>
<td>Ambient [CO₂]</td>
<td>23.7 ± 2.1</td>
<td>-39</td>
<td>F(4) = 3.96, p&lt;0.01</td>
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<td></td>
<td></td>
<td>LGM [CO₂]</td>
<td>14.4 ± 1.5</td>
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<td></td>
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<tr>
<td></td>
<td>Year 2</td>
<td>Bog</td>
<td>Ambient [CO₂]</td>
<td>27.2 ± 1.7</td>
<td>-42</td>
<td>F(4.17) = 16.9, p&lt;0.01</td>
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<td>LGM [CO₂]</td>
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<td>Fen</td>
<td>Ambient [CO₂]</td>
<td>25.7 ± 1.2</td>
<td>-49</td>
<td>F(5.09) = 13.7, p&lt;0.01</td>
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<td>LGM [CO₂]</td>
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<td>Dissolved Organic Carbon (DOC)</td>
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<td>Bog</td>
<td>Ambient [CO₂]</td>
<td>67.7 ± 5.6</td>
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<td>F(2.34) = 7.98, p&lt;0.05</td>
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<td>LGM [CO₂]</td>
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<td>Fen</td>
<td>Ambient [CO₂]</td>
<td>82.1 ± 6.6</td>
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<td>F(2.10) = 75.7, p&lt;0.01</td>
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<td>LGM [CO₂]</td>
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<tr>
<td></td>
<td>Year 2</td>
<td>Bog</td>
<td>Ambient [CO₂]</td>
<td>51.2 ± 3.4</td>
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<td>F(2.51) = 5.25, p&lt;0.01</td>
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<td></td>
<td>LGM [CO₂]</td>
<td>44.7 ± 2.5</td>
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<tr>
<td></td>
<td></td>
<td>Fen</td>
<td>Ambient [CO₂]</td>
<td>56.5 ± 3.1</td>
<td>-12</td>
<td>F(3.89) = 15.0, p&lt;0.01</td>
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<td>LGM [CO₂]</td>
<td>49.5 ± 3.0</td>
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Appendix C - Bog acetate concentrations recorded in year 1 and 2.

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<tr>
<th>Year</th>
<th>Period</th>
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<th>Mean (± 1 S.E.)</th>
<th>Difference between treatments (%)</th>
<th>Stats</th>
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<tr>
<td>1</td>
<td>Spring</td>
<td>Ambient [CO₂]</td>
<td>31.3 ± 7.2</td>
<td>-41</td>
<td>T (14) = 1.35, p&gt;0.05</td>
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<tr>
<td></td>
<td></td>
<td>LGM [CO₂]</td>
<td>18.5 ± 6.3</td>
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<tr>
<td></td>
<td>Summer</td>
<td>Ambient [CO₂]</td>
<td>2.2 ± 0.64</td>
<td>45</td>
<td>T (14) = -0.97, p&gt;0.05</td>
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<td>LGM [CO₂]</td>
<td>3.2 ± 0.64</td>
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<tr>
<td></td>
<td>Autumn</td>
<td>Ambient [CO₂]</td>
<td>1.2 ± 0.17</td>
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<td>T (14) = -2.12, p&lt;0.05</td>
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<td>LGM [CO₂]</td>
<td>2.4 ± 0.65</td>
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<tr>
<td></td>
<td>Winter</td>
<td>Ambient [CO₂]</td>
<td>0.8 ± 0.09</td>
<td>38</td>
<td>T (9.42) = -0.82, p&gt;0.05</td>
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<td>LGM [CO₂]</td>
<td>1.1 ± 0.22</td>
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<td>2</td>
<td>Spring</td>
<td>Ambient [CO₂]</td>
<td>6.7 ± 4.23</td>
<td>-48</td>
<td>Z = -0.70, p&gt;0.05</td>
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<td>LGM [CO₂]</td>
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<td>Ambient [CO₂]</td>
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<td>Z = -2.21, p&lt;0.05</td>
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<td>LGM [CO₂]</td>
<td>1.4 ± 0.36</td>
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<td></td>
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<td></td>
<td>Autumn</td>
<td>Ambient [CO₂]</td>
<td>2.3 ± 0.79</td>
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<td>Z = -1.06, p&lt;0.05</td>
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<td>LGM [CO₂]</td>
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<td>Winter</td>
<td>Ambient [CO₂]</td>
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<td>LGM [CO₂]</td>
<td>1.6 ± 0.07</td>
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Fen acetate concentrations recorded in year 1 and 2.

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<th>Treatment</th>
<th>Mean (± 1 S.E.)</th>
<th>Difference between treatments (%)</th>
<th>Stats</th>
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<td>Spring</td>
<td>Ambient [CO₂]</td>
<td>0.8 ± 0.22</td>
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<td>LGM [CO₂]</td>
<td>18.0 ± 7.65</td>
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<td>Summer</td>
<td>Ambient [CO₂]</td>
<td>3.9 ± 2.36</td>
<td>-41</td>
<td>Z = -0.64, p&gt;0.05</td>
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<td>LGM [CO₂]</td>
<td>2.3 ± 2.36</td>
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<td></td>
<td>Autumn</td>
<td>Ambient [CO₂]</td>
<td>3.6 ± 1.79</td>
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<td>Winter</td>
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<td>Ambient [CO₂]</td>
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<td>LGM [CO₂]</td>
<td>7.1 ± 3.79</td>
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<td></td>
<td>Summer</td>
<td>Ambient [CO₂]</td>
<td>1.5 ± 0.06</td>
<td>-20</td>
<td>Z = -1.91, p&gt;0.05</td>
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<tr>
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<td>LGM [CO₂]</td>
<td>1.2 ± 0.16</td>
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<td>Autumn</td>
<td>Ambient [CO₂]</td>
<td>1.1 ± 0.03</td>
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<td>LGM [CO₂]</td>
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<td>Z = -2.08, p&lt;0.05</td>
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<td>LGM [CO₂]</td>
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Appendix D - Acetate concentrations and statistical analysis

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<th>Period</th>
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<th>Treatment</th>
<th>Mean (µg l⁻¹ ± 1 S.E.)</th>
<th>Difference between treatment and control experimental groups (%)</th>
<th>Within-subject effects (time)</th>
<th>Between subject effect (treatment)</th>
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</thead>
<tbody>
<tr>
<td>Year 1</td>
<td>Bog</td>
<td>Ambient [CO₂]</td>
<td>8.9 ± 2.89</td>
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<td>X² (3) = 17.6, p&lt;0.01</td>
<td>H (1) = 0.76, p&gt;0.05</td>
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<td>LGM [CO₂]</td>
<td>6.3 ± 1.97</td>
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<td>X² (3) = 15.5, p&lt;0.01</td>
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<td>Fen</td>
<td>Ambient [CO₂]</td>
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<td>LGM [CO₂]</td>
<td>5.8 ± 2.22</td>
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<td>X² (3) = 19.1, p&lt;0.01</td>
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<td>Year 2</td>
<td>Bog</td>
<td>Ambient [CO₂]</td>
<td>3.4 ± 1.13</td>
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<td>X² (3) = 10.4, p&lt;0.05</td>
<td>H (1) = 2.37, p&gt;0.05</td>
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<td>LGM [CO₂]</td>
<td>1.9 ± 0.21</td>
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<tr>
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<td>Fen</td>
<td>Ambient [CO₂]</td>
<td>1.4 ± 0.06</td>
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<td>X² (3) = 6.75, p&gt;0.05</td>
<td>H (1) = 0.23, p&gt;0.05</td>
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<td>LGM [CO₂]</td>
<td>2.8 ± 1.01</td>
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<td>X² (3) = 13.1, p&lt;0.01</td>
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</table>
Appendix E

pH values measured from (A) bog and (B) fen mesocosm during 9 seasons in the experiment. Each bar represents the median of 8 replicates. P-T is an abbreviation for pre-treatment and represents a period when both control and treatment mesocosms were maintained in the same ambient [CO$_2$]$_{atm}$. Error bars show the 95% confidence limits.
Appendix F

Conductivity values measured from (A) bog and (B) fen mesocosm during 9 seasons in the experiment. Each bar show the average of 8 replicates, except in the P-T (pre-treatment) where bog control (n)=5, bog treatment (n)=7, fen control (n)=8 and fen treatment (n)=7. Error bars represent the 95 % confidence limits.
NO CD/DVD ATTACHED

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