Doctoral Thesis

Influence of plants upon methane emissions from wetlands

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INFLUENCE OF PLANTS UPON METHANE EMISSIONS FROM WETLANDS

A B H A N D L U N G
zur Erlangung des Titels

DOKTOR DER WISSENSCHAFTEN
der

ETH ZÜRICH

vorgelegt von

ALBERT KÖLBENER

Dipl. Umwelt-Natw. ETH

geboren am 02. Februar 1978
von Appenzell, Appenzell Innerrhoden

Angenommen auf Antrag von

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Dr. Pascal A. Niklaus
Prof. Dr. Mark Gessner

2010
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Thesis Summary

Deutsche Zusammenfassung


CH$_4$ wird von anaeroben Mikroben produziert. Dennoch können Pflanzen die Produktion, den Verbrauch und den Transport von CH$_4$ beeinflussen. In manchen Fällen konnten 90-98% des CH$_4$-Flusses aus überschwemmten Gebieten mit Gefässpflanzen in Verbindung gebracht werden. Es gibt drei Mechanismen mit welchen Gefässpflanzen die Produktion, den Verbrauch und den Transport von CH$_4$ beeinflussen:

(I) Gefässpflanzen scheiden über ihre Wurzeln eine Vielfalt an Kohlenstoffverbindungen aus - z.B. organische Säuren, Zucker, Ektoenzyme, Phenole und Aminosäuren. Diese Wurzelexudate dienen als Quelle für die CH$_4$-Produktion.

(II) Gefässpflanzen bilden Aerenchymstrukturen aus. Durch diese kann CH$_4$ aus anaeroben Bereichen im Boden direkt in die Atmosphäre gelangen. Dadurch wird verhindert, dass CH$_4$ in den aeroben Bereichen der Erde oxidiert wird. (III) Gefässpflanzen können aber auch CH$_4$-Emissionen vermindern. Sie
können über ihr Wurzelsystem Sauerstoff in ansonsten anaerobe Bereiche des Bodens bringen und dort die CH$_4$ Oxidation fördern.

Aktuell wird über einen vierten Mechanismus diskutiert: Pflanzen sollen selber aerob CH$_4$ produzieren. Verglichen mit den anderen drei Mechanismen scheint dieser Mechanismus (sollte er vorkommen) in Feuchtgebieten relativ unwichtig zu sein.

Feuchtgebiete unterscheiden sich sowohl bezüglich der Struktur als auch der Artenzusammensetzung und beinhalten eine grosse Bandbreite an verschiedenen, funktionellen Pflanzentypen. Viele Feuchtgebiete wurden durch anthropogene Einflüsse verändert und der Einfluss der Klimaerwärmung wird in vielen Regionen sichtbar (z.B. das Auftauen von Permafrostböden). Diese Veränderungen können einen wichtigen Einfluss auf das weltweite CH$_4$-Gleichgewicht haben. Feuchtgebiete spielen eine dominante Rolle als natürliche Methanquelle und die artspezifischen Unterschiede im Einfluss auf Methanemissionen sind sehr gross. Demnach könnte eine Verschiebung der Artenzusammensetzung in Feuchtgebieten einen grossen Einfluss auf die CH$_4$-Emissionen haben. Deshalb benötigen wir ein besseres, allgemeines Verständnis wie verschiedene Pflanzenarten CH$_4$-Emissionen von Feuchtgebieten beeinflussen.


In einem Gewächshausexperiment verglich ich acht Gefäßpflanzenarten aus meso- bis eutrophen Feuchtgebieten bezüglich ihres Einflusses auf die Methanemission von Torfkernen - dies mit niedriger und hoher N-Verfügbarkeit. In einem zusätzlichen Experiment bestimmte ich die Produktion leichter, or-
organischer Säuren (LOS) dieser acht Arten - ebenfalls mit niedriger und hoher N-Verfügbarkeit.


In einem Klimakammerexperiment verglich ich CH$_4$-Emisionen von *Sphagnum*torfblöcken mit geringer und solchen mit hoher Dichte der Gefäßpflanze *Eriophorum vaginatum*. Ausserdem untersuchte ich bei diesen Torfblöcken den Anteil der Emissionen in der Form von CH$_4$-Bläschen.

Zwischen den beiden Typen von Torfblöcken gab es keine signifikanten Unterschiede bezüglich der CH$_4$-Gesamtemissionen. Der Anteil an CH$_4$-Bläschen bewegte sich zwischen 0.2% bei Torfblöcken mit vielen Gefäßpflanzen und 13% bei Torfblöcken mit nur wenigen Gefäßpflanzen.

Es gab einen deutlichen Tagesverlauf sowohl bezüglich der kontinuierlichen Methanemissionen als auch den Emissionen als Bläschen. Zwischen fünf und fünfzehn Stunden nachdem das Licht eingeschaltet und die Lufttemperatur von 14 auf 18 Grad erhöht wurde, erreichten die kontinuierlichen Emissionen ihr Maximum. Obwohl die CH$_4$-Emission zwischen den verschiedenen Torfblöcken stark differierten, waren die Emissionen zwischen dunklen und hellen Bedin-


In beiden Torfblocktypen wurde ¹⁴C-markiertes Methan emittiert. Gleichzeitig stieg die Menge des ¹⁴CH₄ und des ¹⁴CO₂ mit zunehmender Menge an verabreichtem Acetat. Acetat war also ganz klar eine Quelle für Methanogenese - auch in Sphagnum-dominiertem Torfboden. Die Deckung mit E. vaginatum hatte keinen Einfluss, weder auf die Emission noch auf die Oxidation von sowohl ¹⁴C-markiertem als auch nicht markiertem CH₄ oder CO₂. Dies deutet darauf hin, dass E. vaginatum unter diesen experimentellen Bedingungen weder die Oxidation im Wurzelbereich beeinflusste, noch dass es die Durchleitung von CH₄ verstärkt hätte.

Of the long-lived greenhouse gases, methane (CH$_4$) has the second-largest anthropogenic radiative forcing after CO$_2$, and is estimated to have contributed around 20% to the increase in atmospheric radiative forcing over the past 200 years. The sources of atmospheric CH$_4$ are both natural and anthropogenic. Natural sources consist of CH$_4$ emissions from wetlands, forests, oceans, fire, geological sources, CH$_4$ hydrates and termites. Of these, wetlands - although covering only 3% of the world’s land surface - are the main natural source of CH$_4$ and account for 70% of 'natural' emissions and one quarter of total emissions. In wetlands, CH$_4$ can reach the atmosphere in three ways: (i) by diffusion, (ii) by conductive flow through vascular plants, and (iii) in bubbles issuing from the soil (ebullition). Once in the atmosphere, CH$_4$ is estimated to persist for between 7 and 10 years.

Although CH$_4$ is produced by microbes under anaerobic conditions, plants can influence its production, consumption and transport in wetland ecosystems, and therefore have strong influence upon the quantities emitted to the atmosphere. In some cases, as much as 90-98% of the CH$_4$ efflux from inundated sites has been associated with vascular plants. There are three mechanisms by which these plants influence formation, consumption and transport of CH$_4$ from wetlands. First, they release a wide range of carbon compounds - organic acids, sugars, ectoenzymes, phenolic and amino acids - into the rhizosphere, providing a substrate for CH$_4$ production. Second, plant aerenchyma may form a conduit by which CH$_4$ is conducted from the anoxic soil directly to the atmosphere, thereby escaping oxidation in the aerobic topsoil. Third, plants may reduce CH$_4$ emission because their roots oxidise the rhizosphere. A fourth, controversial possibility is that plants produce CH$_4$ themselves (aerobically). However, even if this process occurs, it is probably unimportant compared to the other mechanisms operating in wetlands.

Wetlands vary greatly both in structure and species composition, and contain a wide range of plant functional types. Many wetland ecosystems have already been transformed by anthropogenic activities, and in some regions im-
pacts of climate change are now becoming evident, for example due to the thawing of sub-arctic permafrost. These changes can be expected to have important impacts on the global CH$_4$ budget. Considering the large variation among species in their effects on methane emission, shifts in species composition in wetlands may have a large effect on this emission. Therefore, we need a better general understanding of how different wetland plants affect CH$_4$ emissions.

The aims of this dissertation were to (i) improve our understanding of how vascular plants influence CH$_4$ emissions from wetlands via the release of carbon compounds into the rhizosphere, and (ii) to investigate the role of acetate as a source for methanogenesis in Sphagnum peat soils in the presence and absence of vascular plants. Many authors consider acetate to be a substrate for methanogenesis. However, some found that in northern peatlands acetate was the end product of anaerobic decomposition, especially in Sphagnum dominated vegetation with few vascular plants.

In a greenhouse experiment, I compared the influence of eight vascular plant species from mesotrophic to eutrophic wetlands on CH$_4$ emissions from peat cores, under low and high N supply. In an additional experiment, I measured the production of low-molecular-weight organic acids (LOA) by the same species, also at low and high N supply. There were considerable differences amongst species in their effects upon rates of CH$_4$ emission. Six species increased emissions - by up to five times compared to control cores without plants - whereas two species had no effect. There was a weak negative correlation between plant biomass and CH$_4$ emission, but N addition had no significant effect. LOA production varied considerably among species, and tended to be highest in species from mesotrophic habitats; LOA production was stimulated by N addition. I conclude that some species from mesotrophic wetlands cause higher CH$_4$ emissions than species from eutrophic wetlands. This pattern - which contradicts the traditional view of a positive correlation between wetland productivity and methane emission - may reflect higher LOA production rates by species adapted to less productive habitats.
In a mesocosm experiment in a climate chamber, I compared CH$_4$ emissions from Sphagnum peat between monoliths with a low and with a high cover of the vascular plant Eriophorum vaginatum, and investigated the contribution of CH$_4$ in the form of bubbles (ebullition) to overall emissions. The total CH$_4$ emissions did not differ significantly between these two types of monoliths, but the proportion due to ebullition ranged from 0.2% in monoliths with many vascular plants to 13% in monoliths with few vascular plants. There was a clear diurnal pattern in steady CH$_4$ emission and ebullition. Steady emissions peaked 5-15 hours after the lights were switched on and air temperature was increased from 14 to 18 °C. Although total CH$_4$ emission varied greatly amongst monoliths, the ‘diurnal’ variation was rather constant (around 20 µg CH$_4$/h), which could have been due to the temperature regime or to photosynthetic activity. In conclusion, this study suggests that CH$_4$ emission through ebullition could be important in Sphagnum dominated peat bogs, but is likely to be negligible where vascular plants such as E. vaginatum are abundant.

In the same mesocosm experiment, I investigated whether (14C-labelled) acetate was a source for CH$_4$ formation in peat monoliths dominated by Sphagnum, and whether the presence of Eriophorum vaginatum plants influenced the consumption of CH$_4$. In both types of monoliths, 14C-labelled methane was emitted, and the emissions of 14CH$_4$ and 14CO$_2$ increased with the supply of acetate. Hence, acetate was clearly a source for methanogenesis - also in Sphagnum dominated peat. Furthermore, the quantity of E. vaginatum present had no significant effect upon the emission or oxidation of 14C labelled CH$_4$ or CO$_2$, nor upon total CH$_4$ oxidation and emission. This suggests that under these experimental conditions E. vaginatum did not significantly affect methane dynamics, either by oxidising CH$_4$ in the rhizosphere or by increasing the conduction of methane to the atmosphere.

Two main conclusions can be drawn from these greenhouse experiments: 1.) vascular plants may change the form of methane emissions (i.e. fewer bubbles, more continuous flow), and 2.) plants adapted to low nutrient availability may enhance CH$_4$ formation and emission. At the same time it seems that physical factors such as light intensity or soil and air temperature may overlay
the plant’s effects on CH$_4$-fluxes. To be able to predict effects of vegetation changes on greenhouse gas emissions and global warming, it is important to better understand the interactions of vascular plants with methanogenic and methanotrophic communities in natural wetlands, and how these are affected by physical environmental factors.
0 General Introduction

0.1 Methane - sinks and sources

Methane (CH$_4$) has the second-largest radiative forcing of long-lived greenhouse gases (Ramaswamy et al. 2001) - after CO$_2$ - and is estimated to have contributed around 20% to the total trace gas-induced increase in atmospheric radiative forcing over the past 200 years (Bartlett and Harriss 1993; Forster et al. 2007). Once in the atmosphere, CH$_4$ is estimated to persist between 7 and 10 years (Denman et al. 2007). In 2005 the global average concentration of CH$_4$ in both hemispheres was 1'774 ± 1.8 ppb (Forster et al. 2007). This is a much higher value than occurred during the previous 10’000 years (Fig. 0.1), when concentrations as determined from ice cores ranged between 320 ppb during glacial periods to 790 ppb during interglacials (Spahni et al. 2005). However, during the last 250 years the global concentrations of CH$_4$

![Figure 0.1: Atmospheric CH$_4$ concentration in the last 10’000 years (IPCC 2007).](image-url)
have increased from around 700 ppb in 1750 (Flückiger et al. 2002) to 1’774 in 2005. Between 1960 and 1999 the mean atmospheric CH$_4$ concentration increased at least six times faster than during any 40-year’s period in the 2000 years before 1800 (Denman et al. 2007). This increase was almost certainly caused by human activities (Forster et al. 2007).

The sources of CH$_4$ in the atmosphere are both biogenic and non-biogenic. The latter include emissions from mining fossil fuel and burning of natural gas, petroleum and coal, as well as waste treatment, biomass burning and geological sources such as geothermal and volcanic CH$_4$ or fossil CH$_4$ from natural gas seepage in sedimentary basins. Biogenic sources of CH$_4$ include wetlands, forests, oceans, rice fields, ruminant animals such as cattle or sheep, landfills, and termites. Today, these biogenic sources account for more than 70% of global emissions (Table 0.1).

Sources can also be divided into natural and anthropogenic. The latter include livestock, rice agriculture, waste treatment, landfills, and burning of both fossil fuel and biomass. Natural sources consist of CH$_4$ emissions from wetlands, forests, oceans, fire, geological sources, CH$_4$ hydrates and termites (Table 0.1). Although wetlands cover 5.5 x 10$^{12}$ m$^2$ (Bartlett and Harriss 1993; Limpens et al. 2008), or only 3% of the world’s land surface, they are the main natural source of CH$_4$ to the atmosphere and account for two thirds of ‘natural’ emissions and one quarter of total emissions (Table 0.1).

There are three major sinks for CH$_4$: tropospheric oxidation by OH radicals, loss of CH$_4$ to the stratosphere, and biological CH$_4$ oxidation in dry soils (Denman et al. 2007). Although all the important sources and sinks of CH$_4$ seem to be identified at present time, the contributions of each source or sink to the balance of global atmospheric CH$_4$ concentrations are not precisely known; as a result, estimates of global CH$_4$ emissions from wetlands vary between 100 and 231 Tg CH$_4$ yr$^{-1}$ (Table 0.1).
Table 0.1: Mean of estimated contribution of different sinks and sources (Tg CH₄ per year) to the global CH₄ budget as presented in the Fourth Assessment Report of the Intergovernmental Panel on Climate Change (Denman et al. 2007).

<table>
<thead>
<tr>
<th>Sources:</th>
<th>Lowest / highest estimate</th>
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<tbody>
<tr>
<td><strong>Natural:</strong></td>
<td></td>
</tr>
<tr>
<td>Wetlands</td>
<td>100-231</td>
</tr>
<tr>
<td>Termites</td>
<td>20-29</td>
</tr>
<tr>
<td>Ocean</td>
<td>4-15</td>
</tr>
<tr>
<td>Geological</td>
<td>4-14</td>
</tr>
<tr>
<td>Other</td>
<td>6-25</td>
</tr>
<tr>
<td><strong>Anthropogenic:</strong></td>
<td></td>
</tr>
<tr>
<td>Energy production</td>
<td>82-175</td>
</tr>
<tr>
<td>Landfills &amp; waste</td>
<td>35-69</td>
</tr>
<tr>
<td>Ruminants</td>
<td>76-189</td>
</tr>
<tr>
<td>Rice agriculture</td>
<td>31-112</td>
</tr>
<tr>
<td>Biomass burning</td>
<td>14-88</td>
</tr>
<tr>
<td><strong>Total¹</strong></td>
<td><strong>582</strong>*</td>
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<table>
<thead>
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<td>Soils</td>
<td>26-34</td>
</tr>
<tr>
<td>Tropospheric OH</td>
<td>428-511</td>
</tr>
<tr>
<td>Stratospheric loss</td>
<td>30-45</td>
</tr>
<tr>
<td><strong>Total¹</strong></td>
<td><strong>581</strong>*</td>
</tr>
</tbody>
</table>

*Total of CH₄ sources and sinks estimated and used in the Fourth Assessment Report of the IPCC

0.2 Formation and emission of CH₄ in wetlands

In wetlands under typically anaerobic conditions with low sulphate and nitrate concentrations, organic matter is transformed into CH₄ and CO₂ through methanogenic bacteria. The process can be expressed in a simplified form as:

\[ C_6H_{12}O_6 \rightarrow 3CO_2 + 3CH_4. \]

This transformation requires successive actions of four types of microbes: (i) hydrolysis of biological polymers into monomers, (ii) acidogenesis from monomeric and intermediary fermentation compounds, (iii) acetogenesis from the previous metabolites, and (iv) methanogenesis from the simple compounds (particularly H₂ / CO₂, and acetate) that can be used by methanogens. The two major pathways of CH₄ production in submerged wetland soils are acetotrophy and CO₂ reduction by H₂ (Conrad et al. 1989; Schütz et al. 1989; Takai 1970). Although only 14% of methanogenic species
can use acetate as a C and energy source, acetotrophy is considered responsible for two-thirds of CH$_4$ produced (Le Mer and Roger 2001). Under aerobic conditions, CH$_4$ is oxidised by methanotrophic bacteria via one of two pathways: (i) high affinity oxidation (CH$_4$ concentration < 12 ppm) which is estimated to contribute 10% of total CH$_4$ consumption, and (ii) methanotrophy sensu stricto, low affinity oxidation (CH$_4$ concentration > 40 ppm) by methanotrophic bacteria (Jones and Nedwell 1993; King et al. 1990; Whalen et al. 1990). Methanotrophs occur in the oxidised topsoil, in the aerobic rhizosphere of plants having aerenchymatous structures, and also inside roots and bryophytes (Le Mer and Roger 2001; Raghoebarsing et al. 2005).

Several physico-chemical factors influence the formation and consumption of CH$_4$. Whereas methanogenesis is a strictly anaerobic microbial process and requires low redox potentials (Eh < 200 mV), methanotrophy is limited by the availability of oxygen (Stralis-Pavese et al. 2006). The depth of the water table therefore influences whether or not a wetland soil is a CH$_4$ source. A high water table decreases the aerobic area in the soil and hence hampers CH$_4$

![Figure 0.2: Schematic picture of production and consumption of CH$_4$ and CH$_4$ transport to the atmosphere (Le Mer and Roger 2001).](image-url)
oxidation and at the same time increases CH$_4$ formation (Klinger et al. 1994; Rosenberry et al. 2006; Shannon and White 1994). CH$_4$ formation is strongly correlated with the availability of carbon compounds, and carbon recently fixed by plants is an important source for methanogens (Ström et al. 2003).

Climatic factors also influence the formation and consumption of CH$_4$. The optimal temperature for methanogenesis is between 30 and 40 °C, and seasonal or diurnal differences of CH$_4$ emissions in temperate or cold regions can partly be attributed to varying soil-temperatures (Klinger et al. 1994). Methanotrophy appears to be less temperature sensitive than methanogenesis. However, in temperate and subarctic peat soils, temperatures between 20 and 30 °C have been reported as optimal for both processes (Dunfield et al. 1993; Le Mer and Roger 2001).

CH$_4$ can reach the atmosphere in three different ways: (i) by diffusion, (ii) by conductive flow through vascular plants and (iii) in bubbles issuing from the soil (ebullition) (Whalen 2005). The contribution of ebullition to CH$_4$ emission from soils varies widely among studies, and ranges from 1-50% in subarctic tundra (Christensen et al. 2003; Martens et al. 1992; Minkkinen et al. 1997) to 45-85% in the Amazon flood plain or tropical swamps (Bartlett et al. 1988; Devol et al. 1988; Happell and Chanton 1993; Wassmann et al. 1992). This variation depends upon environmental conditions, as described above, and may also be influenced by the vegetation. If there are vascular plants with a well-developed aerenchyma system, for example, CH$_4$ emission might take place mainly through conductive flow through the plant, decreasing the importance of ebullition (Brix et al. 2001; Sheppard et al. 2007; Sorrell and Boon 1992; 1994)

0.3 Influence of vascular plants on CH$_4$ production, consumption and transport

Although CH$_4$ is produced primarily by microbial activity, plants have a strong effect upon CH$_4$ emissions from wetland ecosystems because they influence the production, consumption and transport of CH$_4$ in the soil (Christensen et al.
In some cases, as much as 90-98% of the \( \text{CH}_4 \) efflux from inundated sites has been associated with vascular plants (Joabsson et al. 1999; Verville et al. 1998), though much smaller effects have also been reported (Table 0.2). There are four pathways by which vascular plants influence formation, consumption and transport of \( \text{CH}_4 \):

I.) Release of carbon compounds into the rhizosphere

II.) Aerenchymatous structures serving as conduits

III.) Oxygen transport into the rhizosphere

IV.) \( \text{CH}_4 \) production by plants

The first two and the fourth pathways enhance \( \text{CH}_4 \) fluxes from wetlands, whereas the third pathway reduces \( \text{CH}_4 \) emissions to the atmosphere.

**Table 0.2:** Percentage of \( \text{CH}_4 \) emission from wetlands attributed to plant effects.

<table>
<thead>
<tr>
<th>Plant species</th>
<th>( \text{CH}_4 ) emissions (% of total)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carex spp.</td>
<td>25</td>
<td>(Morrissey et al. 1991)</td>
</tr>
<tr>
<td>Deyeuxia angustifolia</td>
<td>28-31</td>
<td>(Ding et al. 2005)</td>
</tr>
<tr>
<td>Eriophorum and Carex spp.</td>
<td>36</td>
<td>(Schimel 1995)</td>
</tr>
<tr>
<td>Eriophorum angustifolium</td>
<td>48</td>
<td>(Christensen et al. 2003)</td>
</tr>
<tr>
<td>Eriophorum and Carex spp.</td>
<td>55-85</td>
<td>(Waddington et al. 1996)</td>
</tr>
<tr>
<td>Carex spp.</td>
<td>60</td>
<td>(King et al. 1998)</td>
</tr>
<tr>
<td>Phragmites australis</td>
<td>62</td>
<td>(Grünfeld and Brix 1999)</td>
</tr>
<tr>
<td>Carex lasiocarpa</td>
<td>73-82</td>
<td>(Ding et al. 2005)</td>
</tr>
<tr>
<td>Carex meyeriana</td>
<td>75-86</td>
<td>(Ding et al. 2005)</td>
</tr>
<tr>
<td>Carex spp.</td>
<td>89</td>
<td>(Verville et al. 1998)</td>
</tr>
<tr>
<td>Fen vegetation</td>
<td>90</td>
<td>(Whiting and Chanton 1992)</td>
</tr>
<tr>
<td>Scheuzeria palustris</td>
<td>97</td>
<td>(Frenzel and Karofeld 2000)</td>
</tr>
<tr>
<td>Tundra vegetation</td>
<td>&gt; 97</td>
<td>(Bartlett et al. 1992)</td>
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</tbody>
</table>
Pathway I: Release of carbon compounds into the rhizosphere stimulating methanogenesis

Vascular plants release a wide range of carbon compounds into the rhizosphere - i.e. organic acids, sugars, ectoenzymes, phenolic and amino acids (Bais et al. 2006). The functions of these compounds for the plants are thought to be related to interactions with other plants and microorganisms, for example, altering soil chemical and microbial processes to increase soil nutrients and other resources (Bais et al. 2006; Ström et al. 2005b). Several authors have suggested that acetate produced through the fermentation of organic compounds (e.g. released by plants) is an important substrate for methanogenesis (Avery et al. 2003; Bellisario et al. 1999; Boone 1991; Ferry 1997), and studies using $^{14}$C-acetate have supported this idea (Chasar et al. 2000; Ström et al. 2003; Ström et al. 2005a). Generally, peatlands contain large soil C-pools. Nevertheless, the readily decomposable carbon compounds excreted by vascular plants are important substrates for methanogenesis since the soil C-pool largely consists of recalcitrant material (Christensen et al. 1999). The quantity of carbon compounds released by plants varies greatly among species, from less than 10% of net carbon assimilation to as much as 44% (Bais et al. 2006). Nutrient availability, in particular phosphorus availability also determines the quantity of root exudates (Bais et al. 2006; Hinsinger 2001; Neumann and Römheld 1999). Several plant species increase the rhizospheric release of organic acids substantially under conditions of phosphorus deficiency, and for some species (e.g. Lupines spp) this increase coincides with the formation of proteoid roots (Bais et al. 2006; Dakora and Phillips 2002; Neumann and Römheld 1999). The quantity and quality of carbon compounds also varies between calcifuge and acidifuge plants species; for example, the former release more acetic acids whereas the latter tend to produce more citric acid (Ström 1997; Ström et al. 1994).
**Pathway II: Aerenchymatous structures in vascular plants serving as conduits for CH$_4$ transport**

CH$_4$ can be transported from the anoxic soil directly to the atmosphere through aerenchymatous structures in vascular plants, avoiding oxidation in the aerobic topsoil (Bellisario et al. 1999; Frenzel and Rudolph 1998). The aerenchyma system of plants plays an important role in the transport of CH$_4$ and other gases, as demonstrated for some rice cultivars (Aulakh et al. 2000). Roots of many (but not all) wetland species contain large amounts of aerenchyma, and up to 90% of CH$_4$ emission from water logged soils may occur via this plant-mediated conduit (Shannon et al. 1996). Temperature influences the conductance of CH$_4$ through plants (Hosono and Nouchi 1997) and also rates of pressurised convective flow (Chanton et al. 1993; Van der Nat et al. 1998).

**Pathway III: Enhanced CH$_4$ oxidation through O2 transport into the rhizosphere**

Oxygen diffuses from plant roots in the rhizosphere where it can fuel CH$_4$ oxidation (Ström et al. 2005a; Van der Nat and Middelburg 1998a; b). Up to 30-40% of the oxygen transported through the aerenchyma may diffuse into the soil (Armstrong 1979). Hence, plants are able to oxidise their rhizosphere, enabling CH$_4$ oxidising bacteria (methanotrophs) to consume CH$_4$ before it can be emitted. There are indications that plant species vary widely in their effect on CH$_4$ oxidation, and therefore its emission from wetlands (Ström et al. 2005a).

**Pathway IV: CH$_4$ production by plants**

Until recently, methanogenesis was assumed to be the only significant source of CH$_4$ from ecosystems. However, Keppler et al. (2006) demonstrated that plants themselves may emit CH$_4$, and that the emission of CH$_4$ was light and temperature dependent. Moreover, they suggested that vegetation could account directly for a substantial proportion of the global CH$_4$ budget. This publication raised much discussion (i.e. Kirschbaum et al. 2006). Dueck et al. (2007) found no evidence of a substantial emission of $^{13}$CH$_4$ under aerobic
conditions from terrestrial plants grown in a $^{13}$C atmosphere, but Vigano et al. (2008) showed that UV light was the responsible factor for CH$_4$ formation in plants, being the reason that Dueck et al. (2007) did not measure CH$_4$ formation under their UV-free light conditions. However, for wetland ecosystems the direct CH$_4$ emission from plants is likely negligible compared to the plant’s effects on bacterial processes in the soil. The CH$_4$ production rates caused by plants are likely to be 10 to 100-fold lower than those of bacterial activity in the soil.

### 0.4 Aim of the study

The vegetation of wetlands is extremely diverse, both in structure and species composition, and contains a wide range of plant functional types. Subtle gradients in vegetation are characteristic of wetlands, reflecting sensitive adjustments of species composition to local hydrological and nutrient conditions. These conditions are readily modified, for example by changes in flow regime or eutrophication, and this often leads to changes in the species composition (Olde Venterink et al. 2002). Many wetland ecosystems have already been transformed by anthropogenic activities such as drainage and changed land-use. Impacts of climate change are becoming evident in many regions e.g. thawing of subarctic permafrost (Christensen et al. 2004). These changes might have an important impact on the global CH$_4$ budget. The dominant role of wetlands as a natural source for CH$_4$, the shift of species composition in wetlands promoted by e.g. climate change, and the potentially large differences in how plant species affect CH$_4$ emissions (Ding et al. 2005; Joabsson and Christensen 2001; Van der Nat and Middelburg 1998a) urge a better general understanding of how plant effect CH$_4$ emissions from wetlands. This knowledge could help to improve the weak estimates of the contribution of wetland ecosystems to the global CH$_4$ budget (Table 0.1).

In this study, I focused on root exudation as an important mechanism by which plants influence CH$_4$ formation in wetland soils (pathway I). As mentioned above, many authors consider acetate to be a substrate for methano-
genesis (Avery et al. 2003; Bellisario et al. 1999; Boone 1991; Chasar et al. 2000; Ferry 1997; Ström et al. 2003; Ström et al. 2005a). However, Hines et al. (2008), working in northern peatlands, found that acetate was the end product of anaerobic decomposition, especially if the vegetation was composed of *Sphagnum* mosses with no vascular plants. The aims of this study were to (i) improve our understanding of how vascular plants influence CH$_4$ emissions from wetlands via the release of carbon compounds into the rhizosphere, and (ii) to investigate role of acetate as a source for methanogenesis in *Sphagnum* peat soils in the presence and absence of vascular plants.

I focused only on the microbial formation of CH$_4$. The CH$_4$ production by plants (pathway IV) remains controversial, and is certainly negligible in wetland ecosystems compared to the production of CH$_4$ originating in microbial processes.

### 0.5 Outline of the thesis

In Chapter 1 - Plant species from mesotrophic wetlands cause relatively high CH$_4$ emissions from peat soil - results of a greenhouse experiment are presented in which eight vascular plant species from meso- to eutrophic wetlands are compared for their influence upon CH$_4$ emissions, under low and high N supply. Additionally, results from a second experiment are presented, which show differences in the production of low-molecular-weight organic acids (LOA) by the same eight species (Pathway I). The objective of chapter 1 is to evaluate the effects of differences in plant biomass production, fertility of the natural habitat, as well as root LOA production of the eight species, on CH$_4$ emission from peat soil.

In Chapter 2 - Importance of the vascular plant *Eriophorum vaginatum* on CH$_4$ emission from *Sphagnum* peat – CH$_4$ emission rates and diurnal patterns from *Sphagnum* peat are compared between peat monoliths with low or high cover of the vascular plant *Eriophorum vaginatum*. Also, the contribution of ebullition to the total CH$_4$ emission, and how this was affected by the presence of vascular plants, is quantified.
Chapter 3 - $^{14}$C-acetate as a source for methanogenesis in Sphagnum peat in presence and absence of Eriophorum vaginatum plants - investigates whether acetate is a source for CH$_4$ formation in peat monoliths vegetated with Sphagnum sp. only, and in monoliths dominated by the vascular plant Eriophorum vaginatum (pathway I). Furthermore, using the fate of $^{14}$C-labelled acetate, differences in CH$_4$ oxidation (pathways III) are compared between monoliths with and without the vascular plant Eriophorum vaginatum

### 0.6 Literature cited


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1 Plant species from mesotrophic wetlands cause relatively high methane emissions from peat soil

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1.1 Abstract

Plants can influence methane emissions from wetland ecosystems by altering its production, consumption and transport in the soil. The aim of this study was to investigate how eight vascular plant species from mesotrophic to eutrophic wetlands vary in their influence on CH₄ emissions from peat cores, under low and high N supply. Additionally, we measured the production of low-molecular-weight organic acids (LOA) by the same species (also at low and high N supply), because LOA form a substrate for methanogenesis. There were considerable differences among species in their effects upon rates of CH₄ emission. Six of the species (Eriophorum latifolium Hoppe, Potentilla palustris (L.) Scop., Anthoxanthum odoratum (L.) s. str., Carex rostrata Stokes, Carex elata All., Carex acutiformis (Ehrh.) increased CH₄ emissions up to five times compared to control peat cores without plants, whereas two species (Phalaris arundinacea L., Phragmites australis (Cav.) Trin. ex Steud.) had no effect.
There was a weak negative correlation between plant biomass and CH$_4$ emission. N addition had no significant general effect upon CH$_4$ emission. LOA production varied considerably among species, and tended to be highest for species from mesotrophic habitats. LOA production was stimulated by N addition. We conclude that some species from mesotrophic wetlands tend to cause higher CH$_4$ emissions than species from eutrophic wetlands. This pattern, which contradicts what is often mentioned in literature, may be explained by the higher LOA production rates of species adapted to less productive habitats.

1.2 Introduction

Methane (CH$_4$) is an important greenhouse gas, estimated to be responsible for almost 20% of the radiative forcing from long-lived and globally mixed greenhouse gases (IPCC 2007). Natural wetlands are among the most important sources of CH$_4$, estimated to account for 70% of natural emissions and one third of total emissions (IPCC 2007).

Plants can have a strong effect upon the emission of CH$_4$ from wetland ecosystems, apparently because they influence the production, consumption and transport of CH$_4$ in the soil (Christensen et al. 2003; Joabsson et al. 1999; Saarnio et al. 2004; Ström et al. 2003; Whiting and Chanton 1992). In some cases, as much as 90-98% of the CH$_4$ efflux from inundated sites has been associated with vascular plants (Joabsson et al. 1999; Verville et al. 1998). Three main mechanisms are known through which vascular plants influence CH$_4$ emissions from wetland soils: (1) plant roots continuously release organic compounds into the rhizosphere, which are easily available C-sources for methanogenesis (Ström et al. 2003); (2) CH$_4$ moves through the aerenchyma tissue of the roots, rhizomes and dead or living stems to the atmosphere, thereby escaping oxidation in aerobic soil layers - the conduit or chimney effect (Verville et al. 1998); (3) plants bring oxygen into the rhizosphere, which inhibits methanogenic archaea, and fuels CH$_4$ oxidation (Van der Nat and Middelburg 1998). Only a few plant species have been studied for their influence on CH$_4$ emission; however, these have shown large variation (Ding et al.
suggesting that changes in the species composition of wetlands - either as a consequence of climate change or through some other process - could have a considerable effect on CH$_4$ emissions (Christensen et al. 2004; Johansson et al. 2006). But to be able to predict such effects, more species need to be investigated and the relative importance of the various mechanisms by which plants influence CH$_4$ emissions need to be better understood.

Many wetlands throughout the world are subjected to nutrient enrichment, which may increase the productivity of the vegetation and lead to changes in species composition (Bedford et al 1999, Olde Venterink et al 2002, Galloway et al 2004, Bragazza et al 2006). Some authors have concluded that CH$_4$ emissions tend to increase with increasing wetland productivity because rates of root exudation, as well as and gas exchange via the conduit effect, are likely to be larger for big plants than for small ones and hence to be positively related to plant biomass (cf. Ding et al. 2003; Joabsson and Christensen 2001; Waddington et al. 1996; Whiting and Chanton 1993). However, it could also be argued that, because plant roots tend to produce more exudates under nutrient-poor conditions as a mechanism to increase phosphorus availability (Hinsinger 2001; Lu et al. 1999; Marschner 1998; Neumann and Römheld 1999; Ström 1997), CH$_4$ emissions are likely to be higher in nutrient poor wetlands. In view of the different mechanisms by which plants may influence CH$_4$ emissions, it is unclear whether there is a general tendency for species of eutrophic sites to enhance CH$_4$ emission more than species from mesotrophic sites, or vice versa.

The main aim of this study was to investigate how eight vascular plant species from meso- to eutrophic wetlands vary in their influence upon CH$_4$ emissions. Additionally, we investigated whether N fertilisation influences the effects of plants on CH$_4$ emission. In a separate experiment we investigated how the selected plant species differ in root production of low-molecular-weight organic acids (LOA). It was our objective to evaluate the effects of differences in plant biomass production, fertility of the natural habitat, as well as root LOA production of the species. Our hypotheses were:
I.) Vascular plants increase CH\(_4\) emissions from peat.

II.) N fertilisation results in higher CH\(_4\) emission rates because it stimulates plant productivity and it may also increase root exudation because of induced P-limitation.

III.) Plant species from eutrophic wetlands have a larger effect upon CH\(_4\) emission rates than species from less productive wetlands. This hypothesis is based on the assumption that plant size has a larger effect upon emissions than inherent species differences in production of root exudates.

1.3 Materials and methods

Experiment I: Influence of plant species on CH\(_4\) emission from peat cores

This first experiment was designed to compare the influence of eight plant species on CH\(_4\) emission from peat soil. We compared the following species with each other and with a control (bare soil): Eriophorum latifolium Hoppe, Potentilla palustris (L.) Scop., Carex rostrata Stokes, Anthoxanthum odoratum (L.) s. str., Carex elata All., Carex acutiformis Ehrh., Phragmites australis (Cav.) Trin. ex Steud., and Phalaris arundinacea L. The species differ with respect to fertility of the habitats in which they normally occur, varying from mesotrophic to eutrophic wetlands. We ordered the species according to the fertility indication values of Ellenberg et al. (1991) and Bakkenes et al. (2002) (see Appendix A). Anthoxanthum odoratum, Phalaris arundinacea and Phragmites australis were grown from seeds collected in the field (NE Switzerland), Carex rostrata, Carex elata and Carex acutiformis were propagated from tillers collected in the field. Wild types of Potentilla palustris and Eriophorum latifolium were bought commercially (Die Wildstaudengärtnerei, Eschenbach, Switzerland).

Plants were grown in undisturbed peat cores (one plant per core, or in the case of Eriophorum latifolium two small plants). Peat for the experiment was taken from a wet, mesotrophic fen in Nussbaumen near Frauenfeld, Switzerland, where atmospheric N-deposition is 15-20 kg ha\(^{-1}\) y\(^{-1}\) (BUWAL 2005).
Although it would have been convenient to use a homogenised peat-substrate, the processes of sieving and mixing of peat would have severely altered the microbial community and increased carbon mineralization. We therefore decided to grow the plants in intact peat cores, accepting that inhomogeneities in the substrate would probably lead to variation among replicates. To minimize this variation, all material was collected from a small (10 m$^2$) area where the top 40 cm of the soil had been removed the week before. Peat cores (2.2 l) were collected by driving PVC cylinders (19 cm depth, 12.2 cm diameter) into the peat, taking care not to compact the material, and closing the bottoms with gas-tight lids.

Half of the cores in the experiment were fertilized with N (100 mg N in the form of NH$_4$NO$_3$ per pot during the experiment), while the other half remained unfertilized (equally distributed among plant species and bare soil). We fertilized with N because it was growth-limiting: in a preliminary experiment using the same peat material, Anthoxanthum odoratum plants were supplied with N, P, N+P, and growth was compared with an unfertilized control. Only the addition of N caused a significant increase in biomass (ANOVA: $F_{(1,17)} = 23.48; P = 0.0015$). At the end of the experiment NH$_4$, NO$_3$, PO$_4$ and K concentrations were measured in the soil solution of five fertilised and five unfertilised cores. Nitrate was below the detection limit, whereas NH$_4$, PO$_4$ and K concentrations were on average 0.13, 0.03 and 0.54 mg/l in unfertilised, and 0.20, 0.02 and 0.50 mg/l in the fertilised treatments respectively.

The experiment was carried out in a greenhouse in Zurich, Switzerland, between June and October 2006. The water level in the cylinder was maintained at 2 cm below the peat surface, throughout the experiment with deionised water. This could be monitored from outside by means of a tube connected to the base of the peat core. There was no visible growth of algae. All treatments were replicated ten times, yielding a total of 180 pots (eight species + bare control soil x two (with/without N) x 10 replicates).

Methane emissions were measured using a closed chamber technique. Pots were placed in water-filled vessels (32 cm in diameter; 4 cm high) twelve hours before sampling. In order to take air samples, Plexiglas chambers were placed
on top of the vessels immediately before the sampling. Chambers of two different volumes were used (15.2 or 27.1 litre), depending on the size of the plants. Head-space samples were taken with syringes through a rubber stopper with an inbuilt three-way stopcock. Samples were instantly transferred into evacuated vials with rubber septa. Four samples were taken from each pot at six-minute intervals. Methane concentrations were determined using a gas chromatograph (6890N, Agilent Technologies, California, USA) equipped with a Porapak Q column (80/120 mesh) and a flame ionization detector. Samples were injected using an auto sampler (222xl Sample Changer, Gilson, Wisconsin, USA) and calibrated with several calibration gases injected every nine to 12 samples. The CH$_4$ emission rate of each pot was determined by linear regression of the four repeated headspace CH$_4$ concentrations in the chamber. In case of significant methane emission rates R$^2$-values were high (generally > 0.7). If emission rates were very low or negligible, R$^2$-values could also be low (<0.5) due to bias around zero. The sampling of one full replicate (eight species + one control times two N treatments = 18 pots) took half a day; thus all 180 pots were sampled over five consecutive days. We varied the sample time of different species throughout the day. The CH$_4$ emission rates of all cores were measured for all cores between 25 and 30 September 2006.

After the CH$_4$ measurements, the roots and shoots of all plants were harvested, dried at 70 °C for 48 hours, and weighed.

**Experiment II: Organic acid production of plant species**

In a second experiment, we grew the same eight plant species in nutrient solutions to determined interspecific differences in the production of low molecular weight organic acids (LOA) such as acetate, formate and oxalate. These LOAs may either be exuded directly by roots or, more likely, result in the fermentation of larger acids and carbohydrates from plant root exudates (Dessureault-Rompré et al. 2007). The plants used for this experiment had been grown on peat cores collected from the same 10 m$^2$ as in Experiment I. They had been growing in the same greenhouse and for the same period (five months) as the plants in Experiment I. At the time for harvesting of Experiment I, we
transferred the plants into nutrient solutions. Plants were grown in a low-N or a high-N nutrient solution to simulate differences in N availability, just as in Experiment I. The low-N solution was a 1/10 dilution of a full strength solution containing 57 g/l Ca(NO$_3$)$_2$·4H$_2$O, 12.7 g/l NH$_4$NO$_3$, 13.2 g/l HK$_2$PO$_4$, 26.6 g/l MgSO$_4$·7H$_2$O, 0.1 g/l ZnSO$_4$·7H$_2$O, 0.02 g/l CuSO$_4$·5H$_2$O, 0.5 g/l H$_3$BO$_3$, 0.02 g/l Na$_2$MoO$_4$·H$_2$O and 4.4 g/l Cl$_3$Fe (Modified Hoagland solution after Steiner 1961). The high-N solution contained twice as much NH$_4$NO$_3$ (and therefore had a N:P ratio of 10 instead of 5). All other nutrients were the same in both N-treatments. Both N treatments were replicated four times in a full factorial design, yielding 64 plants (eight species x two N treatments x four replicates). The plants grew in the nutrient solutions for three weeks; the solutions were exchanged weekly. After three weeks, all plants had produced new roots and the nutrient solutions were exchanged for the last time. Subsequently after three days of incubation, samples were taken and analysed for acetate, formate, oxalate, citrate, malate, lactate and propionate by ion chromatography (Dionex autosampler system, AS 11 column, eluent generator: potassium hydroxide (1 to 60 mM), flow: 1.5 ml min$^{-1}$). Unfortunately, all plants of Potentilla palustris died in the nutrient solution, so that no data were obtained for this species.

Statistics

Differences in CH$_4$ emission and LOA production rates were tested by means of one-way ANOVAs. All data were log-transformed. ANOVAs were carried out with species and nitrogen as factors and root biomass and/or shoot biomass as co-variables. For analyses that included the pots without plants (control), or target variables calculated per gram biomass (root or shoot), only the factors species and nitrogen were fitted. All models were determined by the AICc criterion (Burnham and Anderson 2002). Multiple comparisons between pairs of means were carried out with the Tukey test (Zar 1999). All analyses were performed using the statistical software R, version 2.6.2 (R Development Core Team 2006).
Figure 1.1: Mean root and shoot biomass (dry) of the eight wetland plant species of the experiment with plants in peat cores. Species are ordered according to the fertility index (N-values) of Ellenberg et al. (1991) increasing from left to right (see Appendix A). White bars are unfertilised plants, shaded bars are N-fertilised. Error bars show SE of 10 replicates. Stars indicate significant differences in biomass of a species due to N fertilisation (* $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$). Letters show significant differences between species ($P \leq 0.05$).

1.4 Results

Variation in biomass among plant species and effect of N addition (Experiment I)

Biomass varied widely among the eight plant species (Fig. 1.1), with *Carex elata*, *Carex acutiformis*, *Phragmites australis* and *Phalaris arundinacea* having significantly higher shoot and root biomasses than *Eriophorum latifolium*,...
Figure 1.2: Mean CH$_4$ emission rates from peat cores with different plant species of European wetlands, as calculated per pot (A), or per g total dry plant biomass (B). Species are ranked according to the fertility index (N-values) of Ellenberg et al. (1991); values shown in brackets after species name (see Appendix A). Error bars show SE of 10 replicates. In A: Stars indicate whether CH$_4$ emission rates of a species were significantly higher compared to the control pots (peat cores without plants) (* P ≤ 0.05; ** P ≤ 0.01; *** P ≤ 0.001). Letters indicate significant (P ≤ 0.05) differences in CH$_4$ emission rates between species (the N treatment had no significant effect on CH$_4$ emission rates).

Potentilla palustris and Anthoxanthum odoratum. However, it was not always the species from mesotrophic habitats (Eriophorum latifolium, Potentilla palustris and Carex rostrata) that produced the lowest biomass (Fig. 1.1). N addition significantly increased the shoot biomass of Anthoxanthum odoratum, Carex acutiformis and Phalaris arundinacea ($F_{(1,144)} = 53.42$, $P < 0.001$), and also the root biomass of Anthoxanthum odoratum ($F_{(1,140)} = 5.81$, $P < 0.05$).

Effects of vascular plants on CH$_4$ emission (Experiment I)

CH$_4$ emissions were up to five times higher from peat cores with Eriophorum latifolium, Potentilla palustris, Carex rostrata, Anthoxanthum odoratum, Carex elata and Carex acutiformis than from control cores with no plant (Fig. 1.2A, Table 1.1). However, the emission rates of two species - Phragmites australis and Phalaris arundinacea - were not significantly higher than the control. CH$_4$ emission rates tended to be higher with species from mesotrophic wet-
Table 1.1: Effects of species, nitrogen and biomass on CH$_4$ log transformed emission rates (ten replicates per treatment). The results from three ANOVAs either include or exclude control (soil without plant) treatments. Model evaluations were based on the AICc criterion (Burnham and Anderson 2002).

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*P ≤ 0.05; ** P ≤ 0.01; *** P ≤ 0.001

lands (species with Ellenberg N values 2-5) than with species from eutrophic wetlands (species with Ellenberg N=7) (Fig. 1.2A). Also, plant species from mesotrophic wetlands, such as *Eriophorum latifolium* and *Potentilla palustris*, had significantly higher CH$_4$ emission rates per gram total biomass than *Phragmites australis* and *Phalaris arundinacea* (Fig. 1.2B, Table 1.1).

*Phalaris arundinacea* was the only species for which there was a significant positive relationship ($R^2 = 0.302$) between CH$_4$ emissions and shoot biomass (Fig. 1.3, interaction effects Table 1.1). The combined data for all species showed a weakly negative relationship (Fig. 1.3). Correlation between root biomass and CH$_4$ emission showed a similar pattern as for shoot biomass (data not shown).

The N treatment had no significant consistent effect on CH$_4$ emission rates (Table 1.1), either on a per plant or per unit biomass basis, although N application increased the biomass of some species.
Figure 1.3: Methane emission rates plotted against shoot biomass, for eight plant species. Since the N treatment had no significant effect on CH$_4$ emission rates, data from both fertilized and unfertilized treatment were included.

Variation in rhizospheric LOA production among species and upon N addition (Experiment II)

Rhizospheric production of low molecular weight organic acids (LOA) differed considerably among plant species (Fig. 1.4A; Table 1.2). Carex elata had by far the highest LOA production, but Eriophorum latifolium and Carex rostrata with high-N supply also had a higher production of LOA than Carex acutiformis, Phalaris arundinacea and Phragmites australis (Fig. 1.4A). In the low N treatment, Carex rostrata, Anthoxanthum odoratum and Carex elata had higher production rates of LOA than Phalaris arundinacea, Phragmites australis. Generally, species from mesotrophic wetlands tended to produce more LOA than species from eutrophic wetlands, both in absolute terms and per gram plant or root biomass (Fig. 1.4). Independently of species, total LOA production was not correlated with biomass (Table 1.2).
Figure 1.4: Mean total of rhizospheric production (± SE) of low molecular weight organic acids (LOA) of eight wetland species in nutrient solution during three days, as calculated per plant (A), per gram total dry biomass (B), or per gram dry root biomass (C). Species are ordered according to the fertility index (N-values) of Ellenberg et al. (1991) increasing from left to right (see Appendix A). LOA is expressed here as the cumulative carbon release by the plants. Error bars show SE of 4 replicates. Significant effects of N addition are indicated with stars (* P ≤ 0.05; ** P ≤ 0.01; *** P ≤ 0.001). Letters show differences among the plant species. The letters on the right side compare plant species exposed to the high N treatment, the letters of the left side compare the species from the low N treatment.

Overall, a high N addition caused a significantly higher LOA production than the low N treatment (Table 1.2). Acetate and formate were the most important organic acids. Whereas production of both acids was similar in the low N treatment, acetate production was significantly higher than formate production in the high N treatment (Appendix B). Whereas species-specific effects on acetate were very similar to those on total LOA production, there were no species effects on formate production.

1.5 Discussion

Vascular plants can influence CH$_4$ emissions from wetlands by altering the production, consumption and transport of CH$_4$ in the soil (Christensen et al. 2003; Joabsson et al. 1999; Saarnio et al. 2004; Ström et al. 2003; Whiting and Chanton 1992), but different species have been found to vary in the extent and how they influence CH$_4$ emissions (Ström et al. 2005; Verville et al. 1998). Our experiment supports our first hypothesis - that plants strongly influence the
rate of CH$_4$ emission from wetland soils, and they also confirm that there are large differences among species in this respect: whereas six of the eight species significantly increased CH$_4$ emissions compared to bare peat, two species had no detectable effect (Fig. 1.2A). We found indications that the difference among species is related to variation in root exudate production, and hence to the influence of the plants on production of CH$_4$, as we will discuss below.

Whiting and Chanton (1993) found evidence that CH$_4$ emission rates increase as the net production of wetland vegetation increases. Similarly, Chanton et al. (1997) found a strong positive correlation between CH$_4$ emission and living aboveground biomass of rice plants. These positive relationships have been interpreted as indicating that CH$_4$ emissions increase with the increasing primary productivity (Bouchard et al. 2007; Dacey et al. 1994; Greenup et al. 2000). We only found a positive correlation between shoot-biomass and CH$_4$ emission rates for one species (Phalaris arundinacea), and no significant relationships for the other seven (Fig. 1.3), just as Joabsson and Christensen (2001) and Ström et al. (2005) did for some other wetland species. The combined data for all our species actually showed a weak negative correlation between aboveground biomass and CH$_4$ emission (Fig. 1.3). Similarly, Bouchard et al. (2007) found decreasing CH$_4$ emission rates with increasing biomass production of emergent plants in a mesocosm experiment, and such a negative relationship is also evident in the data of Van der Nat and Middleburg (1998), albeit with only two species. If biomass were strictly correlated with CH$_4$ emission, we would expect a constant CH$_4$ emission per gram biomass for all plant species (Fig. 1.2B), but our experiment revealed that the two species with the lowest biomass - Potentilla palustris and Anthoxanthum odoratum - had much higher CH$_4$ emissions per unit of biomass than other species (Fig. 1.1). Overall, therefore, our results are more consistent with those of Bouchard et al. (2007), who found a negative relationship between CH$_4$ emission and biomass, than with those of Whiting and Chanton (1993). We conclude that in wetland ecosystems biomass alone is not a reliable predictor of CH$_4$ emissions because some species enhance CH$_4$ emissions more strongly than others with similar or higher biomass (Joabsson et al. 1999; Ström et al. 2003). Possible
Chapter I

Table 1.2: ANOVA tables for the effects of species and the nitrogen treatment on rhizospheric production of low molecular weight organic acids (log transformed) of seven wetland plant species. The target variable was either LOA production per plant, LOA production calculated per gram dry total biomass or LOA production per gram dry root biomass.

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>df</th>
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<td>11.7</td>
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<td>215.9</td>
<td>***</td>
<td>1</td>
<td>9.3</td>
<td>60.5</td>
<td>***</td>
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<td>Shoot biomass</td>
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<td>0.0001</td>
<td>0.002</td>
<td>nf</td>
<td>nf</td>
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<tr>
<td>Root biomass</td>
<td>1</td>
<td>0.12</td>
<td>1.5</td>
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<td>nf</td>
<td>1.5</td>
</tr>
<tr>
<td>Species x Nitrogen</td>
<td>6</td>
<td>0.93</td>
<td>11.7</td>
<td>***</td>
<td>6</td>
<td>1.7</td>
<td>11.1</td>
<td>***</td>
<td>6</td>
</tr>
<tr>
<td>Residuals</td>
<td>41</td>
<td>0.08</td>
<td>0.15</td>
<td></td>
<td>42</td>
<td>0.15</td>
<td></td>
<td>42</td>
<td>0.20</td>
</tr>
</tbody>
</table>

*P ≤ 0.05; **P ≤ 0.01; ***P ≤ 0.001

reasons for this are that some species supply more substrate for methanogenic archaea than others, or provide a more effective conduit for CH$_4$ transport (Greenup et al. 2000); and some species may even reduce CH$_4$ emissions by causing strong rhizospheric oxidation because of high belowground biomass (Hirota et al. 2004).

Contrary to our hypothesis, species from mesotrophic wetlands tended to show higher CH$_4$ emission rates than species from eutrophic wetlands (Fig. 1.2). Moreover, in Experiment II there was a tendency for species causing high CH$_4$ emission rates to have high rates of LOA production as well (Fig. 1.5). Although we have to be cautious in linking the two experiments - for example, because nutrient availabilities and LOA production in soil may not be the same as in a nutrient solution (Jones 1998) - our results suggest that CH$_4$ emissions are strongly influenced by the differing rates of root exudation from wetland plants (Chanton et al. 1995; Ström et al. 2003). Root exudation in plants is likely to be influenced not only by plant size - with bigger plants producing more exudates in absolute terms (Jones et al. 2004) - but also by the ecological characteristics of a species. Plants adapted to low nutrient availability, particularly of phosphorus, may produce more exudates (Bais et
Figure 1.5: Mean CH$_4$ emission rates per gram plant root (Experiment I) versus mean rhizospheric production of low molecular weight organic acids per gram plant root (Experiment II) after three days. Data points are average values of the two nitrogen treatments (with/without N, and low/high N). Error bars represent SE of 8 and 20 replicates for LOA-production and CH$_4$ emission, respectively.

...al. 2006; Hinsinger 2001; Lu et al. 1999; Marschner 1998; Neumann and Römheld 1999). In absolute terms, total production of organic acids per plant was not correlated with shoot biomass in our experiment (Table 1.2). Species adapted to mesotrophic habitats had higher LOA production than species of eutrophic habitats, particularly in the N-fertilized treatment (i.e. likely P-limited conditions) both per gram of root as well as in absolute terms (Fig. 1.4). Thus, the assumption implicit in our third hypothesis - that plant size is more important than habitat adaptation - was incorrect. We note, however, that besides this general pattern, some species like Carex elata in our study may have exceptionally high exudation rates under specific environmental (e.g. P-limited) conditions. We do not have an explanation why this specific species showed such a high exudation rate.
The reason that plants tend to produce more exudates in nutrient-poor conditions is thought to be in order to increase phosphorus availability (Bais et al. 2006; Hinsinger 2001; Lu et al. 1999; Marschner 1998; Neumann and Römheld 1999; Ström 1997). This idea is consistent with the finding that N fertilisation stimulates root exudation (Henry et al. 2005) since this causes a shift in the relative availabilities of N and P in the direction of P limitation. This might have stimulated some species in Experiment II to enhanced production of root exudates. Consequently, we also found that N addition increased the production of organic acids in some species in this experiment. However, we did not observe any significant effect of N addition upon CH$_4$ emission rates in Experiment I although the underlying mechanisms of a higher biomass and higher LOA production were observed. Hence, our hypothesis predicting a higher CH$_4$ emission upon N-addition has to be rejected. Similar to our results, Silvola et al. (2003) found no significant effect of NH$_4$NO$_3$ on CH$_4$-emissions from oligotrophic peatlands. Effects of chemical N-fertilisers on CH$_4$ formation, consumption and emission are complex and often contradictory. In other studies, negative and positive effects of NO$_3$ and/or NH$_4$ additions on CH$_4$ emissions were observed (cf. Le Mer and Roger 2001).

The finding that there was no difference in CH$_4$ emissions between *Phalaris arundinacea* and *Phragmites australis* and bare soil is surprising, since these species revealed high CH$_4$ emission rates under field conditions (Kankaala et al. 2004; Wilson et al. 2008). This inconsistency may be due to differences between the experimental conditions and those in the field, for example in plant size, rooting depths and age. To make a direct comparison between different plants species, and to show mechanistic differences among these species, we chose relatively young plants and kept conditions such as water table and peat volume the same for all species. Perennial plants might act differently when they grow older, and the water table (2 cm below surface) might not have been optimal for all species. Also, LOA production may differ seasonally and this may vary among species. Furthermore, we noted that the CH$_4$ emission rates in our peat cores (0.4 - 1.5 mg m$^{-2}$ hr$^{-1}$) were rather low compared to reported rates from other peat soils, and were in the order of
magnitude observed (on average below 1.7 mg m$^{-2}$ h$^{-1}$) for ombrotrophic peatlands (Greenup et al. 2000). Therefore the effect of plants on CH$_4$ emission through the chimney effect or soil oxidation may have been smaller than in wetlands with higher CH$_4$ production rates in the soil. In contrast, the effect of plants through rhizospheric exudation of C compounds may have been relatively larger than in other peat soils with higher concentrations of available carbon for methanogenesis. Hence, our peat cores were particularly suited for testing differences among plants with respect to the latter mechanism, but care should be taken in transferring our results to plants growing under field conditions.

Despite intensive research, global estimates of CH$_4$ emissions from wetlands vary widely, from 100 to 231 Tg CH$_4$ / year (IPCC 2007). Our results show that the species composition of wetland vegetations are important in differentiating CH$_4$ emissions, just as shown in other studies (Ström et al. 2005, Bouchard et al. 2007). Ecological traits of plants may be more important than absolute biomass in determining the influence of wetland plants upon CH$_4$ emissions. Taking into account the functional properties of plants offers scope to improve global estimates of CH$_4$ emissions from wetlands. Our results suggest that emission rates tend to be relatively high under environmental conditions promoting a high production of root exudates, such as P-limitation or alkaline soil conditions (Dakora and Phillips 2002; Jones et al. 2004).

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Jersey
1.7 Appendices

**Appendix A:** The eight plant species used in the experiments with their Ellenberg- and MOVE N indicator values (Bakkenes et al. 2002; Ellenberg et al. 1991). MOVE values are based on Ellenberg values. A dataset of 100'000 vegetation relevees from The Netherlands served as a base for the calculation of these values.

<table>
<thead>
<tr>
<th>Species</th>
<th>Ellenberg N-value</th>
<th>MOVE N-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Eriophorum latifolium</em></td>
<td>2</td>
<td></td>
</tr>
<tr>
<td><em>Potentilla palustris</em></td>
<td>2</td>
<td>4.07±0.96</td>
</tr>
<tr>
<td><em>Carex rostrata</em></td>
<td>3</td>
<td>4.02±1.17</td>
</tr>
<tr>
<td><em>Anthoxanthum odoratum</em></td>
<td>-</td>
<td>4.47±1.06</td>
</tr>
<tr>
<td><em>Carex elata</em></td>
<td>5</td>
<td>4.93±1.00</td>
</tr>
<tr>
<td><em>Carex acutiformis</em></td>
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<td>5.14±0.97</td>
</tr>
<tr>
<td><em>Phragmites australis</em></td>
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<td>5.52±1.22</td>
</tr>
<tr>
<td><em>Phalaris arundinacea</em></td>
<td>7</td>
<td>6.14±0.72</td>
</tr>
</tbody>
</table>

**Appendix B:** Mean total acetate (A) and formate (B) production (± SE) per plant of eight wetland species in nutrient solution during three days. Species are ordered according to the fertility index (N-values) of Ellenberg et al. (1991) increasing from left to right (see Appendix A). Notice the different scales in A and B.
2 Importance of the vascular plant *Eriophorum vaginatum* and ebullition on methane emission from *Sphagnum* peat

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2.1 Abstract

Plants influence methane emissions from peat soils by altering the rates at which CH$_4$ is produced and consumed, and the pathways by which it is transported to the atmosphere. We investigated CH$_4$ emissions from *Sphagnum* peat monoliths with and without the vascular plant *Eriophorum vaginatum*. The monoliths were taken from an ombrotrophic bog in Southern Sweden and transferred to a climate chamber with a diurnal dark/light regime (12 h dark at 14 °C; 12 h light at 18 °C), where fluxes of CH$_4$ and CO$_2$ were measured continuously for 60 days. *Eriophorum vaginatum* had no significant effect upon total CH$_4$ emissions, but the contribution due to ebullition (which ranged from 0.2% to 13% of the total) was lower in the presence of vascular plants. There was a clear diurnal pattern in steady CH$_4$ emission and ebullition. Steady emissions peaked 5-15 hours after the lights were switched on and air temperature was increased from 14 to 18 °C. Although total CH$_4$ emissions varied considerably among monoliths, the difference between rates in the dark and light was rather constant (around 20 µg CH$_4$/h), and probably reflected diurnal changes in temperature or in photosynthetic activity. We conclude that CH$_4$ emission through ebullition may be important in *Sphagnum*-dominated peat bogs, but is negligible in bogs with vascular plants such as *E. vaginatum*. 
Chapter II

The role of temperature and photosynthetic activity by mosses and vascular plants for \( \text{CH}_4 \) emission from peat requires further study.

2.2 Introduction

Methane (\( \text{CH}_4 \)) is responsible for almost 20% of the radiative forcing from long-lived and globally mixed greenhouse gases (IPCC 2007). The most important natural sources for \( \text{CH}_4 \) are wetlands, which contribute an estimated 70% of natural \( \text{CH}_4 \) emissions and one third of total emissions (IPCC 2007). By influencing the production, consumption and transport of \( \text{CH}_4 \) in the soil, plants can have a strong effect upon the emission of \( \text{CH}_4 \) from wetland ecosystems (Christensen et al. 2003; Joabsson et al. 1999; Saarnio et al. 2004; Ström et al. 2003; Whiting and Chanton 1992). In some cases, as much as 90-98% of the \( \text{CH}_4 \) efflux from inundated sites has been associated with vascular plants (Joabsson et al. 1999; Verville et al. 1998). In a previous experiment we observed that some vascular plant species - particularly those adapted to unproductive habitats - increased \( \text{CH}_4 \) emission, while others did not (Koelbener et al. 2010).

There are three main pathways by which methane can reach the atmosphere - by diffusion, by conductive flow through vascular plants, and through ebullition (i.e. small bubbles emitted from the soil) (Baird et al. 2004; Whalen 2005). The proportion of total \( \text{CH}_4 \) emissions due to ebullition varies greatly among studies, from 1-50% in subarctic tundra (Christensen et al. 2003; Martens et al. 1992; Minkkinen et al. 1997) to 45-85% in the Amazon flood plain and tropical swamps (Bartlett et al. 1988; Devol et al. 1988; Happell and Chanton 1993; Wassmann et al. 1992). This variation partly depends on environmental conditions such as temperature, water table and changes in atmospheric pressure (Rosenberry et al. 2006; Waddington et al. 2009), and probably also on plant species composition. It has been suggested that in wetlands with a high proportion of vascular plants, particularly species with well developed aerenchyma systems, more \( \text{CH}_4 \) is conducted internally through plants (Brix et al. 2001; Sheppard et al. 2007; Sorrell and Boon 1992; 1994).
However, whether this plant effect significantly alters CH$_4$ emissions due to ebullition is unknown.

CH$_4$ emissions from wetlands follow seasonal and diurnal patterns. In temperate regions, CH$_4$ emission is generally higher in summer than in winter, as a consequence of seasonal differences in temperature, water table, radiation and vegetation development (Kim et al. 1999; Koch et al. 2007; Le Mer and Roger 2001; Shannon and White 1994). There are also diurnal patterns in methane emissions from wetlands, which can be attributed to changes in sediment or air temperature (Huang et al. 2005; Mikkela et al. 1995; Zhu et al. 2007) and light intensity (Brix et al. 2001; Chanton et al. 1993; Whiting and Chanton 1996). Various studies have recorded a peak in CH$_4$ emissions around mid-day or early afternoon, when temperature and radiation are highest (Altor and Mitsch 2006; Kim et al. 1999; Koch et al. 2007; Zhu et al. 2007). Mikkelä et al (1995) observed a time lag between the maximum temperature and the daily peak of CH$_4$ emissions, which they partly explained by photosynthetic activity of plants followed by root exudation of carbon compounds. Chanton et al (1993) observed that the diurnal rhythm of CH$_4$ emissions varied among sites with different vascular plant species; thus, sites with two Typha species had a peak in the morning and sites with Cladium jamaicense showed generally lower CH$_4$ emission rates and no diurnal pattern. The existence of a diurnal rhythm could reflect either higher temperatures during the day - causing a shift from diffusive to pressure-driven methane flow (Brix et al. 1996; Chanton and Whiting 1996; Van der Nat and Middelburg 1998) - or CH$_4$ production coupled to photosynthesis (Brix et al. 2001; Chanton et al. 1995; Chanton et al. 1993; Minoda and Kimura 1994). However, from the data available it is not possible to distinguish between these mechanisms.

The aim of this study was to investigate CH$_4$ emissions and diurnal emission patterns from Sphagnum peat with and without vascular plants. Also, we aimed with quantifying the contribution of ebullition to the total CH$_4$ emission, and how this was affected by the presence of vascular plants. Previous studies have shown that CH$_4$ emission rates are higher in the presence of Eriophorum
vaginatum compared to Sphagnum peat without vascular plants (Frenzel and Rudolph 1998; Greenup et al. 2000). We hypothesised that:

I.) The total emission of CH$_4$ from peat is higher from Sphagnum peat with a high cover of Eriophorum vaginatum than from peat without this vascular plant (or with only a low cover).

II.) The relative contribution of ebullition to the total CH$_4$ emission is negatively correlated with the cover of Eriophorum vaginatum.

III.) Both total CH$_4$ emission and the contribution of ebullition are higher during day than during night.

2.3 Materials and methods

The study was carried out with six undisturbed peat monoliths taken from an ombrotrophic peat-forming bog in southern Sweden (56°16’ N, 13°33’ E). The same Sphagnum species - mainly Sphagnum magellanicum and Sphagnum capillifolium subsp. Rubellum - were present in all monoliths. However, three of the monoliths had a high cover (>90% cover) of Eriophorum vaginatum L. (referred to as ‘Eriophorum monoliths’), while the other three contained at most only a few tillers of this species (< 1% cover; referred to as ‘Sphagnum monoliths’). None of the monoliths contained any other vascular plants. The monoliths were collected at the start of the growing season (16 March 2007). Aluminium frames (length: 25 cm; width: 25 cm; depth: 40 cm) were inserted into the ground. Surrounding peat was removed and the frames containing the undisturbed monoliths were carefully lifted. The monoliths were then transferred into aluminium containers, with the same measures as frames, and filled with bog water.

Immediately after collection, the samples were transported to a climate room in Lund, Sweden, with a 12 h light/dark rhythm (light intensity PAR 1297 ± 18 µmol m$^{-2}$ s$^{-1}$; photosynthetically active radiation). The air temperature was 18 °C during the light period and 14 °C during the dark period, while the soil was kept at 14 °C using a cooling bath. These temperatures correspond
to the mean values measured at the field site in summer 2006. Throughout
the experiment, the water table was kept 4 cm below the surface by adding
distilled water as necessary.

To monitor CO$_2$ and CH$_4$ fluxes, transparent Plexiglas chambers were
fixed over the aluminium containers, producing a hermetic seal. The setup
was successfully used in previous experiments (Christensen et al. 2003; Ström
et al. 2003; Ström et al. 2005). The chambers had a volume of 13 litres and
were flushed with ambient air flowing at 1 litre min$^{-1}$. Water humidity was
kept constant by bubbling the influx air through water. Flowstat, rotameters
and solenoid valves controlled the airflow. Ventilators were installed in the
chambers in order to continuously mix the air.

Flux measurements of CO$_2$ and CH$_4$ started after an acclimation period
of four weeks in the growth chamber, and lasted for 60 days. A EGM-4 (PP
Systems, UK) gas analyser and a DLT-200 Fast Methane Analyzer (Los Gatos
Research, USA) operated continuously and measured CO$_2$ and CH$_4$
concentrations, respectively, of the input and output air. We used a computer for
data logging and for controlling the solenoid valves (Christensen et al. 2003).
The continuously measured fluxes represented average emissions over a period
of about one hour, this being the turnover time of air in the chambers (air flux
1 litre min$^{-1}$). From these data, we calculated total emissions of CH$_4$ from the
flux data over 60 days.

When CH$_4$ was released as a bubble this was visible in the CH$_4$-flux data
by an instant increase of the CH$_4$ flux followed by a steep decrease to previous
fluxes within the turnover-time of the chambers (1 hour). In order to identify
those peaks, we calculated a baseline drift for each of the six monoliths based
on the respective emission rate data. This baseline drift represents the con-
tinuous CH$_4$ flux of a monolith, which shifted strongly during the experiment.
The baseline was determined by an adapted median filter method used for
chromatography (Moore and Jorgenson 1993). The bubbles were then visually
determined based on the calculated baseline (Appendix A) and the amount of
CH$_4$ emitted as bubbles was calculated.
Figure 2.1: Total CH$_4$ emission of the monoliths with Eriophorum and monoliths with Sphagnum, over 60 days (A), CH$_4$ emitted through ebullition in total (B) and during light and dark periods (C), and amount of CH$_4$ per bubble in the Eriophorum and Sphagnum monoliths (D). Standard errors in A - C are calculated from three replicates. Significant differences are indicated with asterisks (*P<0.05; ** P < 0.01; ns: not significant). Note that the unit in D is different than the unit in A, B and C.

We calculated the night-time net ecosystem exchange (NEE-dark) or night-time respiration from the CO$_2$ flux measurements during the dark periods. The data for the first hour after the lights were switched off were not used, since it took the monoliths about an hour to adjust to the sudden change of light conditions. From CO$_2$ uptake fluxes during the light periods we calculated day-time net ecosystem exchange (NEE-light). For the same reason as for NEE-dark, we omitted the data for the first hour after the lights were turned on. Total net ecosystem exchange (NEE-24h) was calculated from the difference between NEE-light and NEE-dark.

The results were analysed by the means of ANOVA and repeated measures ANOVA. All analyses were performed using the statistical software R, version 2.6.2 (R-Development-Core-Team 2008).

2.4 Results

Total CH$_4$ emission and CO$_2$ fluxes

Methane emission varied considerably among monoliths, and also over time (Table 2.1). All monoliths except monolith F, showed a linear increase in methane emission during the first 20 days of measurements. After this period of adaptation, all emission rates were either steady or only increased slightly until
Table 2.1: Fluxes of CH$_4$ and CO$_2$ under light and dark conditions during 60 days and the total number of CH$_4$ number of bubbles in the same period of time is shown. CO$_2$ fluxes are divided in Net Ecosystem Exchange (NEE) during light and dark periods. Negative values mean, that input of CO$_2$ into the system was greater than CO$_2$ output.

<table>
<thead>
<tr>
<th></th>
<th>Total CH$_4$ emission [mg CH$_4$]</th>
<th>Total number of CH$_4$ bubbles</th>
<th>CO$_2$ fluxes [g CO$_2$]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>light</td>
<td>dark</td>
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<tr>
<td><strong>Eriophorum:</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Monolith A</td>
<td>197</td>
<td>193</td>
<td>19</td>
</tr>
<tr>
<td>Monolith B</td>
<td>153</td>
<td>159</td>
<td>36</td>
</tr>
<tr>
<td>Monolith C</td>
<td>321</td>
<td>313</td>
<td>92</td>
</tr>
<tr>
<td><strong>Sphagnum:</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Monolith D</td>
<td>143</td>
<td>135</td>
<td>165</td>
</tr>
<tr>
<td>Monolith E</td>
<td>223</td>
<td>205</td>
<td>215</td>
</tr>
<tr>
<td>Monolith F</td>
<td>31</td>
<td>23</td>
<td>101</td>
</tr>
</tbody>
</table>

The end of the experiment (Appendix B). Monolith F showed a constant, low emission rate during the whole experiment. The *Eriophorum* monoliths tended to have slightly higher total CH$_4$ emissions than the *Sphagnum* monoliths, but this difference was not significant (Fig. 2.1A).

All six monoliths showed a positive, estimated net ecosystem production during the complete experimental period (NEE during light periods was more negative than respiration during dark periods; Table 2.1). Net ecosystem carbon balance, which allows for both NEE and CH$_4$ emissions, did not differ significantly between *Eriophorum* and *Sphagnum* monoliths, although the dark respiration of CO$_2$ was significantly higher in *Eriophorum* than *Sphagnum* monoliths. CO$_2$ fixation usually reached a maximum during the first three hours of the light period, after which it decreased somewhat and then stabilised. There was a similar temporal pattern in dark respiration rates, which peaked two to three hours after the light was switched off and then declined.
CH$_4$ emission rates were closely correlated with NEE-light ($R^2=0.79$) and less so with NEE-dark ($R^2=0.41$) rates (Fig. 2.2). The total CH$_4$ emitted amounted to 3% ± 0.5 of NEE.

**CH$_4$ ebullition**

Ebullition accounted for <0.2% of total CH$_4$ emissions in *Eriophorum* monoliths, but 2-13% in *Sphagnum* monoliths (Table 2.1). On average, three times as many bubbles and 13 times as much CH$_4$ were released from *Sphagnum* monoliths bubbles as from *Eriophorum* monoliths (Fig. 2.1B). These differences between the two types of monoliths were significant. In addition, the mean amount of CH$_4$ released per bubble was significantly ($F=38.0$, $P<0.01$) larger in *Sphagnum* than in *Eriophorum* monoliths (Fig. 2.1D).

**Diel patterns of CH$_4$ emission**

There was a clear ‘diurnal’ rhythm in CH$_4$ emission. (Fig. 2.3), with all monoliths showing increasing emissions after the lights were turned on, and most monoliths showed decreasing emissions after the lights were switched off. *Eriophorum* monolith B showed a lag of about three hours before CH$_4$ emission...
rates decreased. At the same time this monolith showed a time lag of 3 hours after the lights were turned on, before CH$_4$ emission rates started to increase (Fig. 2.3B). *Sphagnum* monolith F showed a peak in CH$_4$ emissions after 6 hours of light. Switching off the light stabilised the emission rates and after 6 hours of darkness, emission rates decreased to a minimum (Fig. 2.3F). With the exception of one monolith, the total amount of CH$_4$ emitted during the light period was significantly (p < 0.05) higher than that emitted during the dark period (Table 2.1, Fig. 2.3B). The absolute differences of maximal and minimal CH$_4$ emission rates were quite constant in all of the six monoliths. The differences were 20-30 µg/h independent of the general level of CH$_4$ emission of a particular monolith (varying from 25 to 440 µg/h).

Both the number of bubbles (F=7.63; P<0.05) and the amount of CH$_4$ emitted in this way (F=5.96; P<0.05) were significantly higher in the light than in the dark; in all monoliths these values were up to 6-11 times higher in the light (Fig. 2.1C, Table 2.1). Overall, our data suggest that ebullition was only a significant pathway for methane in the absence of vascular plants, and then only in the light.

### 2.5 Discussion

Field studies have shown that more CH$_4$ is emitted from wetlands with *Eriophorum vaginatum* than from *Sphagnum*. Possible reasons are that plants of *E. vaginatum* serve as a conduit for CH$_4$ (Frenzel and Rudolph 1998; Greenup et al. 2000), supply C substrates to methanogens (Saarnio et al. 2004), or have a very low potential to rhizospheric CH$_4$ oxidation (Frenzel and Rudolph 1998). However, we did not detect a significant difference in CH$_4$ emission between monoliths with *Sphagnum* only and those with a high cover of *Eriophorum vaginatum*, and hence our first hypothesis was not supported. One possible explanation is that the water table was kept 4 cm below the soil surface, which could have favoured CH$_4$ oxidation (possibly enhanced by methanotrophic symbionts in *Sphagnum* (Raghoebarsing et al. 2005). If so, it suggests that the conduit effect of the *Eriophorum* plants did not enhance methane
Figure 2.3: ‘Diurnal’ patterns of CH$_4$ emissions in monoliths with Eriophorum (A-C) and with Sphagnum (D-F). Each point shows the CH$_4$ emission during a particular one-hour interval of the day, averaged for the 60 days of the experiment. The left half of the x-axis shows emission rates during the light, the right half of the axis the emission during the dark period. Standard errors of the means are represented by the dotted lines. The black lines represent the total sum of bubbles that occurred during the same one-hour intervals. Notice the varying scales of the y-axes among the plots.

emissions from the deeper soil. This conclusion is supported by the results of another experiment in which we applied $^{14}$C labelled acetate to monoliths under the same experimental conditions; in that case, there were no significant
differences in $^{14}$CH$_4$ emissions between the two kinds of monolith (see chapter 3 of this thesis). Although vascular plants have been shown to increase methane emission from wetlands in many studies (Koelbener et al. 2010; Ström et al. 2005), there are also examples in which they did not (Koelbener et al. 2010). Hence, this effect partly depends on specific plant, soil and other physical properties (e.g. temperature or light intensity).

We observed clear differences in both NEE and CH$_4$ emissions between light and dark periods (Fig. 2.2). The negative NEE under light conditions was to be expected and reflected photosynthetic CO$_2$ fixation by Eriophorum and Sphagnum plants. The positive correlation between net CO$_2$ fixation and CH$_4$ emission (Fig 2.2A) agrees with previous findings that increasing photosynthetic activity may lead to higher rhizospheric release of C and more carbon substrate for methanogenesis cf. (Joabsson and Christensen 2001) and the positive correlation between NEE (dark) and CH$_4$ emission (Fig 2.2B) is consistent with results of Greenup et al. (2000). However, NEE (light), NEE (dark) as well as the total NEE during the experiment did not significantly differ between Sphagnum and Eriophorum monoliths (Table 2.1). This may indicate that Eriophorum plants did not increase C substrate in the soil compared to the Sphagnum monoliths, and hence explains why Eriophorum plants did not significantly increase CH$_4$ emissions due to the mechanism of increased C substrate for methanogenesis.

CH$_4$ emission rates were lowest at the end of the dark period, and peaked between 5-15 hours after the light was switched on and temperature was increased from 14 to 18 °C. Although total CH$_4$ emission rates differed greatly among the monoliths, the difference in CH$_4$ emission between the ‘day’ and the ‘night’ conditions were rather constant (around 20 µg CH$_4$/h) (Fig. 2.3). This constant difference may reflect photosynthetic activity. In relative terms this attributed between 7-75% to the total CH$_4$ emission from the soil. Another explanation could be, that difference in CH$_4$-emission was caused by temperature difference in the soil. Unfortunately soil temperature was not measured during the experiment, though air temperatures in the climate chamber increased from 14 °C during the dark period to 18 °C in the light period. However, if
the observed diurnal variation were a temperature effect only, we would expect the degree of in/decrease to be in proportion to the total CH$_4$ emission rate of each monolith - rather than a constant difference of about 20 µg CH$_4$/h for all monoliths. We therefore think that at least part of the daily variation was caused by photosynthetic activity. Labelling experiments have shown that, according to vegetation type, it takes between 2 and 24 hours until fixed CO$_2$-C can be detected as CH$_4$-C (King and Reeburgh 2002; King et al. 2002). In our experiment, however, CH$_4$ emission rates increased 1-2 hours after the lights were turned on, suggesting that additional carbon substrates became available rather quickly. The increase in CH$_4$ emission rates tended to be slower and less steep in *Eriophorum* than in *Sphagnum* monoliths (Fig. 2.3), perhaps because recently fixed carbon is released more slowly from vascular plants than from bryophytes.

There were interesting differences between monoliths in the time-course for CH$_4$ emissions. One *Sphagnum* monolith (Fig. 2.3F) was exceptional in showing a peak in emissions 6 hours after the lights were turned on. In field studies, peaks in CH$_4$ emission after sunrise, mostly in the presence of vascular plants with large aerenchyma systems, have been attributed to pressurised convective flow (Chanton et al. 1993). However, in this particular monolith the soil temperature was kept as constant as possible and there were no vascular plants that could have served as conduits. Therefore, pressurized convective flow seems improbable, though we have no alternative explanation. In another monolith, *Eriophorum* monolith B (Fig. 2.3B), maximum of CH$_4$ emission rates were reached 3 hours after the lights were turned off, after which emission rates continued to decline until 3 hours after turning on the lights. In this case, it seems the transfer of recently fixed carbon from the plants into the soil, and perhaps also methanogenesis, took place rather slowly. These two cases suggest that methane emissions from peat soil are likely to vary greatly among sites.

Our hypothesis that methane emission through ebullition is less important in the presence of a higher plant was supported. CH$_4$ emission through bubbles was 13 times higher in *Sphagnum* than in *Eriophorum* monoliths (Fig. 2.1). The release of CH$_4$ as bubbles depends on the absence of vascular plants: with
vascular plants CH$_4$ emission might take place mainly through conductive flow through the plants, decreasing the importance of ebullition and diffusion (Brix et al. 2001; Rosenberry et al. 2006; Sheppard et al. 2007; Sorrell and Boon 1994). In our _Eriophorum_ monoliths, the cover of _Eriophorum vaginatum_ was more than 90%, hence there were many _Eriophorum_ tillers available to serve as conduits for CH$_4$ emission. In monoliths where only _Sphagnum_ was present root structures were absent and CH$_4$ bubbles could be formed and escape as such. The contribution of ebullition to CH$_4$ emission varies largely and ranges from 1-50% in subarctic tundra (Christensen et al. 2003; Martens et al. 1992; Minkkinen et al. 1997). In our study the overall importance of bubbling was smaller than expected. Ebullition of CH$_4$ was low compared to the total emissions (< 0.2% in _Eriophorum_; 2-13% in _Sphagnum_).

The CH$_4$ emission through ebullition also followed a distinct ‘diurnal’ pattern, being significantly higher in the light than in the dark, as postulated in our third hypothesis. As far as we know, this is the first study to show this pattern under controlled conditions. Just as for total CH$_4$ emissions, this diurnal variation in ebullition could be due to either change in soil temperature, to photosynthetic activity, or to both factors. Higher temperatures increase CH$_4$ emissions (Koch et al. 2007) but on the other hand, the increasing CO$_2$ fixation increases the C substrate availability for methanogenesis in the rhizosphere. If the CH$_4$ is not released through convective flow in the presence of vascular plants the formation and release of bubbles will also increase.

This study provides insight in the mechanisms by which plants influence CH$_4$ formation and emission, albeit under artificial conditions. In the field light intensity would be much higher, and there would be diurnal variation of radiation and temperature, and seasonally changing water tables; hence many factors complicate extrapolating our results to field conditions. We conclude that the large variability in the relative importance of temperature and photosynthesis to CH$_4$ formation (between 7 and 75%) in our experiment pleas for further studying this importance at both controlled and field conditions. We also conclude that the mechanism of CH$_4$ emission through ebullition might be of some importance (up to 13% according to our results) in areas without
vascular plants, such as bogs or tundra, but is of minor importance in wetlands covered with vascular plants.

2.6 Literature cited


King J Y and Reeburgh W S 2002 A pulse-labeling experiment to determine the contribution of recent plant photosynthates to net methane emission in arctic wet sedge tundra. Soil Biology & Biochemistry 34, 173-180.


2.7 Appendices

Appendix A:
Gas ebullition (GE) can be defined as the positive deviation from the ’steady CH$_4$ emission’ (SE). Hence, total CH$_4$ emission (TE) is the sum of SE and GE. We determined a baseline of the emission data (Appendix B) by the mean of an adapted median filter method used for chromatography (Moore and Jorgenson 1993). This baseline we defined as the average SE.

We determined bubbles by eye in the continuous emission data. The occurrence of a bubble could be observed by an instant increase of the CH$_4$ emission followed by a steep decrease back to steady emission rates. We searched the emission data of all six monoliths for this typical pattern and determined the number of bubbles and noted the time, when they were released. The amount of CH$_4$ emitted as bubbles was then calculated by the difference of TE and average SE at the determined points in time. The approach allowed counting the bubbles and attributing them to an exact time during the light/dark periods.
Appendix B: CH$_4$ emission rates from monoliths with *Eriophorum* (A-C) and monoliths with *Sphagnum* (D-F) during 60 days. The first measurement (day zero) took place 31 days after the monoliths were collected from the field.
Appendix C: CO₂ fluxes from monoliths with *Eriophorum* (A-C) and monoliths with *Sphagnum* (D-F) during 60 days. Negative values indicate fixation during the day, positive values show the respiration during the night. The first measurement (day zero) took place 31 days after the monoliths were collected from the field.
3 Acetate as a source for methanogenesis in 
*Sphagnum* peat in presence and absence of 
*Eriophorum vaginatum*.

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3.1 Abstract

The general significance of acetate as a source for methanogenesis in wetlands remains uncertain. Some authors have suggested that acetoclastic methanogenesis is unimportant, especially in peatlands dominated by *Sphagnum* mosses and with only a low biomass of vascular plants. To investigate this question, we recorded the fate of $^{14}$C-labelled acetate injected in peat monoliths containing either *Sphagnum* sp. alone, or both *Sphagnum* sp. and the vascular plant *Eriophorum vaginatum*. Both types of monolith were found to emit $^{14}$C-labelled methane, and emission of $^{14}$CH$_4$ and $^{14}$CO$_2$ increased with the supply of $^{14}$C-acetate. We conclude that acetate was a source for methanogenesis, regardless of whether *E. vaginatum* was present. There were no significant differences between the monolith types in CH$_4$ formation or oxidation, suggesting that under these experimental conditions *E. vaginatum* had no significant effect upon methane dynamics, either through rhizosphere oxidation or by providing a conduit for CH$_4$. However, depth profiles of acetate in the soil solution differed considerably between the two types of monolith.
3.2 Introduction

Methane (CH$_4$) is an important greenhouse gas, estimated to have contributed around 20% to the total trace gas-induced increase in atmospheric radiative forcing over the past 200 years (Bartlett and Harriss 1993; Forster et al. 2007). Of the natural sources of CH$_4$, almost 70% - representing one third of current emissions (Forster et al. 2007) - is produced by wetlands. Much of the additional CH$_4$ is produced by artificial wetlands, especially rice paddies, and domestic livestock.

Plants in wetland ecosystems can strongly influence the production, consumption and transport of CH$_4$ in the soil (Christensen et al. 2003; Joabsson et al. 1999; Saarnio et al. 2004; Ström et al. 2003; Whiting and Chanton 1992). In some cases, as much as 90-98% of the CH$_4$ efflux from inundated sites has been associated with vascular plants (Joabsson et al. 1999; Verville et al. 1998). Three main mechanisms are known by which vascular plants influence CH$_4$ emissions from wetlands: (i) plant roots release organic compounds that are easily available C-sources for methanogenesis into the rhizosphere (Ström et al. 2003); (ii) CH$_4$ moves through the aerenchyma tissue of the roots, rhizomes and dead or living stems to the atmosphere, thereby escaping oxidation in aerobic soil layers - the conduit or chimney effect (Bellisario et al. 1999; Verville et al. 1998); (iii) plants bring oxygen into the rhizosphere, which inhibits methanogenic archaea and fuels CH$_4$ oxidation (Van der Nat and Middelburg 1998a; b).

Although the relative importance of these processes remains unclear, it is known that vascular plants release a wide range of carbon compounds into the rhizosphere, including organic acids, sugars, ectoenzymes, phenolic and amino acids (Bais et al. 2006). Amongst these compounds, acetate - which is a by-product of the fermentation of various organic compounds - is often mentioned as an important source for methanogenesis (Avery et al. 2003; Bellisario et al. 1999; Boone 1991; Ferry 1997; Ström et al. 2003; Ström et al. 2005). In a comparison of several wetland plant species (chapter I of this thesis), we observed that CH$_4$ emissions from peat cores with plants tended to be higher
for plant species with inherent high exudation rates of organic compounds than for those with low exudation rates. Moreover, \( \text{CH}_4 \) emissions were higher from peat cores containing plant species from mesotrophic habitats than from cores containing eutrophic plant species. However, some studies suggest that acetate is not a source for methanogenesis, at least in northern peatlands, but tends to accumulate until it is degraded by aerobic oxidation (Hines et al. 2001; Rooney-Varga et al. 2007). For example, Hines et al. (2008) found that acetate was an end product of anaerobic decomposition, especially if only *Sphagnum* mosses were present and vascular plants were lacking.

Our aims in this study were to investigate (i) whether acetate is a source for methanogenesis from a wetland soil in both the presence and absence of a vascular plant and, if so, (ii) whether \( \text{CH}_4 \) production increases according to the availability of acetate. To do this, we studied the fate of different quantities of \(^{14}\text{C}\)-labelled acetate injected into intact peat monoliths containing either *Sphagnum* sp. alone or both *Sphagnum* sp. and the vascular plant, *Eriophorum vaginatum* L. It has been shown that *E. vaginatum* has a low capacity for rhizospheric \( \text{CH}_4 \) oxidation (Frenzel and Rudolph 1998) but provides an effective conduit for \( \text{CH}_4 \) to escape to the atmosphere (Greenup et al. 2000). In addition, high rates of acetate formation have been measured in the vicinity of *E. vaginatum* roots (Ström et al. 2003). We hypothesised that:

I.) Acetate is a source for methanogenesis in peat soils.

II.) \( \text{CH}_4 \) production increases with the amount of acetate in the soil.

III.) \( \text{CH}_4 \) emissions are higher in the presence of *E. vaginatum* (assuming that effects of *E. vaginatum* on rhizospheric \( \text{CH}_4 \) oxidation is lower than its role as a conduit (Frenzel and Rudolph 1998).

### 3.3 Materials and methods

**Experimental setup**

The study was carried out with the same six peat monoliths and the same basic experimental set up as described in Chapter 2 (see Chapter 2 for details).
Undisturbed peat monoliths (25 x 25 cm; depth 40 cm) covered with *Sphagnum* mosses (mainly *S. magellanicum* and *S. capillifolium* subsp. *rubellum*) were collected in an ombrotrophic peat-forming bog in southern Sweden (56°16’ N, 13°33’ E), in March 2007. Three of the monoliths - the ‘*Eriophorum* monoliths’ - had a high cover (>90% cover) of *Eriophorum vaginatum*, while the other three - the ‘*Sphagnum* monoliths’ - contained at most a few tillers of this species (< 1% cover). None of the monoliths contained any other vascular plants. After collection, the monoliths were placed in a climate chamber with a 12h light/dark rhythm. The soil temperature of the monoliths was kept at 14 °C and air temperature was 18 °C during the light period and 14 °C during the dark period. During the experiment, the water table was maintained 4 cm below the surface by adding small amounts of distilled water as needed. Air humidity was also kept constant. An air-tight Plexiglas chamber was constructed on top of the monoliths, in which CO$_2$ and CH$_4$ fluxes were measured continuously.

To determine whether acetate was a substrate for CH$_4$ and CO$_2$ formation, we injected $^{14}$C-labelled acetate ($^{14}$CH$_3$COO$^-$) and measured emissions of $^{14}$CH$_4$ and $^{14}$CO$_2$. This technique has been described in detail by Ström et al. (2003) and (2005). The first labelling took place 47 and 48 days after the monoliths were brought into the growth chamber (2 and 3 May 2007). As explained below, the emission of labelled-C could only be measured in three monoliths at one time. Therefore, we labelled three randomly selected monoliths on day 47 after collection, and the other three on day 48. The second labellings were on 14 and 15 May, respectively, and the last on 2 and 3 June. On each occasion, we added 100 ml of $^{14}$C-acetate solution consisting of [2-$^{14}$C] acetic acid Na-salt, and an unlabelled mixture of 100 µM acetic acid and 100 µM Na-acetate. The acetate concentration in the solution corresponded to the mean background concentration of acetate measured in the six monoliths. Before adding $^{14}$C-acetate, the solution was flushed with He for four hours to remove O$_2$.

The acetate solution was injected into the monoliths 20 cm below the surface through two channels closed by septa. An injection tube 22 cm long and 1.5 mm in diameter was inserted into each channel, and 2.4 ml $^{14}$C-acetate
solution was injected every 1 cm over a distance of 20 cm. The total quantities of $^{14}$C applied in the first, second and third labelling were 111 kBq, 444 kBq and 1776 kBq (acetic acid Na-salt), respectively. The mean pH values of *Eriophorum* and *Sphagnum* monoliths immediately before the first labelling were $3.80 \pm 0.01$ (standard deviation) and $3.94 \pm 0.03$ respectively.

**Measurements**

We measured the emissions of $^{14}$CH$_4$ and $^{14}$CO$_2$ in two steps (both as $^{14}$CO$_2$), using the method previously applied by (Christensen et al. 2003; Ström et al. 2003; Ström et al. 2005). First, the $^{14}$CO$_2$ of 10% of the outflow air from each monolith was held for 24 hours in a glass vessel containing 100 ml of 0.1 M NaOH solution. $^{14}$CO$_2$ was trapped as Na$_2^{14}$CO$_3$ or NaH$^{14}$CO$_3$. Previous studies with the same setup and pre-experimental tests have shown that this volume of NaOH solution is sufficient to completely trap $^{14}$CO$_2$ during the required 24 hours (Ström et al. 2003; Ström et al. 2005). After removing $^{14}$CO$_2$, the air was channelled through a furnace (820 °C) in which any $^{14}$CH$_4$ was oxidised to $^{14}$CO$_2$. This newly formed $^{14}$CO$_2$ was then trapped by passing the air through another glass vessel with 100 ml of 0.1 M NaOH. The traps were exchanged after 24 hours and immediately measured for radioactivity by liquid scintillation (Packard Tri-Carb 2100TR liquid scintillation analyser) using alkali compatible scintillation cocktail (OptiPhase, ‘HiSafe’3, Wallac). The technical setup of the furnace only allowed the monitoring of three peat monoliths at one time. Since our study included six monoliths we randomly selected two sets of three monoliths and alternately monitored the two sets. Thus each monolith was monitored for a 24-hour period every other day.

To monitor background concentrations of organic acids and the radioactivity in the soil solution, pore water samples (2 ml) were taken in the middle of the monoliths at 5, 20 and 35 cm below the surface, using pre-installed stainless steel tubes. Samples were taken before the beginning of the labelling experiment (for the first time 31 days after monolith collection), and two days after each of the three labelling events. After collection, the samples were immediately filtered with rinsed Acrodisc PF 0.8 µm/0.2 µm filters and analysed
Figure 3.1: Mean emissions of $^{14}$C in the form of $^{14}$CH$_4$ and $^{14}$CO$_2$ from three peat monoliths dominated by Eriophorum vaginatum (A) and three monoliths without vascular plants and only covered by Sphagnum sp. (B), during a period of 41 days. Injection of 111 kBq, 444 kBq and 1776 kBq labelled acetate took place on days 0, 12 and 31 (see arrows). SE of $^{14}$CH$_4$ is shown with dotted, and SE of $^{14}$CO$_2$ is shown with dashed lines.

for acetate, oxalate, formate and other organic acids with an anion exchange HPLC system equipped with a column system from Dionex, including the analytical column AS11 (4mm, P/N 044076). A more detailed description of the HPLC system and method can be found in Ström and Christensen (2007). Radioactivity of the soil solution samples was measured using the method described above.

Calculations and statistics

Each monolith was monitored every other day over an 11-day period. In order to obtain the total emissions of $^{14}$CH$_4$ and $^{14}$CO$_2$, we estimated the missing days as the means of the adjacent values. For technical reasons, the last measurement after the second labelling was delayed for eight days; as a result, the calculated quantities of $^{14}$CH$_4$ and $^{14}$CO$_2$ emitted after the second labelling are underestimated (Fig 3.1).

A labelled [2-$^{14}$C]-acetate molecule ($^{14}$CH$_3$–COO$^-$) can potentially end up in one $^{14}$CH$_4$ from the methyl group (acetoclastic reaction (Boone 1991)), and one unlabelled CH$_4$ from the carboxylic group (via H$_2$ oxidation). $^{14}$CH$_4$-oxidation was calculated as the fraction of $^{14}$CO$_2$ measured in the outflow
Figure 3.2: Mean sums of $^{14}$C-emissions ($^{14}$CH$_4$ and $^{14}$CO$_2$) during 11 days after every $^{14}$C-acetate labelling (A) and separately shown for $^{14}$CH$_4$- (B) and $^{14}$CO$_2$-emissions (C). Mean sums in B and C are plotted against the amount of $^{14}$C-acetate injected in the respective labelling event. D: Mean $^{14}$CH$_4$ oxidation to $^{14}$CO$_2$ during the whole experiment. The presented data are separated for monoliths dominated by Eriophorum vaginatum and monoliths only covered by Sphagnum sp. SE are shown for three replicates.

air compared to the total of trapped $^{14}$C, and therefore only represents the oxidation of $^{14}$CH$_4$ originating from the acetoclastic reaction. We assumed that $^{14}$C-acetate oxidation in the aerobic topsoil was negligible.

We used ANOVAs to test for differences in total emissions of $^{14}$CH$_4$ and $^{14}$CO$_2$ between the three Sphagnum and Eriophorum monoliths. Changes in background concentrations of organic acids over time and the influence of increasing $^{14}$C-acetate application on emissions of $^{14}$CH$_4$ and $^{14}$CO$_2$ were investigated using regression analysis. All analyses were performed using the statistical software R, version 2.8.0 (R-Development-Core-Team 2008).

3.4 Results

Emission of $^{14}$CH$_4$ and $^{14}$CO$_2$

In all six monoliths, $^{14}$CH$_4$ and $^{14}$CO$_2$ were detected within 24h of adding labelled acetate (Fig. 3.1) and the emissions of $^{14}$CH$_4$ and $^{14}$CO$_2$ on average had declined to lower levels by the time the next dose of labelled acetate was injected. These results clearly indicate that the labelled acetate was a source for methanogenesis, both in the presence and absence of E. vaginatum.
Table 3.1: Mean (± SE) measured radioactivity in Bq/ml soil solution of three *Eriophorum* and three *Sphagnum* monoliths. The radioactivity of soil solution was measured in all six monoliths and at three different depths after each of the three labelling events. Labelled acetate was injected at 20 cm.

<table>
<thead>
<tr>
<th>Sampling depth</th>
<th>Mean radioactivity in <em>Eriophorum</em> monoliths [Bq/ml]</th>
<th>Mean radioactivity in <em>Sphagnum</em> monoliths [Bq/ml]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>labelling: I  II  III</td>
<td>labelling: I  II  III</td>
</tr>
<tr>
<td>5 cm</td>
<td>0.0 ± 0.0  1.4 ± 0.7  4.3 ± 1.8</td>
<td>0.1 ± 0.0  0.6 ± 0.0  2.8 ± 0.1</td>
</tr>
<tr>
<td>20cm</td>
<td>0.2 ± 0.1  3.7 ± 0.9  32 ± 16</td>
<td>4.2 ± 1.6  13 ± 5.2  35 ± 20</td>
</tr>
<tr>
<td>35cm</td>
<td>0.0 ± 0.0  0.1 ± 0.1  0.5 ± 0.3</td>
<td>0.0 ± 0.0  0.1 ± 0.1  0.9 ± 0.3</td>
</tr>
</tbody>
</table>

Each fourfold increase of injected $^{14}$C-acetate resulted in a proportional increase in the total quantities of $^{14}$CH$_4$ and $^{14}$CO$_2$ trapped during the following 11 days (Fig. 3.2A/B/C). Thus, the sum of $^{14}$C-emissions significantly increased with increasing amount of injected $^{14}$C-acetate in both the *Eriophorum* and *Sphagnum* monoliths; (*Eriophorum* monoliths: $R^2 = 0.68$, $p = 0.006$; *Sphagnum* monoliths: $R^2 = 0.72$, $p = 0.004$).

The mean total $^{14}$C recaptured ($^{14}$CH$_4$ and $^{14}$CO$_2$) at the end of the experiment was slightly higher for the *Eriophorum* than the *Sphagnum* monoliths (Fig. 3.2A), but the difference was not significant ($F_{(1,4)} = 0.17$, $p = 0.70$). Also, the mean sums of emitted $^{14}$CH$_4$- or $^{14}$CO$_2$ separately (Fig. 3.2B/C) did not significantly differ between *Eriophorum* and *Sphagnum* monoliths ($F_{(1,4)} = 0.81$, $p = 0.42$; $F_{(1,4)} = 0.63$, $p=0.47$ for CO$_2$).

There was some indication that less $^{14}$CH$_4$ was oxidized to $^{14}$CO$_2$ in *Eriophorum* monoliths than in *Sphagnum* monoliths (Fig. 3.2D), but there was large variation among replicates and this difference was not significant ($F_{(1,4)} = 0.97$; $p = 0.38$).

At the end of the experiment, an estimated average of 9% (±3% SE) of the injected radioactivity had escaped, either as $^{14}$CH$_4$ or as $^{14}$CO$_2$, and the monoliths were still emitting radioactivity (Fig. 3.1).
Organic acids and radioactivity in the soil solution

Acetate was by far the most important organic acid in the soil solution. Acetate concentrations were almost two orders of magnitude higher than those of oxalate or formate (Fig. 3.3), which were in turn higher than those of lactate, malate, tartarate, succinate and citrate (data not shown).

The vertical distributions of acetate and formate differed between the two kinds of monoliths. Multiple regression analyses showed that in *Eriophorum* monoliths, mean concentrations of acetate were highest at 5 cm below the surface and significantly lower at 20 cm and 35 cm depths, whereas in the *Sphagnum* monoliths concentrations of these organic acids were equally high at 5 and 20 cm but significantly lower at 35 cm (Fig. 3.3A/D). Furthermore, while the concentrations of these organic acids remained constant in the *Eriophorum* monoliths, they declined significantly in the *Sphagnum* monoliths. Formate showed similar concentration patterns as acetate but the concentrations were much lower (Fig. 3.3C/F). The radioactivity in the soil solution samples was highest at a depth of 20 cm, where the label was injected, and lowest at 35 cm. Levels of radioactivity increased more at 5 cm than at 35 cm but remained far below those in the middle of the monoliths (Table 3.1).

3.5 Discussion

Many authors regard acetate as an important source of CH$_4$ in peatlands (Avery et al. 2003; Bellisario et al. 1999; Ström et al. 2003; Ström et al. 2005). However, others argue that acetate accumulates in peat, and is only decomposed under aerobic conditions in the topsoil where it is consumed by oxidation (Duddleston et al. 2002; Hines et al. 2001; Rooney-Varga et al. 2007). In support of this view, Hines et al. (2008) found that acetate was an end-product of anaerobic decomposition, especially if the peat was covered by *Sphagnum* mosses and vascular plants were absent. In our experiment, however, it was very obvious that acetate was a source for CH$_4$ formation. Thus, within 24 hours of adding $^{14}$C-acetate, CH$_4$ originating from acetoclastic methanogenesis was produced from all monoliths, including those dominated
Figure 3.3: Mean background concentrations of acetate (A,D), oxalate (B,E), and formate (C,F) in the soil solution at three different depths in monoliths dominated by *Eriophorum vaginatum* (A-C) and monoliths without vascular plants and only covered by *Sphagnum* sp. (D-E). SE of three replicates is shown with dotted lines. The first measurement (day zero) took place 31 days after the monoliths were collected from the field. Notice that A and D have a different scale (mM) than the other panels (µM).
by *Sphagnum sp.* (Fig. 3.1). Our results therefore support our first hypothesis and the conclusions of the first group of studies cited above.

In our experiment, we used [2-14C]-acetate (\(^{14}\text{CH}_3\text{-COO}^-\)). Based on the position of the labelled-C within the acetate molecule, we might expect the \(^{14}\text{C}\)-acetate to be degraded to \(^{14}\text{CH}_4\) and \(\text{CO}_2\) by acetoclastic methanogenesis. By this reaction, the labelled methyl group is reduced to \(^{14}\text{CH}_4\) and the non-labelled carboxylic group is oxidised to \(\text{CO}_2\) (Boone 1991). In theory, the \(^{14}\text{CO}_2\) could be produced either through (rhizospheric) oxidation of \(^{14}\text{CH}_4\) from the acetoclastic reaction or through aerobic decomposition of \(^{14}\text{C}\)-acetate. However, the very low levels of radioactivity in the topsoil (Table 3.1) suggest that aerobic decomposition was negligible in our experiment. The data shown in Table 3.1 could result from: (i) a slow diffusion rate of \(^{14}\text{C}\)-acetate to the aerobic top soil, and hence a low amount of \(^{14}\text{C}\)-acetate directly oxidised to \(^{14}\text{CO}_2\) and \(\text{CO}_2\), or (ii) a faster transport of \(^{14}\text{C}\)-acetate to the aerobic top layer, rapid oxidation, and rapid emission of the \(^{14}\text{CO}_2\) thus produced to the air. Since increasing amounts of \(^{14}\text{C}\)-acetate led to increasing emissions of both \(^{14}\text{CH}_4\) and \(^{14}\text{CO}_2\) emissions, the first possibility seems more plausible (Fig. 3.2). Therefore we conclude, that our second hypothesis - a higher acetate supply leads to higher \(\text{CH}_4\) formation in peat with and without *E. vaginatum* - is supported by our results. Nevertheless we cannot distinguish the \(^{14}\text{CH}_4\) / \(^{14}\text{CO}_2\) acetate with the radioactivity measurements of the soil solution (Table 3.1), and thus we cannot exclude the possibility that some \(^{14}\text{C}\)-acetate was oxidised aerobically.

\(^{14}\text{CH}_4\) emissions were not significantly higher in the *Eriophorum* monoliths than in the *Sphagnum* monoliths (Fig. 3.2B), and the unlabelled \(\text{CH}_4\) emissions were also not significantly different between monolith types (see Fig. 3.1A in Chapter 2 of this thesis). Hence our third hypothesis was not supported. In contrast, Frenzel (1998) and Greenup et al. (2000) found that \(\text{CH}_4\) emissions were higher from field sites with *E. vaginatum* than from sites with only *Sphagnum*. Frenzel (1998) found no evidence that *E. vaginatum* increased rhizospheric \(\text{CH}_4\) oxidation in *Sphagnum* peat, and concluded that the higher emissions were due to *E. vaginatum* acting as a conduit. Our results
are consistent with this conclusion in that we also did not observe higher oxidation rates of $^{14}\text{CH}_4$ into $^{14}\text{CO}_2$ in monoliths with \textit{E. vaginatum} than in the \textit{Sphagnum} monoliths; indeed, although not significant, the trend was in the opposite direction (Fig. 3.2D). We note, however, that variation in our monoliths was huge, and that in an experiment similar to ours, Ström et al. (2005) recorded very high CH$_4$ oxidation (>90% of all $^{14}\text{CH}_4$ produced was oxidized) in a single monolith covered by \textit{Sphagnum} and \textit{Eriophorum vaginatum}. Overall, we consider that any differences between our study and field observations (Frenzel and Rudolph 1998; Greenup et al. 2000) in the effect of \textit{E. vaginatum} on CH$_4$ emission are more likely due to differences in the conduit effect than in CH$_4$-oxidation. Greenup et al. (2000) concluded that increased CH$_4$ emissions in the presence of \textit{E. vaginatum} under field conditions was because the plants acted as a conduit for CH$_4$ as well as producing carbon compounds. Our data therefore suggest that there was no enhanced diffusive or convective CH$_4$-flow through plants in laboratory conditions compared to the field. This could be because factors influencing this flow, such soil temperature and radiation, varied much less and changed more slowly than in the field (Mikkela et al. 1995; Whiting and Chanton 1996; Zhu et al. 2007).

Concentrations of acetate in the soil solution were far higher than those of other organic acids in all monoliths (Fig. 3.3). High concentrations of acetate in the soil, together with the finding that acetate was a substrate for methanogenesis, indicate that rate of acetoclastic methanogenesis was probably not limited by acetate availability. Furthermore, the acetate concentrations in the soil remained constant in the \textit{Eriophorum} monoliths, whereas they decreased over time in the \textit{Sphagnum} monoliths (Fig. 3.3). A stable acetate concentration could arise if there was equilibrium between acetate production (e.g. following root exudation or decomposition of organic matter) and acetate consumption by methanogens or aerobic oxidation. In contrast, a declining acetate concentration suggests that the consumption rate of acetate through methanogenesis and oxidation was higher than the supply rates of acetate and other organic acids, and therefore that the \textit{Sphagnum} monoliths had not reached equilibrium. At the start of the measurements (31 days after monolith collection),
acetate concentrations in the 5 and 20 cm depths were higher in Sphagnum monoliths than in the Eriophorum monoliths (Fig. 3.3). These initially high concentrations could reflect a relatively high acetate production after collection of the monoliths from the field (e.g. decomposition of cut organic material), or a relatively low acetate production during the incubation period. However, we cannot explain why either of these processes would have affected only the Sphagnum monoliths.

The concentrations of acetate and formate at 20 cm depths were lower in Eriophorum than in Sphagnum monoliths (Fig 3.3). Ström et al. (2005) also measured a depth profile of acetate concentrations in soil solution for E. vaginatum in a very similar setup. They found the same pattern of high concentrations in the top 5 cm and much lower concentrations in deeper layers. In contrast to Sphagnum, E. vaginatum builds roots reaching down into the monoliths. Plant roots have an important influence on the composition of the microbial community in the soil, for example by exuding organic compounds (Bais et al. 2006; Westover et al. 1997; Zak et al. 2003). Since the recalcitrant content of peat increases with depth, dissolved organic compounds (e.g. recently fixed carbon released by plant roots) may be the only substrate for fermenting microbes (Bais et al. 2006; Christensen et al. 1999). Our pattern of lower acid concentrations at 20 cm in Eriophorum monoliths (Fig. 3.3) therefore perhaps reflect differences in the vertical distribution of the microbial community in the soil and the consumption of organic acids by these microbes in the rhizosphere of E. vaginatum; however, this needs to be verified by further studies.

In conclusion, this study has shown that acetate is a source for methanogenesis, both in presence and absence of the vascular plant E. vaginatum. However, in contrast to what might have been expected from previous field studies (Frenzel and Rudolph 1998; Greenup et al. 2000), E. vaginatum did not significantly influence CH$_4$ dynamics in the monoliths under experimental conditions, either through rhizosphere oxidation or by enhancing CH$_4$ flow-through. However, there were interesting differences in the distribution of acetate in the Sphagnum and Eriophorum monoliths. Further studies about
the differences between the field and controlled conditions are required to understand how vascular plants interact with the methanogenic and the methanotrophic communities in the soil. Understanding these interactions will be crucial for predicting how changes in wetland communities, for example due to plant invasions or eutrophication, affect greenhouse gas emissions and global warming.

3.6 Literature cited


Van der Nat F and Middelburg J J 1998b Seasonal variation in methane oxidation by the rhizosphere of Phragmites australis and Scirpus lacustris. Aquatic Botany 61, 95-110.


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