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THE ROLE OF *SPHAGNUM* MOSSES IN METHANE OXIDATION IN A TEMPERATE FEN

by

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ABSTRACT

Peatlands are a global source of atmospheric methane (CH_4); however oxidation of CH_4 by methanotrophic microbes residing in the moss layer provides the potential for mitigation of CH_4 emissions (Bubier and Moore, 1994). Determining the controls on CH_4 oxidation in *Sphagnum* mosses will increase our understanding of CH_4 dynamics in wetlands, and will allow a better understanding of the influence of climate change on these ecosystems. Studies have shown that the primary controls of oxidation are environmental, i.e. water table and temperature; however little is known about the role of moss species in controlling CH_4 oxidation (Basiliko et al., 2004; Larmola et al., 2010).

A *Sphagnum* transplantation experiment was conducted at Sallie's Fen, a temperate peatland in Barrington, New Hampshire, to observe the effect of environmental conditions on CH_4 oxidation rates in *Sphagnum fallax* and *Sphagnum magellanicum* and to investigate the relative importance of species versus environment in controlling CH_4 oxidation. The mosses were sampled four times over a 28 day period. Triplicate control and transplanted samples were incubated in the lab at two concentrations of CH_4, 1000 parts per million (ppm; limiting to low affinity methanotrophs) and 10,000 ppm (non-limiting). Averages of the potential CH_4 oxidation of all four sampling days show that when CH_4 is limiting there is an interaction between *Sphagnum* species and the environment. The effect of environment on the microbes of *S. magellanicum* was evident when host rates of potential CH_4 oxidation (0.4 μmol g dw^{-1} d^{-1} s.e. 0.10) in the hummock increased significantly ($p < 0.05$) upon transplantation to the hollow (1.8 μmol g dw^{-1} d^{-1} s.e. 0.38). The *Sphagnum* species effect is supported by the response of *S. fallax*, which, after transplantation, exhibited potential CH_4 oxidation rates that were not significantly different ($p > 0.05$) from the host rates (1.5 μmol g dw^{-1} d^{-1} s.e. 0.20) in the hollow. There was no difference between the sites or species when CH_4 was not limiting ($p > 0.05$). These results indicate that there is a joint control of the environment and *Sphagnum* species on CH_4 oxidation; however the particularly dry summer may have confounded the results.

INTRODUCTION

Peatlands

Peatlands are characterized by the presence of peat, partially decayed organic matter that is conserved by a combination of permanent water saturation, low oxygen levels, and high levels of acidity. These terrestrial wetland ecosystems store large amounts of carbon (C) due to the imbalance between C sequestration and decomposition (Weider and Vitt, 2006).

An ecosystem is defined as a peatland once it contains 30–40 cm of peat (Rydin and Jeglum, 2006). The surface of most peatlands has a characteristic microtopography. Hollows and flarks represent depressions in the peat surface where the water table can be consistently above the peat surface. On the other hand, hummocks and strings represent raised areas where the water table fluctuates to varying degrees depending on seasonal precipitation and the degree of snow melt in the spring (Malmer et al., 1994). The reasons that this microtopography exists are unknown; however it is thought that they form either due to preexisting variations in topography, or from differences in the growth of *Sphagnum* species and the growth of vascular species (Malmer et al., 1994).

Peatlands can broadly be classified into bogs or fens (Lai, 2009) and are more specifically categorized by their nutrient levels (Blodau, 2002). The differences between bogs and fens are due to chemical, climatic, nutrient and vegetation differences. Bogs are ombrotrophic, meaning that the only input of water into the ecosystem is from the atmosphere. As a result bogs tend to be

nutrient poor in comparison to fens, which can receive nutrients from ground and surface water as well as from the atmosphere. Depending on the source, fens range from mineral poor to mineral rich environments. Fens often have diverse vegetation consisting of shrubs, graminoids, herbs and mosses, while bogs are often primarily characterized by large expanses of *Sphagnum* moss, and have less diverse shrub, graminoid, and herb communities (Crum, 1992).

Carbon Cycling in Peatlands

Peatlands hold approximately 30% or an estimated 400-600 Pg of the world's soil C, but represent only about 3% of the world's terrestrial land area (Blodau, 2002; Gorham, 1991; Tarnocai et al. 2009). Boreal and sub-arctic peatlands represent about 75-80% of peat worldwide (Frolking et al. 2010). Northern peatlands are long-term sinks for atmospheric carbon dioxide (CO_2) and sources of atmospheric methane (CH_4) (Blodau, 2002). As a result, northern peatland ecosystems have received much of the attention of climate scientists. Most peat has accumulated within the past 10,000-20,000 years, and peat accumulation today is much slower than it used to be (Yu et al. 2010). When undisturbed, today's peatlands are weak sinks of C (Frolking et al., 2010).

Photosynthesis by plants during the growing season is the process that is responsible for capturing C, in the form of CO_2, from the atmosphere. Carbon that is photosynthesized by peatland plants is used to build plant structures, such as stems and leaves, including belowground structures such as roots where a large portion of plant biomass is located (Vasander, 1982). Net primary productivity

(NPP) is a term that refers to the net amount of C captured through photosynthesis. Estimated through direct biomass measurements or as net ecosystem exchange, aboveground net primary productivity (NPP) in northern peatlands ranges from about 5 g m^{-2} a^{-1} dry weight to more than 1000 g m^{-2} a^{-1} (Blodua, 2002). Net primary productivity in northern peatlands is lower than in many other ecosystems (Frolking et al.,1998) but is similar to the NPP of boreal forests (Blodau, 2002). These values, however, do not account for belowground production, which is cited as a significant portion of vascular plant production. However, belowground estimates are harder to estimate and therefore have been studied to a lesser degree (Szumigalski and Bayley, 1996). The NPP in northern peatlands is mainly controlled by the level of photosynthetic radiation (PAR), water table position, and the availability of nitrogen and phosphorus (Blodau, 2002).

Net primary productivity is only one part of the C cycling story of peatlands. Net ecosystem exchange (NEE) is a term that refers to the flux of CO_2 from peatlands. This flux is represented by the balance between production and C mineralization. Carbon mineralization therefore represents the other part of the C cycling story. Carbon mineralization refers to the processes that are responsible for the release of C from peatlands into the atmosphere. These processes depend on the availability of oxygen, the microbial activity in the peat, the soil temperature, the type of vegetation, and the chemical characteristics of the peat (Blodau, 2002). Autotrophic and heterotrophic respiration is responsible for

returning the C that is photosynthesized by peatland plants into CO_2. Decomposition of litter primarily by bacteria in the aerobic layers also leads to the release of CO_2 (Clymo 1984; 1992). In the water-saturated anaerobic part of the peat, decomposition is slow, and a large portion of the total mineralized C is released to the atmosphere as CH_4. Carbon also flows in and out of the peatland in the form of dissolved organic C (DOC) (Vasander, 1982; Moore, 2003). As peatlands have very high C densities, the DOC output from them usually exceeds the DOC input with water inflow (Wieder and Vitt, 2006).

Present NEE measurements indicate that at present peatlands can be both sources and sinks of CO_2 to the atmosphere with large interannual variability (Gorham 1991; Roulet et al., 2007). The C balance in peatlands is controlled by the effect of environmental variables on NEE (i.e. on C mineralization and NPP). Climate change today poses a threat to peatland ecosystems. Higher temperatures and changes in hydrology and vegetation could shift rates of C mineralization and plant production, potentially leading to increases in the release of C from peatlands.

Sphagnum

Sphagnum mosses are the dominant moss species in many peatland ecosystems and play a big role in C cycling. *Sphagna* are responsible for producing the wet, anoxic, acidic, and nutrient poor environment found in peatlands and as a result act as the primary ecosystem engineers in peatlands.

Sphagna are remarkable for their high water holding capacity. This ability is due to their hyaline cells, which seem to exist solely for the function of storing water. *Sphagna* lack rhizoids and internal water conducting tissue, absorbing water through capillary action (Clymo and Hayward, 1982, Malmer et al., 1994). According to van Breemen et al. (1995), around 80% of the plant's volume is these hyaline cells, a statistic that points to their high water retention.

Sphagnum mosses also have a high cation exchange capacity, a feature that lends to their ability to create an acidic environment. As a result, *Sphagnum* mosses are excellent competitors, as a limited number of vascular species can survive in the harsh environment they create. In addition, these mosses effectively utilize nutrients from the atmosphere, further competing with vascular plants for this limited resource. This is because the capitulum, or head of the *Sphagnum* plant, absorbs nutrients at the peat surface before they reach the lower root systems of other plant species. Mosses grow upward, die gradually, and regulate the vertical growth rate of peatlands (Malmer et al., 1994). *Sphagnum* mosses are excellent peat forming plants, because they decompose at very slow rates (Wieder and Vitt, 2006). According to Clymo and Hayward (1982), over half of the world's peat was once *Sphagnum.*

Many *Sphagnum* species are associated with characteristic micro-habitats within wetlands, which suggests that some species are better competitors than others, and that each species may have adapted to the specific environmental conditions of these habitats. Some species are restricted to pools or hollows while

others, such as *S. papillosum,* are often found in lawns or hummocks, not far above the water table (Hayward and Clymo, 1982).

Methane Emissions from Peatlands

Peatlands are a global source of atmospheric CH_4. With a warming capacity twenty times that of CO_2, CH_4 is one of the most potent greenhouse gases (*IPCC*, 2007). Concentrations of CH_4 have more than doubled since pre-industrial times, with a current globally-averaged mixing ratio of around 1750 ppb. The rise in atmospheric CH_4 concentration between 1750 and 1990 has been attributed to the dramatic increase in anthropogenic emissions during that period (Frolking et al., 2010). During the 1980s and 1990s, though, the rate of CH_4 increase slowed steadily by an average of 1 ppb/year/year (Wuebbles and Hayhoe, 2002). Although the reasons for this change remain unknown, CH_4 sources and sinks must play a role (Simpson et al., 2002; Frolking et al., 2010). Understanding the role of wetlands in this change is extremely important, as these ecosystems contribute over 70% of the CH_4 emitted from both natural and anthropogenic sources globally (IPCC, 2007).

Methane fluxes range from slight uptake to emissions of more than 1000 mg m^{-2} d^{-1} (Blodau, 2002). Fluxes are temporally and spatially highly variable (Moore et al., 1998). Fens generally are stronger emitters than bogs because the anaerobic zone is on average closer to the peatland surface (Moore et al. 1990). The CH_4 budget in peatlands is primarily controlled by rates of CH_4 production

and consumption as well as rates of CH_4 transport (Gorham, 1991; Lai, 2009). The environment plays a big role in these processes. The water table position, peat temperature, and substrate quality, microtopography and vegetation distribution are all factors that affect CH_4 flux dynamics in peatlands (Bubier et al., 1995; Bellisario et al., 1999)

Methane Production

Methane is produced at depth in the anaerobic zone (catotelm) of the peat by methanogens (CH_4 producing archaea), that degrade organic matter (Svensson and Sundh, 1992). Methanogenic bacteria can use a limited number of substrates, with acetate and hydrogen the most important ones in fresh water systems (Peters and Conrad 1996; Yavitt and Lang 1990; Segers 1998). CH_4 production is enhanced by the availability of these substrates in combination with an increase in temperature (Blodau, 2002). Maximal CH_4 production has been observed at about 20 cm below the water table (Sundh et al., 1994).

Methane Transport

Methane is liberated from peat via three routes: diffusion, ebullition, and plant mediated transport (Gorham, 1991; Lai, 2009) (Appendix 1). Diffusion, accounts for the smallest portion of emitted CH_4 (Schlesinger, 1997). The literature suggests that the diffusion rate of CH_4 from the peat is controlled in

large part by the gradient of CH_4 in the peat and atmospheric temperature, which often results in a diurnal pattern of emission (Lai, 2009).

Ebullition, in contrast, accounts for a large part of the CH_4 budget (Chanton, 2005). Due to very high rates of methanogenesis, supersaturation of CH_4 can be found in the pore water of deep anaerobic peat layers. Methane gas bubbles form when the partial pressure of all dissolved gases in solution is greater than the hydrostatic pressure in peat (Chanton et al., 1995). After the bubbles have accumulated, they are released. This process is often triggered by a drop in atmospheric pressure, a reduction in hydrostatic pressure, or a rise in temperature (Lai, 2009).

Finally, some vascular plants in peatlands develop an internal gas-space ventilation system called aerenchyma to provide oxygen for submerged plant parts. Some plants, like *Phragmites* and *Typha*, show active gas transport based on pressure differences, while others, like *Carex* spp., have only passive diffusion (Wieder and Vitt, 2006). These pathways allow efficient and direct transport of CH_4 to the atmosphere, bypassing oxic zones in the soil where CH_4 can potentially be oxidized (Christensen et al., 2003; Ström et al., 2005). However, in some vascular species such as *Carex*, the presence of aerenchyma within vascular plants also increases the transfer of oxygen into the soil, which can lead to CH_4 oxidation in the rhizosphere (Frenzel and Rudolph, 1998; Popp et al., 2000; Whalen, 2005). As discussed by Frenzel and Rudolph (1998), vegetation also influences the quality of substrate that is available for oxidation (Ström et al.,

2005). Temperature has been shown to have an effect on the within-plant diffusion rate (Thomas et al., 1996).

Methane Oxidation

Though peatlands are a global source of atmospheric CH_4, up to 90% of the CH_4 produced in peat can be consumed by methanotrophs in the soil (Bubier and Moore, 1994; Whalen, 2005). Therefore these microbes play a huge role in mitigating CH_4 inputs to the atmosphere (Raghoebarsing et al., 2005; Lai, 2009).

Methane is primarily oxidized in the oxic top layer or in the oxic rhizosphere (acrotelm) where oxygen is more readily available to aerobic methanotrophic bacteria that consume or oxidize CH_4 (King, 1996). Oxidation rates depend on CH_4 and oxygen availability, which are connected to peat moisture conditions, temperature, and the activity of CH_4 oxidizing bacteria in the peat (Wieder and Vitt, 2006).

Methanotrophic bacteria oxidize CH_4 sequentially to methanol, formaldehyde, formate and eventually CO_2 (Whalen, 2005). All methanotrophs possess the enzyme CH_4 monooxygenase (MMO), either in soluble cytoplasmic or particulate membrane-bound form, which catalyses the bacterial CH_4 oxidation pathway (Lai, 2009). The literature recognizes two types of methanotrophic activity: high affinity that occurs at low atmospheric CH_4 concentrations and low affinity that occurs at high CH_4 concentrations (Segers, 1998; Lai 2009). According to Segers (1998), when soil CH_4 concentrations are in the range of high affinity CH_4 oxidation, CH_4 emission can only be relatively small for

wetlands. Therefore, in wetlands, CH_4 oxidation is believed to be primarily carried out by low-affinity CH_4 oxidizers.

Environmental Controls of CH_4 Oxidation

The water table is one of the primary controls in CH_4 cycling, as it defines the zones of production and consumption in peatlands (Sundh et al., 1995; Whalen, 2005). Methane oxidation relies on the availability of oxygen. In turn, it also relies on the availability of CH_4. The zone of optimal oxidation therefore corresponds to the boundary between aerobic and anaerobic zones where the ratio of CH_4 to oxygen is optimal (Dedysh, 2002). As demonstrated by Basiliko et al. (2004) and Larmola et al. (2010), the relationship between water level and potential oxidation is represented by a bell shaped curve due to this combined control of CH_4 and oxygen supply. Spatially, the zone of optimal CH_4 oxidation lies within 25cm of the water table, primarily in the upper layers of the soil (Segers, 1998). Methane oxidation can occur in peat soils that were previously anaerobic, suggesting that the responsible microbes are relatively resilient to anaerobic conditions (Moore et al. 1994).

The individuals responsible for oxidizing CH_4 are methanotrophs or CH_4 oxidizing bacteria. These methanotrophs are active in a specific temperature range (Sundh et al., 1995). Kip et al., (2010) demonstrated that potential oxidation rates in *Sphagnum* were highest at 20 °C, much lower at 10 °C and undetectable at 4 °C with one exception. The temperature control has been suggested to be less

important for CH_4 oxidation than for CH_4 production (Dunfield et al. 1993; Segers 1998). Peat acidity can potentially limit methanotrophic activities; the growth of methanotrophs is favored at acidic pH ranging from 4.3 to 5.9 (Lai, 2009).

Methanotrophs have a high potential to oxidize CH_4 that diffuses out of the peat, as the CH_4 travels up through the soil contacting the oxidation zone (Bubier and Moore, 1994; Lai, 2009). In peatland sites where the water table is close to the surface, the moss layer is potentially the only aerobic region where CH_4 can be consumed before escaping to the atmosphere (Basiliko et al., 2004). CH_4 oxidation rates are also controlled by the biomass of methanotrophs in these moss layers (Sundh et al., 1995).

The Role of Sphagnum Mosses in CH_4 Oxidation

Recent investigations of CH_4 flux in peatlands indicate that *Sphagnum* mosses may play a role in controlling CH_4 oxidation. Studies have shown differences in CH_4 flux from sedge-dominated areas versus *Sphagnum* dominated areas (Parmentier et al., 2010), indicating that these microenvironments could be responsible for either the facilitation or mitigation of CH_4 emissions respectively. Studies have further shown that submerged mosses facilitate high levels of oxidation in comparison to un-submerged mosses (Basiliko et al., 2004; Larmola et al., 2010; Kip et al., 2010). Kip et al. (2010) attribute this finding to methanotrophs' stable position along the CH_4 gradient in the water column and

from the oxygen supply by the mosses. However, with respect to the role of *Sphagnum* in CH_4 oxidation, several questions remain unanswered.

What is the relationship between Sphagnum and methanotrophs?

Sphagnum mosses in particular may host a large community of methanotrophs. The large hyaline cells within *Sphagnum* moss can serve as ideal microenvironments for these bacteria (Raghoebarsing et al., 2005). *Sphagnum* hyaline cells have a higher water holding capacity than other mosses. This feature may lend to the ability of *Sphagnum* to host a larger biomass of methanotrophs (Basiliko et al., 2004). However, there have been few studies testing this hypothesis. In a study by Raghoebarsing et al. (2005) on *Sphagnum* mosses, it was found that methanotrophs in *S. cuspidatum* actively grow in the hyaline cells. They define the symbiotic relationship between methanotrophs and *Sphagnum* as "loose" due to the ability of methanotrophs to move from plant to plant through the porous cell walls of hyaline cells. The results of Kettunen et al. (1999) indicate that methanotrophs are attached to peat particles and are not transported by vertical water movements. However, recent investigations by Kip et al. (2010) suggest that there may not be a large amount of free-living methanotrophs due to the fact that oxidation was not observed in peat water samples. However, the contribution of symbiotic versus free-living methanotrophs to CH_4 oxidation in peatlands is uncertain (Chen and Murrell, 2010). Mosses exhibiting low oxidation levels became active after being watered with peat water samples from

a high oxidation environment, suggesting that methanotrophs may be primarily free-living (Larmola et al., 2010).

Despite the debate about the strength of the *Sphagnum*-methanotroph relationship, some level of symbiosis between them is relatively clear. *Sphagnum* provides oxygen, through photosynthesis, for the methanotrophs to utilize in their consumption of CH_4. Once the CH_4 is consumed, the final byproduct, CO_2 ,becomes available to the moss to utilize. This cycle could explain the high C sequestration of *Sphagnum* (Raghoebarsing et al., 2005). However, there is much more information that can be gained about the *Sphagnum*-methanotroph relationship.

Do Sphagnum species vary in their ability to control CH_4 oxidation?

Basiliko et al. (2004) investigated the role of *Sphagnum* mosses in CH_4 oxidation; *S. capillifolium* and *S. magellanicum* were examined under similar environmental conditions. They found that the CH_4 oxidation potential of *S. capillifolium* was greater than that of *S. magellanicum*, indicating that to some degree species may play a role in oxidation. In response to this study's findings Larmola et al. (2010) tested the hypothesis by conducting a transplantation experiment of *Sphagnum* mosses. They found no differences between species *S. rubellum* and *S. balticum* when transplanted in a common hollow. In addition, after one month, all transplants moved from inactive (potential oxidation rates not different from 0) to active sites showed rates of potential oxidation that did not

differ from the host species. These results do not support a species effect, but rather indicate that the environment is the major control in CH_4 oxidation. Therefore, the ability of certain *Sphagnum* species to significantly control CH_4 oxidation remains unknown due to limited study as well as these conflicting results by Basiliko et al. (2004) and Larmola et al. (2010).

Purposes of this study

This study is an extension of the study done by Larmola et al. (2010). The purpose of this companion study is to further investigate the relative importance of species versus the environment in controlling potential CH_4 oxidation in a temperate fen. In order to separate the effect of species and the environment on CH_4 oxidation rates, I conducted a reciprocal transplantation of inactive and active moss patches of *S. fallax* and *S. magellanicum* with the technique used by Larmola et al. (2010).

I hypothesized that the water table would be the major control of CH_4 oxidation and that species would represent some, but a much smaller effect on CH_4 oxidation. I also hypothesized that CH_4 oxidation would vary over the experiment due to the seasonal variability of the environment. I also hypothesized that *S. magellanicum* transplant rates of CH_4 oxidation would gradually increase on each sampling day, to levels similar to host site's *S. fallax* controls due to the favorable conditions found there. I hypothesized the opposite for *S. fallax* species, such that their rates would gradually decrease over the

sampling time due to the unfavorable conditions in the new site. I hypothesized that there would be a different response between the two concentrations of CH_4 that were used.

In regards to the relationship between CH_4 oxidation and environmental variables, I hypothesized that I would see a negative relationship between potential CH_4 oxidation and average depth to the water table, and a positive relationship between potential CH_4 oxidation and 10cm peat temperatures, pore water CH_4, and moss water content separately.

METHODS AND MATERIALS

Site Description

Sallie's Fen is a 1.7 ha poor fen located in Barrington, New Hampshire (43°12.5' N, 71°03.5' W). The fen is minerotrophic and receives water from runoff, rainfall, and a small ephemeral stream, which runs along the north edge. The average annual temperature from 1951 to 1980 was 8.1°C, and average annual precipitation is 1100 mm (Frolking and Crill, 1994). The average growing season temperature is 17.1°C (Treat et al., 2007). The vegetation in the fen is diverse, dominated by *Sphagnum* mosses including *S. fallax* and *S. magellanicum*. The vascular species are represented by both ericaceous and deciduous shrubs. Sedges such as *Carex rostrata* are relatively abundant. The dominant tree species found along the boundaries of the fen is Red maple (*Acer rubrum*)

Summary of Experimental Design

In order to separate the effect of species and the environment on CH_4 oxidation rates, I conducted a reciprocal transplantation of inactive and active moss patches of *Sphagnum fallax* and *Sphagnum magellanicum* at Sallie's Fen, Barrington, NH (Appendix 2). These two species represent a large percentage of the moss cover in Sallie's Fen. *S. fallax* dominates wet, microenvironments, where the peat is floating and usually does not experience large fluctuations in the water table position. These topographic features are hereafter called hollows. In contrast, *S. magellanicum* dominates hummock environments, which are

characterized by a water table that can fluctuate to a large degree depending on precipitation patterns throughout a season (Appendix 3). Experimentation began by getting base oxidation rates of the two chosen sites by completing flask incubations. The reciprocal transplantation was then conducted. The samples were transplanted by hand and placed at least 10 cm apart and at the same level as host moss (Appendix 4). A total of 24 samples of each species were transplanted, 12 of which were replanted within the site (controls) and 12 of which were moved to the other site (transplants).

The sites were sampled 4 times during a 28-day period. Four-day incubations and flux calculations using gas chromatography were performed to calculate the oxidation potential at two concentrations. Temperature, water table, pore-water profiles, and moss water content were measured for each site.

Field Measurements

On the 17th of June 2010, I collected moss samples of *S. magellanicum* and *S. majus*, the dominant species in the two test sites, the hummock and the hollow respectively. Those samples were tested in the lab for potential oxidation of CH_4. *S. magellanicum* proved to be inactive yielding an average of -4.8 μmol g dw^{-1} d^{-1} s.e. 0.06 and 8.08 μmol g dw^{-1} d^{-1} s.e. 7.48 at low and high concentrations of CH_4 additions respectively. In comparison, *S. majus* rates averaged 6.67 μmol g dw^{-1} d^{-1} s.e. 0.37 and 51.5 μmol g dw^{-1} d^{-1} s.e. 12.18 at the low and high concentrations (Figure 1). Statistical analyses of these preliminary

data reveal that there was an effect of both site and concentration on potential oxidation as well as an interaction between site and concentration (Table 1). Further analyses indicate that these results were due to a significant difference between the average rates in the hollow (high concentration) and all other site and concentration averages (Hummock-low, Hollow-low, Hummock-high) (Table 2, Figure 1).

After obtaining the base rates, a reciprocal transplantation followed. I decided to change one of the species to be transplanted. That is, instead of using *S. majus* which was measured in the pretrial, I transplanted *S. fallax*. Closer inspection of the site showed that *S. fallax* was more dominant than *S. majus* and would allow me to have my desired sample size. A previous study conducted in 2009 at Sallie's Fen (T. Larmola, unpublished results) found no significant difference between the rates of CH_4 oxidation of *S. majus* and *S. fallax*. Therefore, I felt that the switch was justified. On June 25th a total of 48 moss samples were transplanted, 24 in each site. Each site was divided into three sub-sites which each held 8 samples. Half of the moss samples from the hummock species, *S. magellanicum* were transplanted in the hollow site and the other half were transplanted within the hummock site acting as controls for that site. The same procedure was done for the hollow site, where half of the *S. fallax* moss samples were transplanted into the hummock site and the remaining samples were transplanted within site.

Transplantation

At each site I removed 24 samples of the host moss *Sphagnum* species by hand and labeled them with string as either transplant or control. The string was tied loosely to ensure that there would be little to no disturbance to the mosses. The samples were carefully placed in cups to avoid contamination. The spaces (holes) that these mosses left within each site would hold the transplanted mosses from the opposite site. Twelve controls of each species were immediately re-transplanted within each site and were planted at least 10 cm apart in 3 sub-sites (Appendix 4). It was ensured that all of the moss samples were placed at the same level as the original site level. The *S. magellanicum* samples were transplanted to the hollow site. The *S. fallax* samples were transplanted to the hummock site.

Sampling

The mosses were sampled on day 3, 7, 14, and 28 after transplantation. On each sampling day a total of 12 moss samples were collected, 6 from each site, 3 transplants and 3 controls. The samples were collected and put in sealed bags ensuring no contamination. The 10 cm peat temperature, water table position and pore water concentration of CH_4 were collected on each sampling day. The mean water table position was obtained in the hummock site using 3 previously installed wells. In the hollow site the water level was close enough to the surface to be measured by hand. Water samples for pore water were collected at 10 cm intervals starting from the peat surface to 60 cm deep. However, the pore water

data are relatively incomplete, due in part to the low water table position in the hummock, as well as difficulties retrieving (pulling) water samples from the deeper depths.

Lab Measurements

CH_4 Oxidation Potential

The techniques used to measure CH_4 oxidation potential were modeled from Larmola et al. 2010 and are described in detail below.

a. Moss Rinsing

A clean workspace was prepared with all materials needed to rinse the mosses. The top 10 cm of each moss sample was cut with scissors to be used for testing and the remaining moss was discarded. Before sorting, approximately 10 grams of the fresh mass of each moss (4-5 strands) were taken out of the sample in order to measure the moss water content of each sample. The fresh weight was taken immediately and the dry weight was taken after 24 hours of being in the oven at about 15.5 °C.

The mosses were sorted to isolate the target species; the litter and roots of other plants were removed and discarded. The mosses were dipped (1-4 strands) in a cup with di/Millipore (nanopure) water using tweezers. After rinsing, the mosses weights were recorded. Once rinsed, the mosses remained in the fridge overnight, losing water in the process. This change in weight was taken into account when considering how much moss to put in jars. On average there was

about a 60% water loss. Each of the 12 moss samples was divided into two in order to test oxidation at two concentrations of CH_4.

The workstation, as well as all tools used in the rinsing process, was cleaned to prevent cross contamination of the mosses or transference of the microbes. After being refrigerated overnight, the mosses were properly prepared to begin the incubation process.

b. Spiking, Sampling, and Incubating

The incubation procedures were based on the methods of Basiliko et al. (2004) and Raghoebarsing et al. (2005). The 473.17 (1pint) ml jars used for the incubation were checked for leaks by sealing them and then submerging them in a tub of water and ensuring that there was not a steady flow of bubbles coming from the lids. I put at least 25g of moss in each jar and closed the jars tightly by hand. The jars were then spiked with CH_4. Half of the jars were spiked with 5 ml of CH_4 100% (10,000 ppm) for the high concentration and half of that (1,000 ppm) was used for the lower concentration. Two concentrations were chosen in order to understand the response of methanotrophs to non-limiting (high) and limiting (low) levels of CH_4. It must be noted that on day 3 of experimentation, the high concentration samples were accidentally spiked with 20,000 instead of 10,000 ppm of CH_4. The impact of this error on the data analysis is discussed later. Compensation air was also added to all the jars in order to raise the pressure in the jars and as a result ease the sampling process.

The jars were incubated in a dark fridge at 15° C and were sampled 6 times over a period of 3 days, at 1, 7, 24, 31, 48 and 72 hours. To sample, the jars were flushed two times with about 10 ml of headspace air (take air in, push back, repeat) to ensure that the headspace air was well mixed. 12 ml samples were taken out in order to have 2 replicates to analyze on a gas chromatograph equipped with a flame ionization detector (GC-FID, Shimadzu GA-14A) within one hour. The average concentration of the two samples was used for the final calculations. For the first sampling after spiking, the concentrations of CH_4 were checked and compared and the jars were re-spiked if needed. After each incubation the volume of the headspace in the jars was determined and the mosses were dried and weighed.

CH_4 Oxidation Flux Calculations

The fluxes of potential CH_4 oxidation were calculated as the slope of the linear regression of CH_4 concentration versus time (Appendix 5). As there was a trend of increased CH_4 concentration in the first seven hours after spiking, the first data point, taken 1 hour after spiking, was excluded from the analysis for all post-transplantation incubations. The next five points (7-72 hrs) were used to calculate the flux, that is, the rate of CH_4 consumption. Fluxes with n=4 were accepted if data were lost due to syringe leakage or malfunction of the GC-FID. No other data points or fluxes were discarded from the analysis.

Data Analysis

Apart from the preliminary potential CH_4 oxidation data, all of the post-transplantation potential CH_4 oxidation data were log-transformed and analyzed with multiple comparison tests using SPSS 16.0 (SPSS Inc.). The log of potential CH_4 oxidation was the dependent variable. Water table depth, 10 cm peat temperature, pore water CH_4 concentration, and water content were treated as independent variables.

Analysis of variance (ANOVA) was conducted to investigate the factors site, species, time, and concentration (Table 1) in a four-way factorial design. A three-way factorial design was also done with site, species, and time as the three factors. Bonferroni confidence intervals were used for post hoc follow up tests to identify significant differences ($\alpha = 0.05$) between the transplants and controls of each species at each concentration (Table 3 and 5). Regression analyses were conducted between the dependent and independent variables except pore water CH_4 concentration at $\alpha = 0.05$.

RESULTS

Average Potential CH_4 Oxidation

Analysis of the potential CH_4 oxidation data revealed that a transformation may be necessary; the distribution of the data did not fit the assumption of normality required by the ANOVA analysis. The log-transformation improved the behavior of the data and therefore I used these transformed data for my final analysis. However, the transformation was not successful in completely normalizing the data within the ANOVA requirements. The results therefore need to be taken with caution.

The 4-Way ANOVA results show that there is a significant effect of concentration, site, species, and time, all four factors investigated on potential CH_4 oxidation ($p<0.05$) (Table 3). There is also a significant interaction between site and time, as well as an interaction between time and concentration ($p<0.05$). These results support the hypothesis that there will be a difference between the potential CH_4 oxidation rates of the low and high concentrations. The species effect confirms the hypothesis that *S. magellanicum* and *S. fallax* will have significantly different potential CH_4 oxidation rates. Similarly, the site effect supports the hypothesis that the hummock and hollow will have different potential CH_4 oxidation rates. As expected, these results also show that there is a significant difference between the potential CH_4 oxidation rates of the four sampling days. Evidence of the concentration effect suggests that the low and high concentration results should be treated as separate data sets. It is possible

that the difference in concentration is driving the other differences found in this ANOVA analysis. Therefore, the rest of the analyses were done for each concentration separately.

Low Concentration

The results of the ANOVA for the low concentration potential CH_4 oxidation rates (Table 4) indicate that there is an effect of species, site, and time on potential CH_4 oxidation ($p<0.05$). There is also an interaction between species and site ($p<0.05$) demonstrating that each species responded differently at each site. Further analysis revealed that on average, *S. magellanicum* control rates (hummock) were significantly lower than the *S. magellanicum* transplant rates (hollow), the *S. fallax* controls (hollow), and *S. fallax* transplants (hummock) ($p<0.05$) (Table 5, Figure 2). That is, on average, the *S. magellanicum* transplant rates increased to a level similar to the hollow rates but *S. fallax* transplants did not decrease to the level of hummock rates.

High Concentration

The results of the ANOVA for the high concentration potential CH_4 oxidation rates (Table 6) indicate that there is only an effect of time as well as an interaction between site and time ($p<0.05$). Further analysis confirms that there was no difference between the controls and transplants of either species (Table 7, Figure 3). The differences that may be driving the time effect and the interaction between site and time are shown below.

Potential CH_4 Oxidation by Sampling Day

Low Concentration

As expected, the potential CH_4 oxidation results for the controls of both species remained relatively constant (Figure 4). The rates for *S. magellanicum* transplants on each day fluctuated and therefore did not gradually increase as expected. Overall, the *S. magellanicum* transplants had an increasing trend over time, increasing from day 3 to 28 but decreasing from day 7 to 1. Though I expected *S. fallax* transplants to decrease over time, the rates fluctuate from day to day. Potential CH_4 oxidation for *S. fallax* transplants increased from day 3 to 28 overall, but decreased from day 7 to 14.

High Concentration

At the high concentration, the controls for both species seemed to follow the general trend of the transplants (Figure 5). The magnitude of change over time for these controls seemed to be smaller than that of the transplants, however it is not clear whether the controls remained constant over the experimentation. Overall, the controls of both species had a decreasing trend over time.

The *S. magellanicum* transplants had a decreasing trend from day 3 to 28 but experienced a slight increase from day 7 to 14. These results therefore do not support the hypothesis that there will be a gradual increase over time for *S.*

magellanicum transplants. The transplants experience the biggest decrease out of all the sampling days from day 3 to 7.

The *S. fallax* transplants behaved similarly to the *S. magellanicum* transplants, but the decreasing trend for this species was expected. The decreasing trends at the high concentration may have to do with the environmental conditions, however it has to be noted that the methodological error on day 3 may have a big effect on the interpretation of what happened over time. Day 3 samples were spiked with 20,000 ppm of CH_4 instead of 10,000 ppm, therefore it is possible that because of this error the potential CH_4 oxidation rates over time may not be accurately reflected. Excluding day 3, the decreasing trend is not as strong for either species.

Environmental Variables

Temporal Patterns

The average depth to the water table experienced over the 28 day sampling period at the hummock site was 27 cm. In contrast the average depth to the water table during this time at the hollow site was 4 cm. The depth to the water table ranged from 23 to 31 cm at the hummock site and from 2 to 6 cm at the hollow site (Figure 6). Over the 28-day sampling period the water table at the hummock site increased from day 3 to day 14 and then did not change on day 28. At the hollow site day 3 was lower the other sampling days.

The 10 cm peat temperature was measured at each sub-site of the hummock and hollow sites. The results show that, for both sites, the temperature ranged from 15.0 °C to 22.5 ° C with an average of 19.8 °C. This range is also the range of the temperatures experienced at the hummock site, which had an average of 19.8 °C. The hollow site experienced temperatures from 17.1 to 22.3 °C with an average of 19.8 °C (Figure 7). I expected the hollow site to have cooler 10 cm peat temperatures than the hummock site, since the hollow site had an average water table position of 3.79 cm, above the temperature measurement zone. However, the 10cm peat temperature data varied between days and sites. Peat temperature at this depth decreased from day 3 to day 7, from about 22 °C to about 16 °C, and increased to about 21 °C on day 14 (Figure 7).

According to the meteorological station at Sallie's Fen, the depth to the water table increased in Sallie's Fen as the 20 cm peat temperature increased (Figure 8). These data more accurately represent conditions at the hummock site because the meteorological station is in closer proximity and has a comparatively raised peat surface.

The moss water content ranged from 0 to 176 mass of water per dry mass of moss (calculated as ratio). The average water content of the mosses at the hummock site (transplants and controls) was 6.5, drier than the mosses at the hollow site, which averaged 34.6 (Figure 9). On average, *S. fallax* had significantly higher water content values than *S. magellanicum* (Table 11).

The results for the pore water CH_4 concentrations are only from the 30cm depth because this depth represented the most complete data set. This is due to the fact that the dry summer conditions especially in the hummock site restricted sample collection to the lower levels of the peat (30 cm and deeper). The pore water concentration of CH_4 at 30cm depth remained around 200,000 nM on day 3, 14, and 28, but increased to almost 900,000 on day 7 (Figure 10).

Potential CH_4 Oxidation Versus Environmental Variables

Low and High Concentrations

The relationships between potential CH_4 oxidation and three of the environmental variables (water table position, 10 cm peat temperature, and water content) were examined (Figures 11-16, Tables 8-10). No relationship was found between potential CH_4 oxidation and water table in either site at the low concentration (Figure 11, Tables 8a-b); these trends were positive contrary to what was expected. There was also no relationship between the two variables in the hollow site at the high concentration; the trend of the data was negative as expected. A significant negative relationship was found in the hummock site at the high concentration supporting my hypothesis about this relationship ($p<0.05$) (Figure 12, Tables 8c-d).

No relationships between potential CH_4 oxidation and 10 cm peat temperature were found in either site or concentration (Figure 13-14, Tables 9a-d). Contrary to what was expected, at the low concentration, the hummock and

hollow yielded negative trends. However, at the high concentration the data yielded positive trends as expected.

A significant positive relationship was found between potential CH_4 oxidation and moss water content in the hummock at the low concentration, supporting my hypothesis (Figure 15, Table 10a). No other significant trends were found at the site or concentration, however, all the trends between the variables were positive as expected (Figure 15-16, Table 10b-d).

DISCUSSION

Summary

The investigation of the relative importance of species versus environment in controlling CH_4 oxidation yielded interesting results. Analysis of the potential CH_4 oxidation data reveals differences in the rates at the two concentrations of CH_4. At the low concentration, there is an effect of site and *Sphagnum* moss species on rates of potential CH_4 oxidation in the transplantation experiment at Sallie's fen. Rates of potential CH_4 oxidation in *S. magellanicum* transplants significantly increased from the average level of the controls to rates that did not significantly differ from the *S. fallax* rates in that site ($p<0.05$). On the contrary, it did not seem to matter which environment *S. fallax* was in, as the rates in both sites did not significantly differ. These results indicate that there may be a joint control of species and environment on potential CH_4 oxidation. At the high concentration, no significant differences were found between the potential CH_4 oxidation of the species in either site suggesting that, regardless of the environment or *Sphagnum* species, the methanotrophic community oxidizes CH_4 at comparable rates. The relative importance of environment and *Sphagnum* species is discussed in more detail later in the discussion.

It must be noted that this investigation at Sallie's fen in 2010 occurred during an unusually hot and dry summer. Over the entire sampling period the water table at the hummock averaged 27 cm ranging from 23 to 31 cm below the peat surface, much lower than levels in the previous summer, which experienced a

high level of precipitation. At the hollow site, the water table ranged from 2 to 6 cm with an average of 4 cm. This site also experienced a water table that was much lower than previous summers, which usually experienced levels at or above the moss layer. The importance of these environmental conditions is also discussed later.

The Role of Species versus Environment

The relative importance of species versus environment in controlling CH_4 oxidation in Sallie's Fen is still unclear. I hypothesized that the environment would be the major control on the rates of potential CH_4 oxidation, but also that the transplantation experiment may reveal that *Sphagnum* species may play a role. The experimental design used in this study presented the opportunity to investigate the effect of transplanting inactive moss samples into sites with high rates of oxidation as well as transplanting active moss samples into sites with little to no methanotroph activity.

Evidence for an environmental control of CH_4 oxidation comes from the response of *S. magellanicum* to the transplantation. On average the rates of potential CH_4 oxidation of this species were not significantly different from the host moss species *S. fallax* in the hollow site (Figure 2). My results over time indicate that the magnitude of potential CH_4 oxidation for controls and transplants in the hollow were similar (overlapping). The controls begin lower on day 3 but are slightly higher on day 28 (Figure 3) indicating that the new environment facilitated higher oxidation rates almost immediately, with no lag time in the

response. These results suggest that the environment is the major control of CH_4 oxidation in this situation. Possible mechanisms for this response are discussed later on, as my study was not able to directly identify them.

While the environment seems to be the major control in the inactive to active portion, the results for active to inactive could suggest that species may be playing a bigger role than the environment. Does *S. fallax* play a significant role in controlling CH_4 oxidation? Or does the environment once again explain the results found? After transplantation, the low concentration data show that rates in *S. fallax* controls did not differ from *S. fallax* transplants. The results of potential CH_4 oxidation over time also show that there was a difference in magnitude of rates for controls and transplants in the hummock site, such that the *S. magellanicum* controls are consistently lower (Figure 4). I hypothesized that once these transplants were put in an environment that did not favor high methanotrophy, the rates of potential CH_4 oxidation would decrease. However, though a decrease took place, *S. fallax* transplants rates on average were not significantly different from controls in the host site (hollow).

At the high concentration, my results show that, regardless of *Sphagnum* species and site, the rates of potential CH_4 oxidation are similar (Figure 3). That is, though *S. magellanicum* transplants were oxidizing at rates similar to *S. fallax* controls in the hollow, these rates were not different from base rates (*S. magellanicum* controls) in the hummock. This could suggest that the CH_4 concentration was at such a high level that the methanotrophs in both species

oxidized at a high capacity regardless of environment. In comparison to other peatland CH_4 oxidation studies these results are particularly interesting. CH_4 oxidation studies in peatlands have by and large measured CH_4 oxidation at a similar high concentration of CH_4, yielding differences at least between hummocks and hollows (Basiliko et al., 2004; Raghoebarsing et al., 2005; Kip et al., 2010; Larmola et al., 2010). This study was modeled after the study by Larmola et al. (2010), and therefore a comparison of the results is relevant. In light of all the similarities in the experimental design of the studies, it seems that the most likely explanation lies with the differences between the study sites, and possibly due to the fact that there may be different methanotroph communities involved in the two studies.

The results at both concentrations provide interesting answers to the main study question and allow for ample discussion of the mechanisms that could be taking place. At the low concentration, the results indicate that there is a joint control of the environment and *Sphagnum* species, as indicated by the distinct behavior of the two species after transplantation. There was no difference between the rates of potential oxidation at the high concentration.

Possible Mechanisms

As stated above, of particular importance to this study is the study by Larmola et al. (2010) where the authors conducted a transplantation of *Sphagnum* in order to investigate CH_4 oxidation and separate the effect of species from environment in a Finnish site. Larmola et al. (2010) transplanted three species of

Sphagnum from low methanotrophic activity, dry, hummock environments to a high activity, wet, hollow environment. They measured potential CH_4 oxidation at one concentration of CH_4, 10,000 ppm, where CH_4 is non-limiting. They found that after one month the transplants began oxidizing at rates not significantly different from the host moss species, as I saw for *S. magellanicum* transplants at the low concentration.

Larmola et al. (2010) took the investigation one step further by studying the methanotrophic communities in their samples. After the month long experimentation, the inactive transplants had a DNA sequence that was not previously found, either because it was not present or because it was below the detection limit. They also found that before transplantation the inactive and active samples had another methanotroph sequence in common. These results point out that I must consider that the methanotroph populations in the hummock and hollow of my study may be fundamentally different, therefore responding differently to the transplantation and yielding different rates of CH_4 oxidation. However, it is also possible that the methanotrophs that were present in inactive moss samples before transplantation became activated once transplanted.

The results of Larmola et al. (2010) also suggest that methanotrophs have a loose symbiosis with *Sphagnum* and are able to move from plant to plant through the water column. Larmola et al. (2010) proposed that methanotroph communities may have a relatively loose association with the mosses. The strongest argument can be observed in unpublished investigations where they

found that inactive *Sphagnum* samples that were watered with unfiltered mire water from an active site became active. In comparison, inactive mosses watered with filtered mire water from the active site did not become active. While Larmola et al. (2010) provide a strong argument other authors are otherwise convinced. Kip et al. (2010) also studied CH_4 oxidation and argued against a large amount of free-living methanotrophs. They base this claim on the fact that they saw no CH_4 oxidation in peat water samples. They concluded that methanotrophs thrive because of the presence of *Sphagnum* especially in certain environmental conditions. Raghoebarsing et al. (2005) also found no CH_4 oxidation in bog water samples, concluding that methanotrophic bacteria reside on or in the living *Sphagnum* tissue. Kip et al. (2010) provided evidence for some level of association or symbiosis with *Sphagnum*; however that level remains unclear since they do not provide any information to quantify the strength of the relationship.

Raghoebarsing et al. (2005) assert that methanotrophs have a strong relationship with *Sphagnum* based on the molecular analysis of the methanotroph community they found in *S cuspidatum*. They found clusters of "tightly bound" methanotrophs within the hyaline cells of the moss that were actively growing throughout their experiment. They do, however, indicate that the hyaline cells have pore spaces that bacteria can move in and out of. Unfortunately Raghoebarsing et al. (2005) were not able to show or quantify the amount of methanotrophic movement that occurs within the moss or within the environment.

As Larmola et al. (2010) point out, the high level of variability in CH_4 oxidation rates with seemingly optimum environmental conditions does not provide convincing evidence for a strong methanotroph-*Sphagnum* relationship. At the very least, it indicates that methanotroph activity is not predictable and possibly that there are environmental controls that scientists do not fully understand or are not yet aware of.

In light of debated relationship between methanotrophs and *Sphagnum*, it is important to further investigate the joint control found between the environment and *Sphagnum* species at the low concentration. My results at the low concentration suggest either that microbial movement took place in the *S. magellanicum* transplants or that the environment in the hollow somehow activated the mosses or microbes. That is, as was seen in Larmola et al. (2010), it is possible that high-activity hollow methanotrophs colonized the *S. magellanicum* transplants, therefore yielding rates comparable with *S. fallax* controls. This colonization would be possible if the methanotrophs are able to freely move throughout the water column, especially since the water table remained within the moss layer in the hollow. Identification of the methanotrophic communities that were studied through microbial analysis would have provided much needed insight on which, if either, of the mechanisms took place. Such an analysis would have allowed me to see whether the methanotroph communities differed in the hummock and hollow, as well as if they changed post-transplantation. These results suggest that, regardless of the type of

methanotrophic community, the capacity of the mosses to facilitate CH_4 oxidation does not differ as long as they are living in a favorable environment.

However, differences in the two *Sphagnum* species studied may be relevant when trying to understand the behavior of the *S. fallax* transplants. It is possible that the size and density of the methanotrophic populations differ, resulting from morphological differences between *S. magellanicum* and *S. fallax*. The size of the hyaline cells could indicate a larger habitat for the methanotrophs to reside in (Raghoebarsing et al., 2005). I was not able to examine the hyaline cells of either species, however, hyaline cell size of *Sphagnum* mosses has been related to the water-holding capacity in Rice et al. (2008). My results indicate that there is a significant positive relationship between potential CH_4 oxidation and moss water content in the hummock at the low concentration (Figure 15, Table 10a). That is, high levels of potential CH_4 oxidation observed in this site were found in mosses that had high water contents. These high rates were found in *S. fallax*, indicating that this species may potentially host a larger population of methanotrophs. Information on the pore size of the hyaline cells could also indicate how easily the microbes can move in and out of the cells of each species. That is, small pore sizes may reduce the movement of methanotrophs in and out of the mosses.

Another possible explanation for the behavior of *S. fallax* exists. If the methanotrophs can move freely in the water column, then mixing of the methanotrophic populations would occur in the hummock site, similar to the

movement in the hollow. However, the hot and dry summer conditions did not perceptibly allow mixing to occur in the hummock. Therefore, the methanotroph population within *S. fallax* transplants could have remained intact over the sampling period due to these conditions. In regards to the results of the high concentration, this explanation would not be relevant if the microbes respond to such high levels of CH_4.

Environmental Controls and Seasonal Patterns

I hypothesized that the environment would be a major control of potential CH_4 oxidation. I examined four environmental variables, average depth to the water table, 10 cm peat temperature, pore water concentration of CH_4, and moss water content. I specifically hypothesized that I would see a negative relationship between potential CH_4 oxidation and average depth to the water table, and a positive relationships between potential CH_4 oxidation and 10cm peat temperatures, pore water CH_4, and moss water content separately. However, the results did not consistently reveal significant relationships and the trends at times were not what I expected. The relationships also varied between the two concentrations.

The major difference observed between the two sites was the water table level, which was close to the moss surface in the hollow but much deeper in the hummock. The water table played a unique role in the potential CH_4 oxidation in the two species of *Sphagnum* moss. The results demonstrate that within hollow sites where the water table is at the moss level, methanotroph activity is much

higher than in drier hummock sites. This relationship seems counterintuitive since a deeper water level should favor CH_4 oxidation. That is, drier sites should theoretically have more oxygen for methanotrophs to consume. Many studies have approached CH_4 oxidation investigations with these expectations (Aerts and Ludwig, 1997; Bubier et al., 1995); however this has been shown to be a misconnection by the numerous studies that have found the opposite result (Kip et. al., 2010; Larmola et al., 2010). Larmola et al. (2010) found that seasonal patterns of potential CH_4 oxidation in their study were related to changes in the water table. The 10cm peat temperature was similar in the two sites but the there was, on average, a higher pore water CH_4 concentration in the hollow site at the 30cm depth. As I was unable to collect water samples from the top 20 cm of peat in the hummock site, I was unable to compare the pore water profiles in each site. The profile for the hollow site, however, has the expected average pattern of increasing concentration with increasing depth.

The dry summer conditions provided a unique opportunity to study the effect of a natural water table drawdown, particularly in the hummock site where the water table ranged remained below 25cm. On a day to day basis, the results do not show any striking links between the rates of potential CH_4 oxidation and the environmental controls. Temperature and pore water concentration seem to explain individual days better than water table. Contrary to what I expected, I observed positive (though weak) relationships in both sites between potential CH_4 oxidation and water table at the low concentration. As stated earlier, CH_4

oxidation has been shown to be highest when the water table is at the moss layer (Basiliko et al., 2004). Therefore, I expected not only that potential CH_4 oxidation would be relatively low in the hummock, but that these rates would decrease with increasing depth to the water table. It is possible that the water table was so low within the peat profile that it had no effect on the potential CH_4 oxidation rates in the top 10 cm of moss that I tested. With respect to the hollow, the depth to the water table also increased but remained within the top 10 cm of the moss. The slight change may have increased the availability of oxygen for the methanotroph population, therefore resulting in a slight positive relationship. Overall, at the high concentration, I found negative relationships between potential CH_4 oxidation and water table at both sites as I expected. I attribute the differences I found between the two concentrations to the differences in the response of the methanotroph communities to limiting and non-limiting levels of CH_4.

Jaatinen at al. (2005) had the opportunity to study CH_4 oxidation in drained and un-drained (pristine) peatlands and found that drainage reduced CH_4 oxidation at high concentration incubation methods. As stated above, I observed this negative relationship between water table and potential CH_4 oxidation in the hummock site at the high concentration. A stronger negative relationship may have resulted if Sallie's Fen had experienced longer-term drainage conditions. Yrjala et al.'s (2011) study is more a recent example that looks at peatlands of varying moisture levels and that demonstrates the same relationship. The lowest rates of both oxidation and production found were lowest in sites that had larger

water level drawdown. These studies indicate that methanotrophs exhibit high potentials in areas where more CH_4 is produced. The study by Kettunen et al. (1999) provides insights on the short-term effect of oxidation on water table manipulations in comparison to the studies by Jaatinen at al. (2005), and Yrjala et al. (2011), which looked at long-term effects of drainage. Despite the time period, these studies suggest the same results: water table draw down can result in reduced CH_4 oxidation.

Potential CH_4 Oxidation and Contribution to Emissions

The rates of CH_4 oxidation found in this study ranged from about 0 to 24 μmol g dw^{-1} d^{-1} at both the high and low concentrations, and are comparable to the rates observed in other sites around the world. Most studies have investigated CH_4 oxidation at high concentrations of CH_4 (10,000 ppm), and therefore the rates that I found at the low concentration, ranging from 0 to 5 μmol g dw^{-1} d^{-1} are less comparable. Kip et al. (2010) found an average rate as high as 80 μmol g dw^{-1} d^{-1}, in the pools of Northern Siberia and an average of about 73 μmol g dw^{-1} d^{-1}in one Argentinean pool. Otherwise, the majority of rates from their other sites (Northern and Western Siberia, Tiera del Fuego, Argentina, the Netherlands, United Kingdom, and Germany) fell between 0 and 15 μmol g dw^{-1} d^{-1}. Larmola et al. (2010) observed rates of potential CH_4 oxidation ranging from 0 to 62 μmol g dw^{-1} d^{-1} in their Finnish field site while Raghoebarsing et al. (2005) found rates ranging from 0 to around 28 μmol g dw^{-1} d^{-1}.

In comparison to the pre-transplantation rates, the post-transplantation rates have a much smaller range and magnitude at the high concentration, but a similar range at the low concentration (Figure 1, 2, and 3). Pre-transplantation, potential CH_4 oxidation rates were as high as 75 μmol g dw^{-1} d^{-1} at the high concentration (Figure 1). It may be that the transplantation had an effect on the capacity of the methanotroph population to oxidize CH_4 at such a high concentration. It is also possible that the summer conditions diminished the methanotroph activity, yet this is not clear since there was no difference in the pre and post-transplantation rates at the low concentration. Either way, it must be noted that both the pre-transplantation and the previous summer's data include different moss species than those measured in the transplantation.

It has been commonly cited that up to 90%, or even 100%, of the CH_4 that is produced in peatlands can be potentially oxidized before it is released into the atmosphere (Bubier and More, 1994; Larmola et al., 2010). CH_4 oxidation has not been studied extensively at Sallie's Fen, and therefore the contribution of this process to CH_4 emissions is unknown. Treat at al. (2007) investigated CH_4 flux at Sallie's Fen over a five year period (2000-2004) finding rates that ranged from 8.7 to 3833.1 mg CH_4 m^{-2} d^{-1} in sites that were dominated by either sedge or leather leaf species. Summer conditions during my study are most comparable to the data found in 2002 of Treat et al. (2007)'s study, when the fen experienced dry conditions. The average water table position for the season was 23.4 cm below the peat surface in 2002 in comparison to 27.36 cm for the hummock site in 2010,

and there was a similar decreasing trend in the water table over the summer seasons in both studies, due in part to low precipitation and warm temperatures. Treat et al. (2007) found that the average CH_4 flux during 2002 was 423 mg CH_4 m^{-2} d^{-1} +/- 581 s.e. At the high concentration, which represents the maximum potential of CH_4 oxidation (non-limiting), CH_4 oxidation ranged from 0 to 238 mg CH_4 m^{-2} d^{-1}. The highest potential CH_4 oxidation rates of the hummock and hollow were 209 and 238 CH_4 m^{-2} d^{-1} respectively. By comparing these CH_4 emission and oxidation results it is possible to estimate the percentage of CH_4 that could potentially be oxidized at Sallie's Fen. (CH_4 oxidized/ CH_4 oxidized + Mean net emission). It is estimated that up to 36% of the CH_4 emitted from Sallie's Fen is potentially oxidized. More specifically, up to 33% and 36 % of the CH_4 emitted is potentially oxidized at the hummock and hollow respectively. These percentages represent CH_4 oxidation in a particularly dry summer and therefore could be higher in years when the water table is closer to the moss surface and more CH_4 available to the microbes. However, Treat et al., (2007) point out that CH_4 emissions from Sallie's Fen increased over the season as temperature increased and water table decreased. The authors suggest that the emission rates are most likely due to an increase in CH_4 production and increased ebullition events, an effect of pressure changes that arise from the falling water table. They concluded that the increased rates of emissions are more than likely due to increased CH_4 production rather than decreased oxidation. This suggests

that at Sallie's Fen, CH_4 production is the dominant process driving CH_4 emissions in dry years. However, this may not be true in wetter years.

Implications for Climate Change

Shifts in Environment

Climate models indicate that northern environments are likely to experience increased temperatures and well as drier summer conditions (Freeman et al., 2002). Even with potentially more summer precipitation, peatland water could be lost from evaporation due to the warmer conditions. These conditions are likely to have an affect on the activity of the microbial communities found in peatland soils.

Climate change forces one to question how the dynamic between production and consumption may change and what that would mean in terms of the role of peatlands in the global CH_4 concentration. Since the literature asserts that methanotrophs are less sensitive to changes in temperature in comparison to methanogens it becomes clear that climate change has the potential to increase CH_4 production but possibly not change CH_4 oxidation at the same rate. Therefore, oxidation could be less efficient at mitigating CH_4 release (Moore et al. 1998). As there are many environmental controls of oxidation, it is difficult but extremely important to figure out the combined effect of these variables. As discussed earlier, a lowered water table is likely to decrease methanotroph activity (Kettunen et al., 1999; Jaatinen at al., 2005; Yrjala et al., 2010). Increased temperatures are also likely to increase the length of the growing season in

northern environments. My results indicate the importance of environment at least in terms of the comparison between the sites. Though I did not find many significant relationships between CH_4 oxidation and the environmental variables, the hummock and hollow site are markedly different in terms of their relationship between water table, temperature, and CH_4 availability.

Shifts in Species Composition

It is particularly important to understand the strength of the *Sphagnum*-methanotroph relationship as warmer temperatures have the potential to shift the species composition in wetlands. The combination of warmer temperatures and drier conditions could shift the *Sphagnum* community to species that are more adapted to a fluctuating water table. It is possible that a species such as the *S. magellanicum* studied in this experiment could have a competitive advantage. These mosses have been shown to have much lower potential CH_4 oxidation rates than submerged mosses (Larmola et al., 2010) and therefore a shift to these species is likely to have a huge impact on CH_4 emissions in peatlands. It must be kept in mind that my results did not find a direct species effect on CH_4 oxidation, therefore shifts to specific *Sphagnum* species may not be as important as environmental shifts such as the water table.

Warmer conditions also have the potential to shift peatlands species composition away from *Sphagnum* species all together, as vascular plants would thrive in these conditions, outcompeting the mosses (Gunnarsson et al., 2004; Heijmans et al., 2008). Such a shift would have huge implications for climate

change as vascular plants such as sedges have been shown to facilitate CH_4 transport (Schlesinger, 1997). In addition, shifts to vascular plant communities could significantly alter the ability of peatlands to store carbon and regulate CH_4 emissions.

Studies such as Parmentier et al. (2010) have shown the importance of *Sphagnum* dominated environments in regulating CH_4 emissions. These authors compared CH_4 fluxes from areas with and without *Sphagnum*. They found that emissions in *Sphagnum* dominated environments are significantly lower than in areas that are sedge dominated and have little *Sphagnum*. However, this is not a clear demonstration of the importance of *Sphagnum* in the regulation process, as sedge environments tend to have high emissions due to their ability to transport CH_4 through aerenchyma. The importance of *Sphagnum* is more clearly demonstrated by comparing the different environments that these mosses inhabit. This study, as well as the study by Larmola et al. (2010) has compared specific *Sphagnum* species and their capacity to facilitate high CH_4 oxidation events. It has been shown that methanotrophs in areas with submerged *Sphagnum* have a much higher potential of oxidizing the CH_4 that is produced at depth. However, it is still unclear how important specific *Sphagnum* species are in this process. The reciprocal transplantation of this study may provide insight into how *Sphagnum*, and therefore CH_4 oxidation, may be affected if drier conditions persist. The ability of *S. fallax* transplants to oxidize at similar rates as its host environment suggests that these species and microbes are relatively resilient (Kettunen et al.,

1999). However it seems as if these results may have been confounded by the environmental conditions experienced during the summer.

In the short term wetter conditions could result from increased temperatures and therefore increased snow and permafrost melting. These conditions may facilitate higher CH_4 consumption as the highest rates of oxidation have been found in submerged mosses. However, wetter conditions would also promote more CH_4 production. Therefore, the balance between CH_4 production and consumption is an important dynamic to understand.

Limitations and Future Research

Though the data were transformed, they were still not completely normalized as is needed to fit the assumptions of the ANOVA analysis. The raw data (Appendix 6) indicate that there was at times high variability in the rates such that replicated subplots exhibited varying rates at times. Larmola et al. (2010) suggest that this variability may have to do with the fact the methanotroph population may not have that strong of a relationship with *Sphagnum* but are rather more free living in the water column. Therefore, the level of diversity and movement of the methanotroph communities will determine how much variability is observed. Another possibility is that the variability is a result of a methodological error, which could occur in the rinsing, spiking or incubating processes. The GC-FID, as well as damaged syringes, could have caused gas samples to get mixed with the outside air.

The reliability of the potential CH_4 oxidation rates may also be affected by the sampling technique. Laboratory incubations could likely be overestimates of the actual CH_4 oxidation potential *in situ*. *In situ* techniques that utilize biological inhibitors are more accurate according to Popp et al. (2000). These techniques measure CH_4 oxidation by comparing the net flux of CH_4 (production and oxidation) to the gross flux of CH_4 oxidation in the absence of oxidation (Moosavi et al., 1998). Using the selective inhibitor methyl fluoride (CH_3F), Moosavi et al. (1998) found CH_4 oxidation rates ranging from 0 to 88.7 mg CH_4 m^2 d^1 in their study of three wet sedge communities on Alaska's North Slope in 1993 and 1995. These rates are lower than the CH_4 oxidation found in my study, however, an accurate comparison of these rates would require a full comparison of the sites studied, specifically in relation to vegetation composition and water table position. Regardless, as pointed out by Popp et al. (2000), *in situ* techniques are not without limitations; partial, instead of complete inhibition of CH_4 oxidation could lead to confounded rates.

My study investigated potential CH_4 oxidation in the upper most 10cm of the peat. However, this study and others have shown that CH_4 oxidation is often highest close to the water table. Therefore, the results at the hummock may not reflect rates that could have been found from peat around 25 cm deep, much closer to the average water table depth (27.36). If methanotrophs are free living in the water column it is safe to assume that as the water table decreases the methanotrophs would follow, existing deeper in the peat.

There are limitations in this study due in part to the dry summer conditions that made the water table remain consistently below 10 cm from the peat surface at the hummock site. Since only the top 10 cm were measured for potential CH_4 oxidation, it was not possible to know what the rates were like closer to the water table level. Therefore it would have been beneficial to take peat cores down to the position of the water table in the hummock site, to see how much oxidation occurred at depth even if it was outside of the green, growing portion of the *Sphagnum*. These results could potentially further inform us about the role of *Sphagnum* in CH_4 oxidation.

In order to further test whether there is a species effect this study could have sought to investigate two or more species living in one site, as was done in Larmola et al., (2010) based on the results of Basiliko et al. (2004). A follow-up study could also be longer, allowing me to investigate and analyze the results to a further degree. If other hummock and hollow species respond in a similarly to the way that *S. magellanicum* and *S. fallax* did upon transplantation, it would suggest that the host environment is the reason for the apparent species effect. A longer study could also indicate whether or not the effect of species would persist, especially in light of the fact that climate change may increase the length of the growing season.

CONCLUSIONS

This study tested the relative importance of *Sphagnum* species versus the environment in controlling CH_4 oxidation. It is not clear that there is an effect of *Sphagnum* species on CH_4 oxidation, however the results suggest that there is a joint control of *Sphagnum* species and the environment. At the low concentration of CH_4 *S. fallax* somehow thrived in its new and relatively unfavorable environment. *S. magellanicum* on the other hand seemed to respond to the environmental conditions of the hollow site in which it was transplanted. The results at the high concentration are less conclusive because it is unclear whether the amount of CH_4 affected the response of the methanotrophs. Overall, it seems that the methanotroph population may be more important controls of CH_4 oxidation than *Sphagnum* species such that they seem to maximize the best scenario that they are in. Therefore, microbial analysis of the methanotroph population in the transplanted mosses could have strengthened the results of this study.

My results indicate how little is known about the mechanisms of CH_4 oxidation in peatlands. One challenge that peatland scientists face is global climate change, which threatens to change peatland dynamics. Therefore, understanding the role of CH_4 oxidation in the peatland CH_4 budget is important as well as understanding how this role may change. Shifts in species composition in particular could limit CH_4 oxidation or the ability of microbes to survive or live

in the environment whether or not specific *Sphagnum* species play a role in the CH_4 oxidation process.

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FIGURES

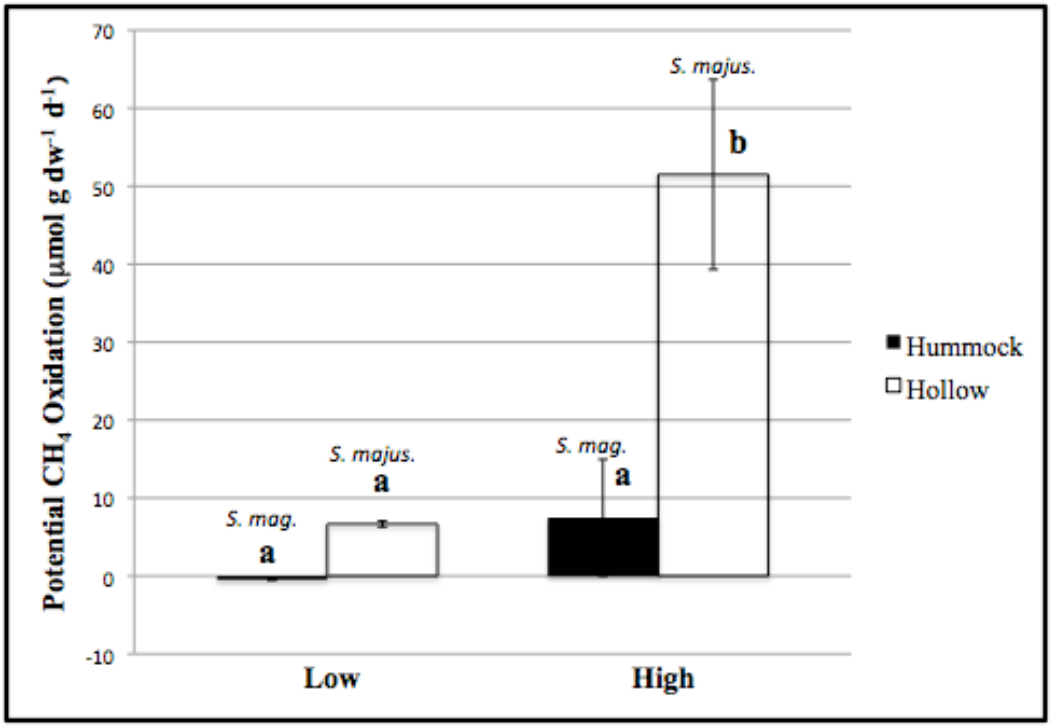


Figure 1. Preliminary data for average potential CH_4 oxidation in the hummock and hollow at each concentration. No letters in common indicate significant differences at $p<0.05$. CH_4 oxidation is expressed in micromoles of CH_4 per gram dry weight per day.

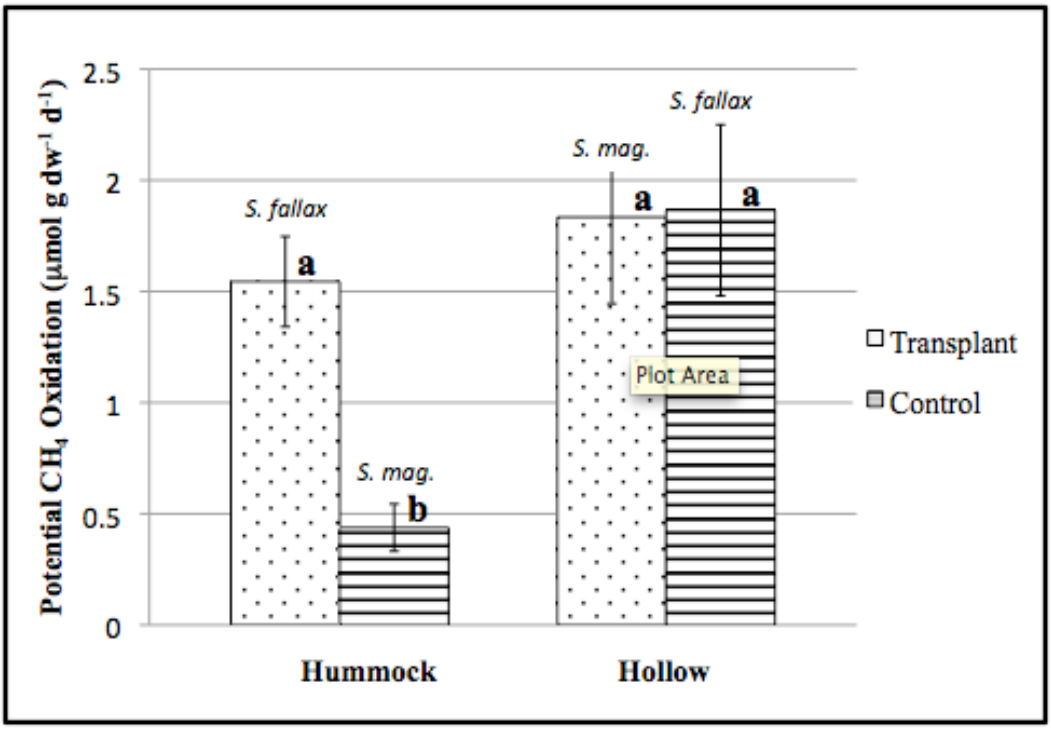


Figure 2. Average potential CH_4 oxidation for transplants and controls at each site for the low concentration. No letters in common indicate significant differences at $p<0.05$. CH_4 oxidation is expressed in micromoles of CH_4 per gram dry weight per day.

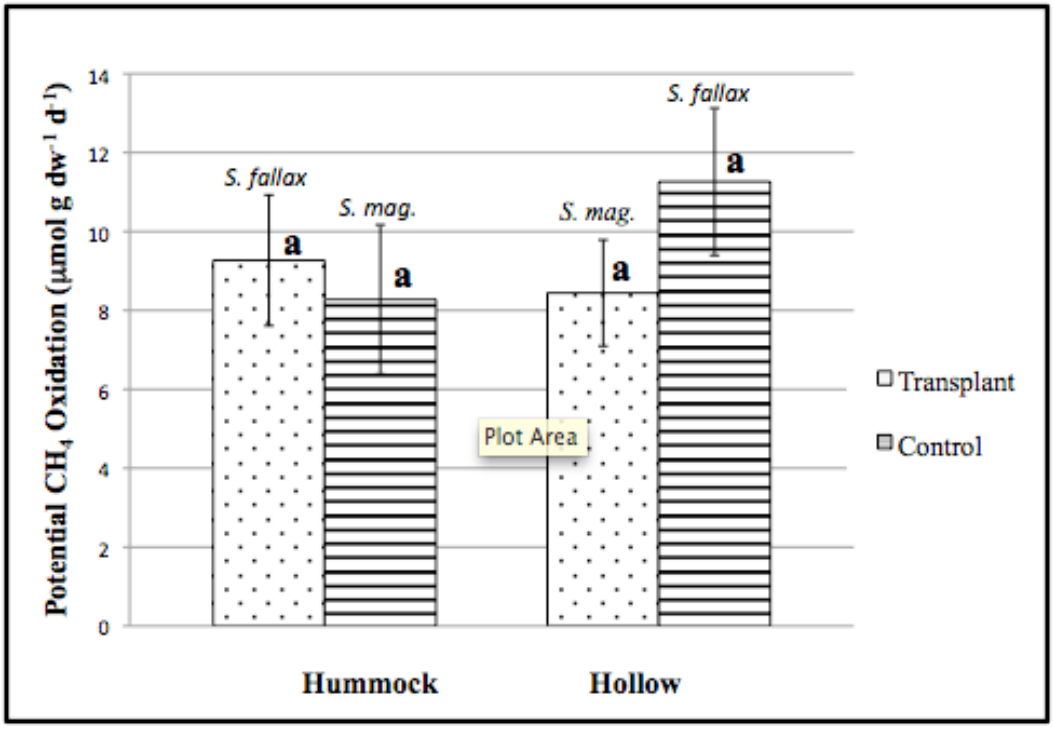


Figure 3. Average potential CH_4 oxidation for transplants and controls in the hummock and hollow at the high concentration. No letters in common indicate significant differences at $p<0.05$. CH_4 oxidation is expressed in micromoles of CH_4 per gram dry weight per day.

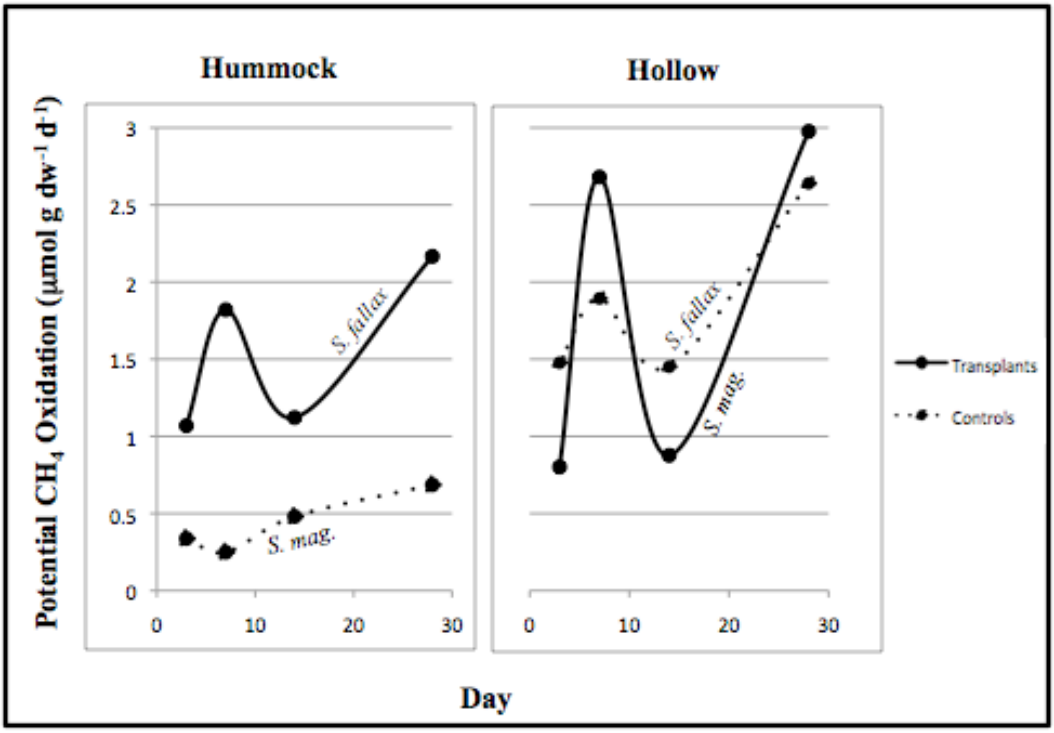


Figure 4. Average potential CH_4 oxidation of transplants and controls for each sampling day in the hummock and hollow at the low concentration, showing an increasing trend over time. CH_4 oxidation is expressed in micromoles of CH_4 per gram dry weight per day.

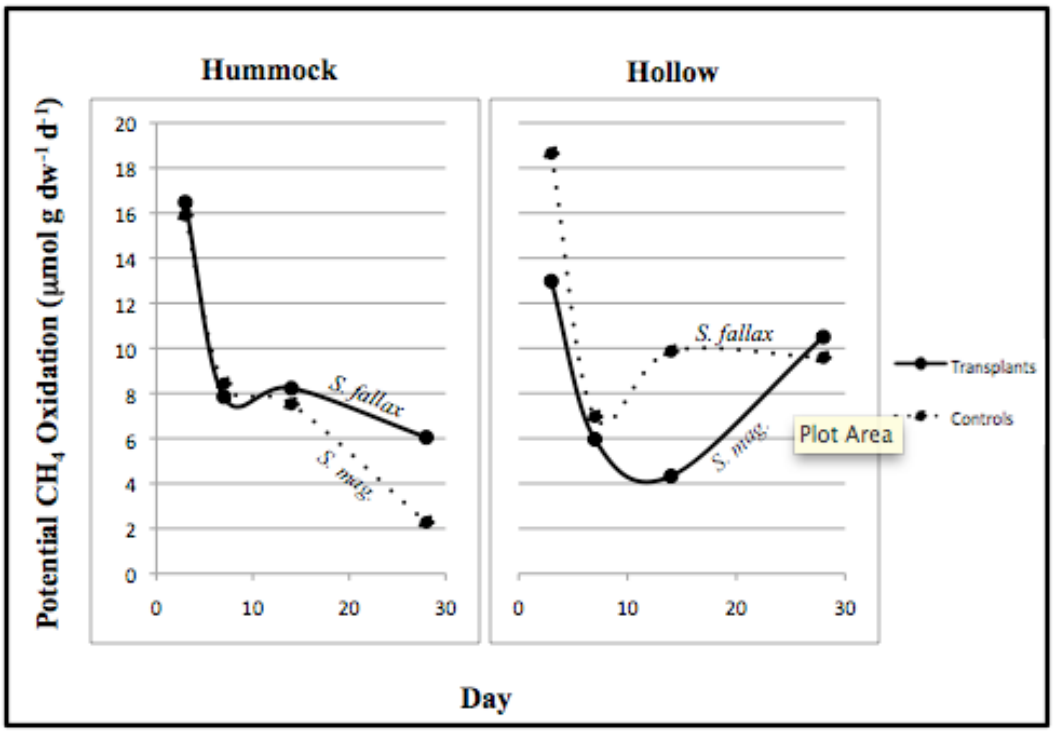


Figure 5. Average potential CH_4 oxidation of transplants and controls for each sampling day in the hummock and hollow at the high concentration, showing a decreasing trend over time. CH_4 oxidation is expressed in micromoles of CH_4 per gram dry weight per day.

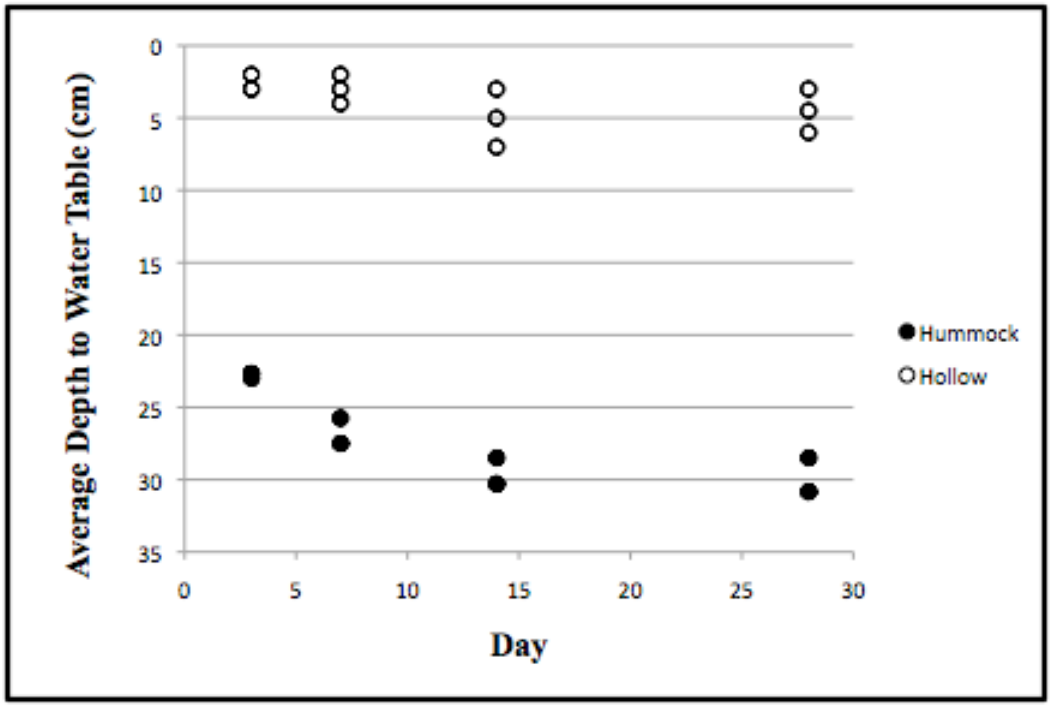


Figure 6. Average depth to the water table in the hummock and hollow at each sampling day.

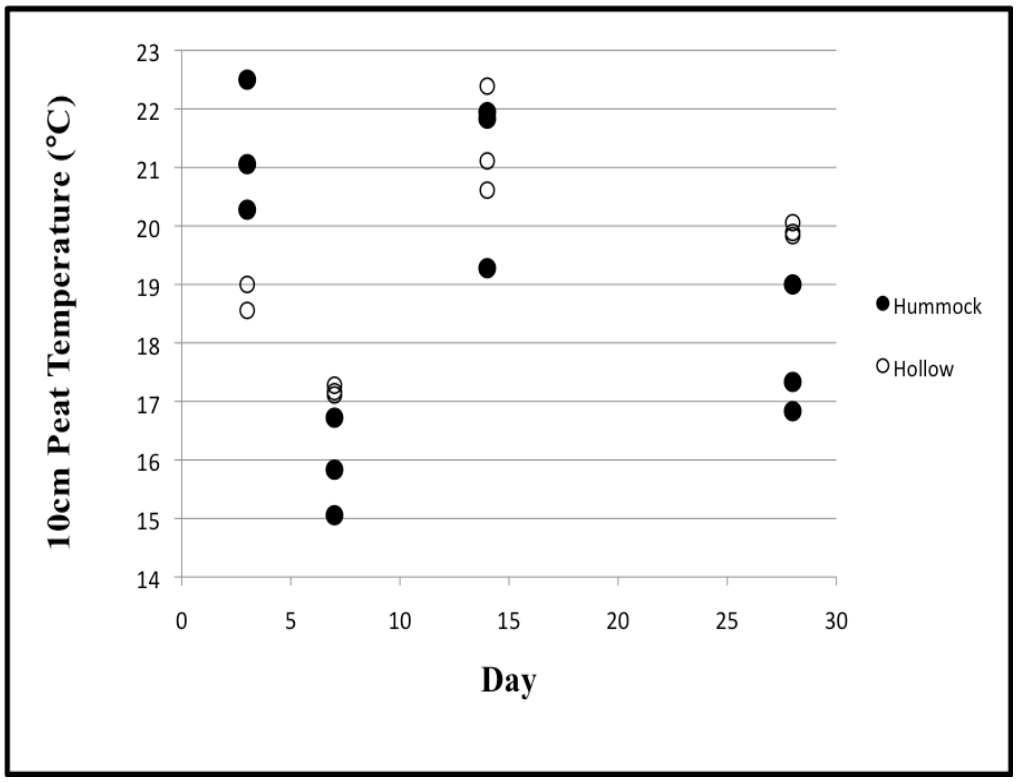


Figure 7. The 10 cm peat temperature in the hummock and hollow sites at each sampling day.

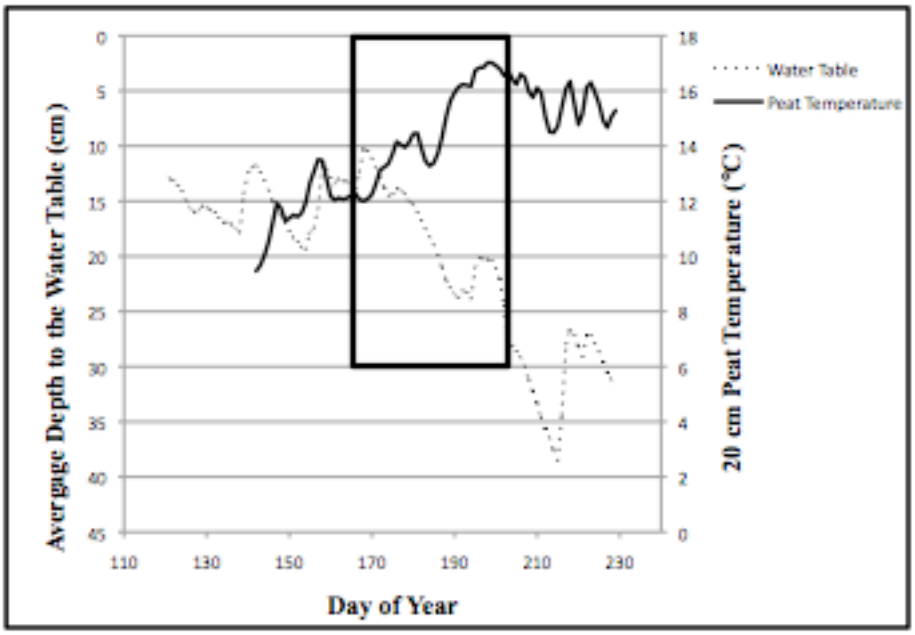


Figure 8. Seasonal trends of water table position and 20 cm peat temperature (°C) at Sallie's Fen, Summer 2010. Data taken from the meteorological station. The box represents the time period of experimentation.

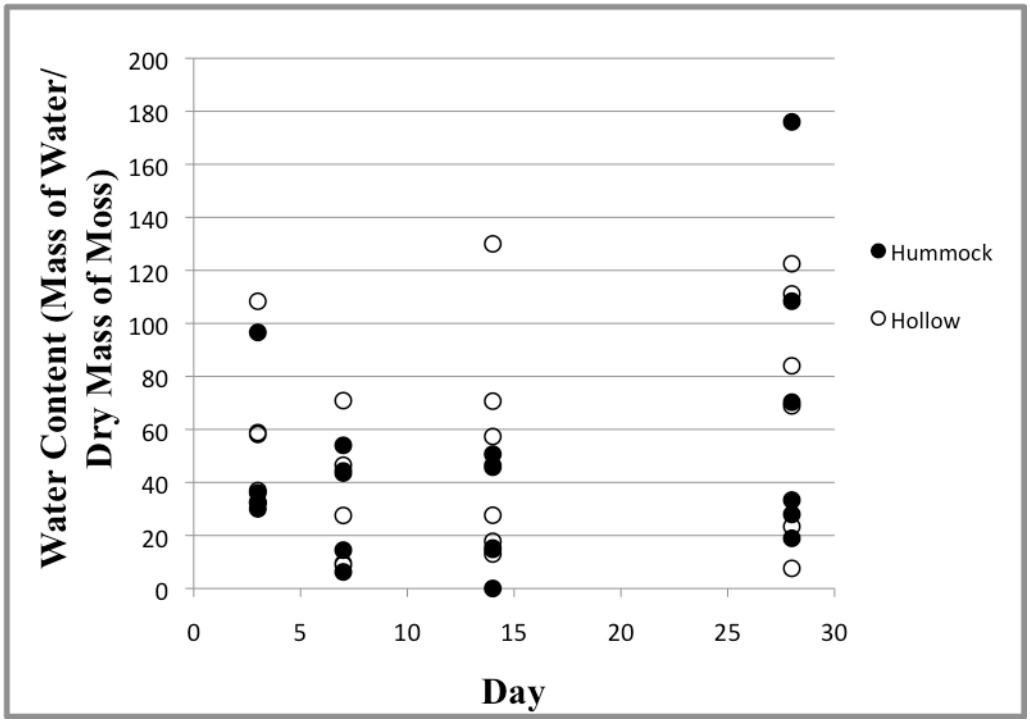


Figure 9. Water content (mass of water/dry mass of moss) over the 28-day sampling period.

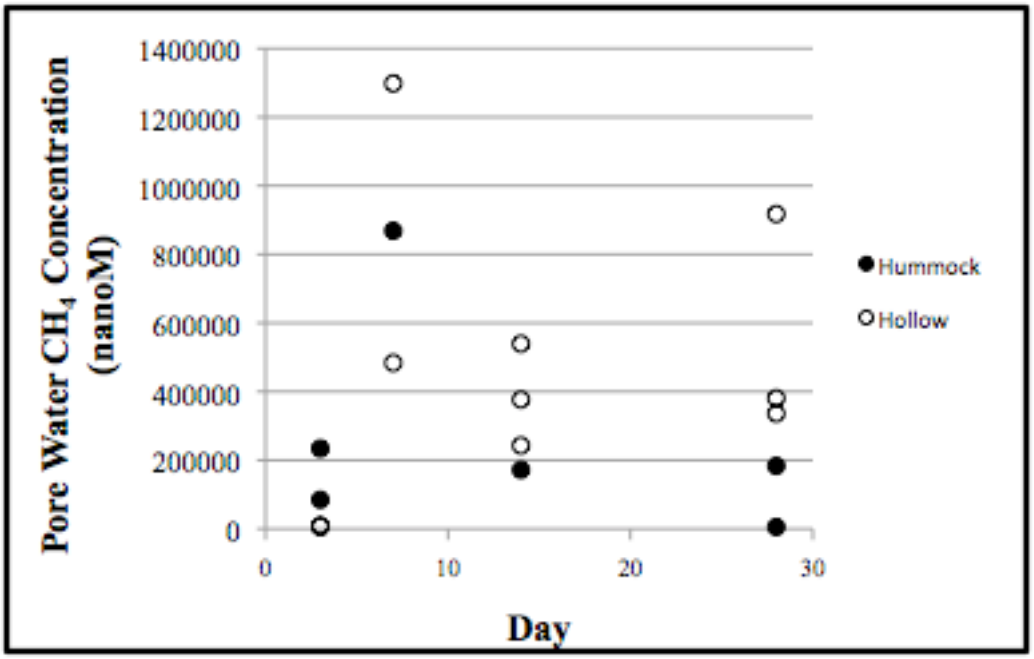


Figure 10. 30 cm pore water CH_4 concentration for each sampling day at each site.

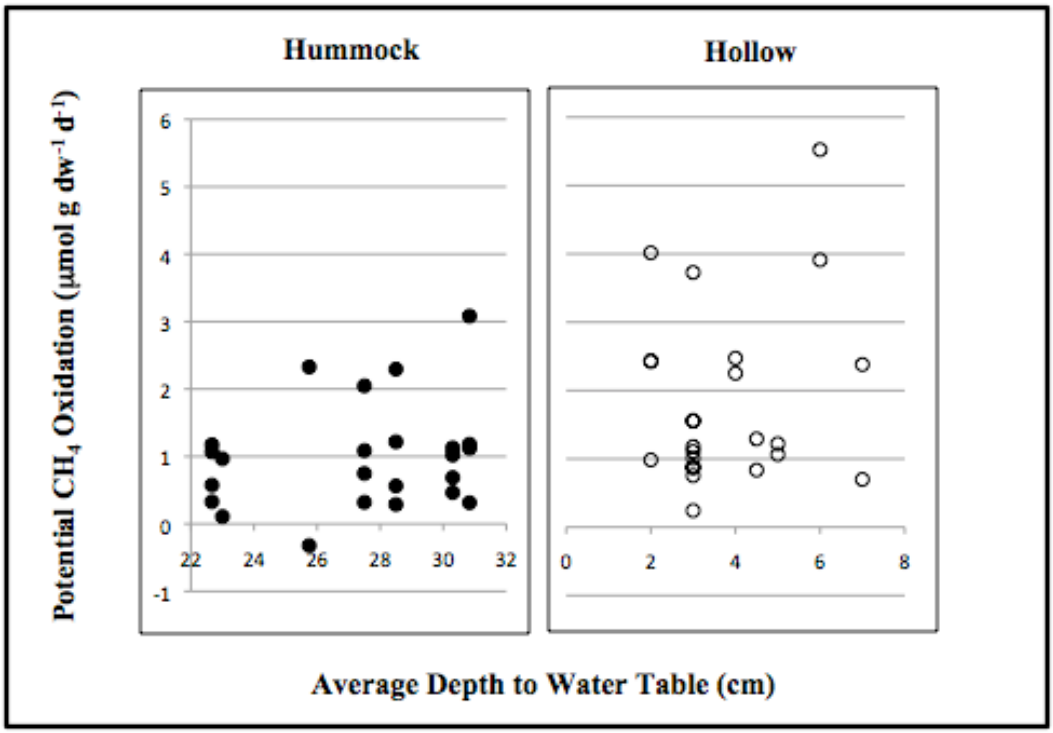


Figure 11. The relationship between potential CH_4 oxidation and average depth to the water table in the hummock and hollow at the low concentration. No significant relationship was found, however, the trends are positive for both sites. CH_4 oxidation is expressed in micromoles of CH_4 per gram dry weight per day.

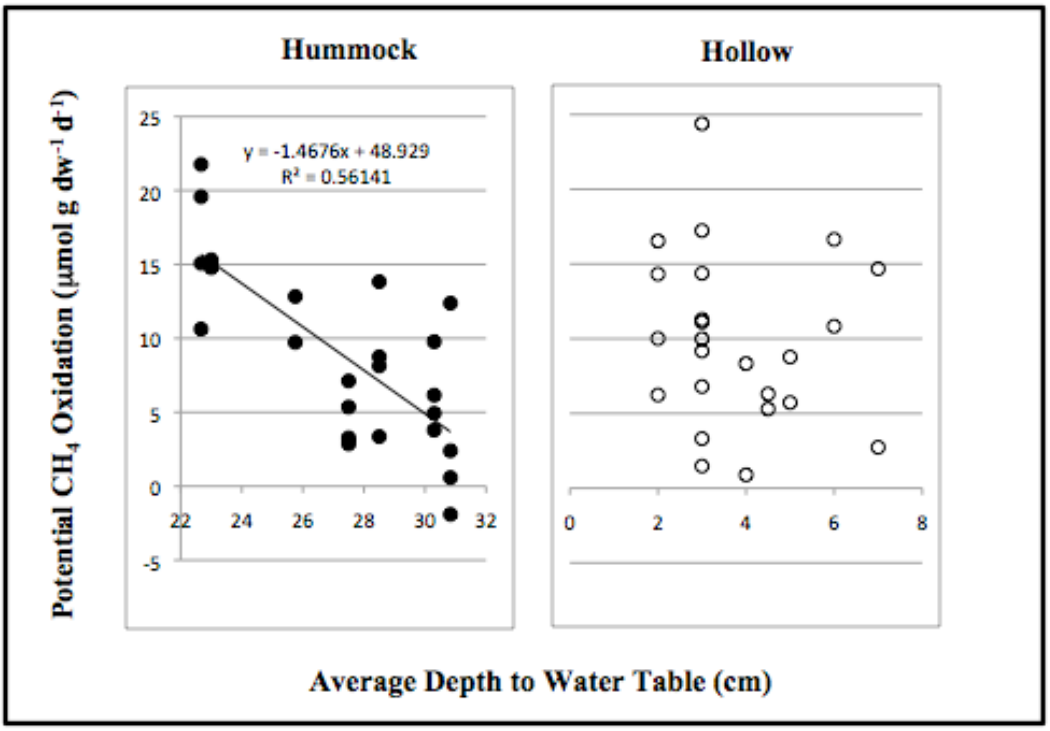


Figure 12. The relationship between potential oxidation and average depth to the water table in the hummock and hollow at the high concentration. There was a significant negative relationship in the hummock at $p<0.05$ and a negative trend in the hollow. CH_4 oxidation is expressed in micromoles of CH_4 per gram dry weight per day.

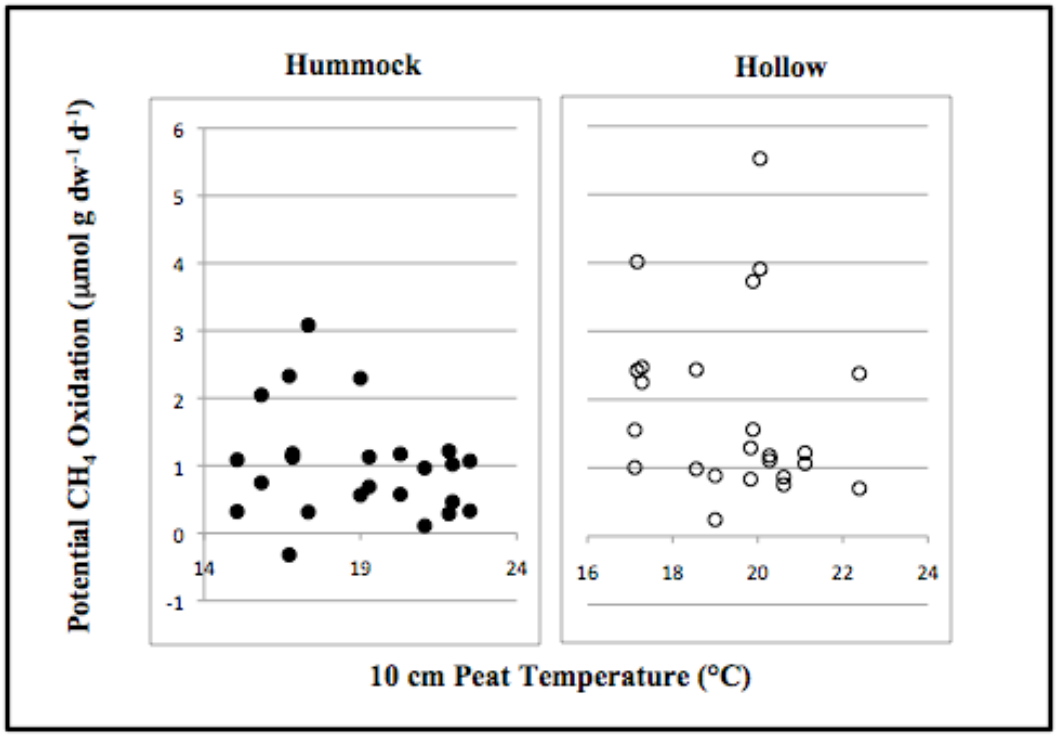


Figure 13. The relationship between potential oxidation and 10 cm peat temperature (°C) in the hummock and hollow at the low concentration. No significant relationships found, however, the trends are negative for both sites. CH_4 oxidation is expressed in micromoles of CH_4 per gram dry weight per day.

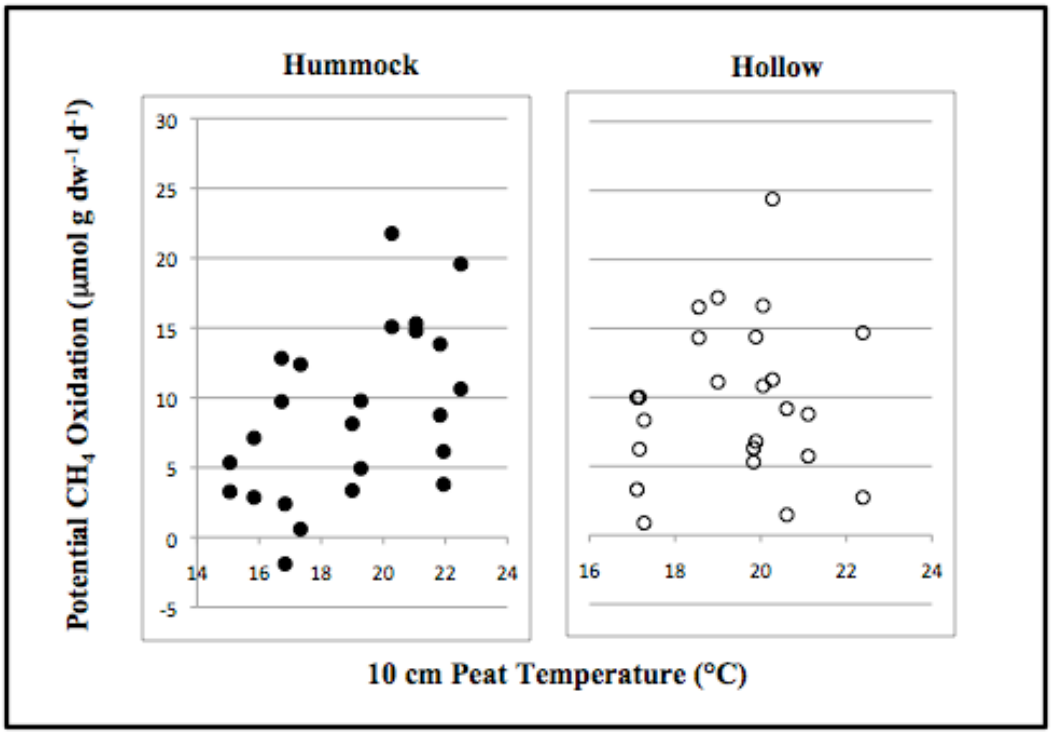


Figure 14. The relationship between potential oxidation and 10 cm peat temperature (°C) in the hollow and hollow at high concentration. No significant relationships were found, however, the trends are positive for both sites. CH_4 oxidation is expressed in micromoles of CH_4 per gram dry weight per day.

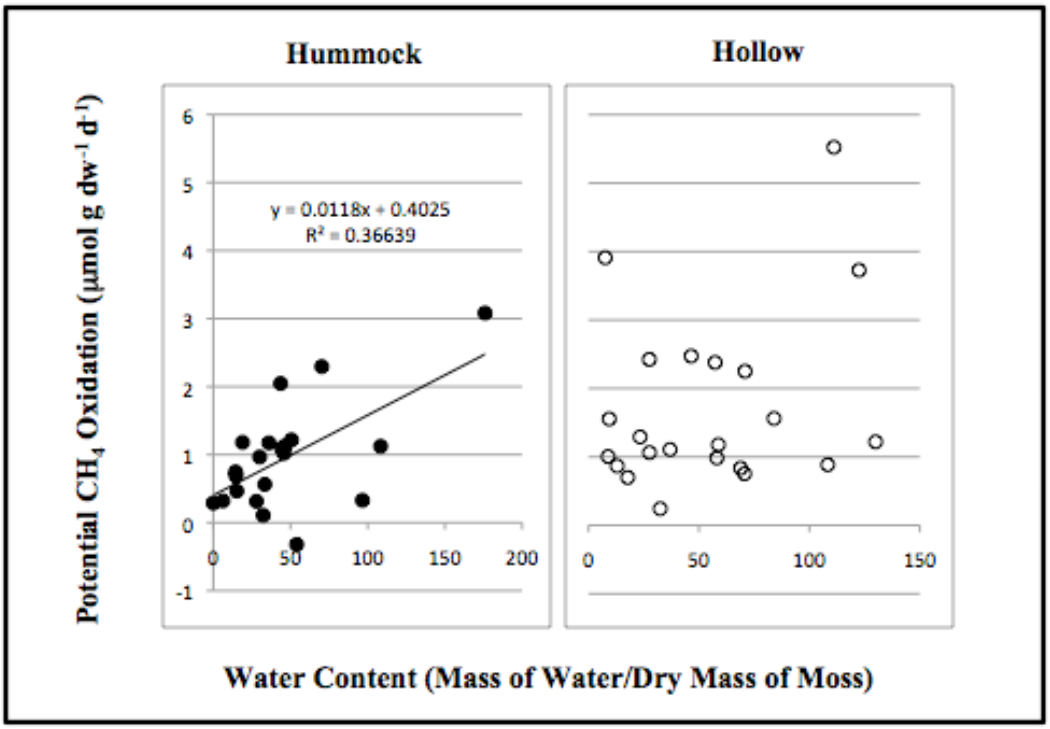


Figure 15. Relationship between potential oxidation and the water content of the mosses in the hummock and hollow at the low concentration. There was a significant positive relationship in the hummock at $p<0.05$ and a positive trend in the hollow. CH_4 oxidation is expressed in micromoles of CH_4 per gram dry weight per day.

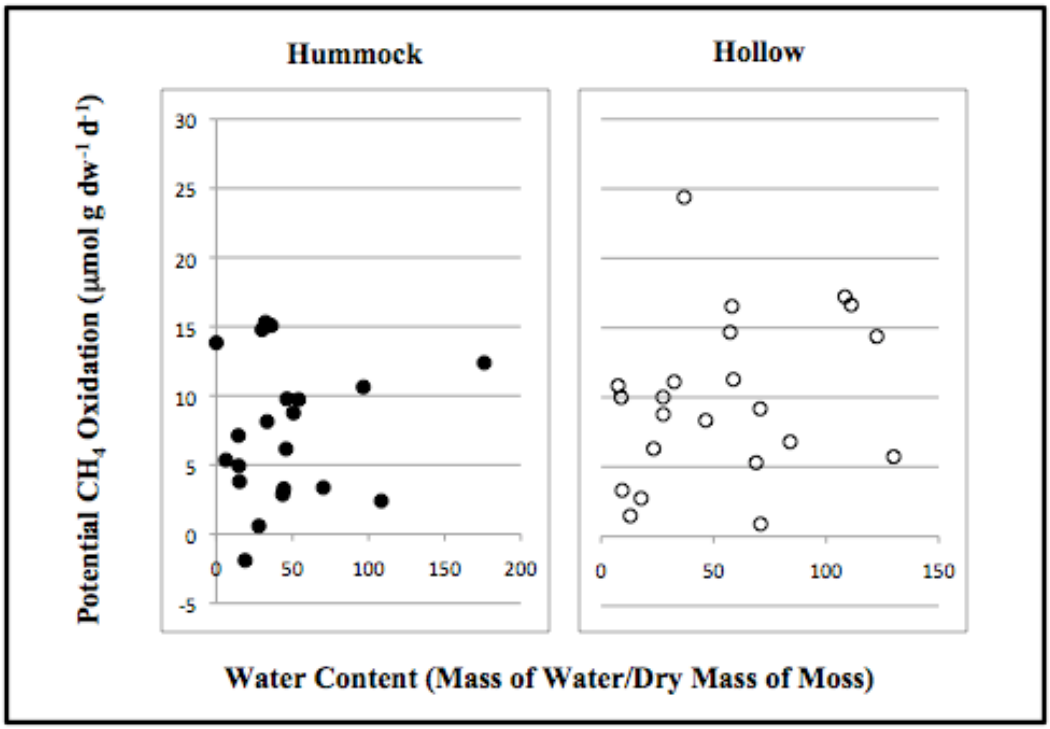


Figure 16. Relationship between potential oxidation and the water content of the mosses in the hummock and hollow at the high concentration. No significant relationships were found, however, the trends are positive in both sites. CH_4 oxidation is expressed in micromoles of CH_4 per gram dry weight per day.

TABLES

Table 1. 2-Way ANOVA of the preliminary average potential CH_4 oxidation data with two factors (site and concentration) showing significant effects of both factors, as well as an interaction between the factors.

Tests of Between-Subjects Effects					
Dependent Variable:Potential_Oxidation					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	5042.726[a]	3	1680.909	10.961	.003
Intercept	3245.969	1	3245.969	21.167	.002
Site	1918.389	1	1918.389	12.510	**.008**
Concentration	2138.558	1	2138.558	13.945	**.006**
Site * Concentration	985.779	1	985.779	6.428	**.035**
Error	1226.828	8	153.353		
Total	9515.523	12			
Corrected Total	6269.554	11			
a. R Squared = .804 (Adjusted R Squared = .731)					

Table 2. Bonferroni post hoc analysis for differences between the preliminary potential CH_4 oxidation data in hummock and hollow at both concentrations.

Bonferroni							
(I) SiteXConcentration			Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
	1	2	-7.1605	10.11116	1	-42.336	28.015
		3	-8.5722	10.11116	1	-43.7477	26.6034
		4	-51.9869*	10.11116	**0.005**	-87.1624	-16.8114
	2	1	7.1605	10.11116	1	-28.015	42.336
		3	-1.4117	10.11116	1	-36.5872	33.7638
		4	-44.8264*	10.11116	**0.013**	-80.002	-9.6509
	3	1	8.5722	10.11116	1	-26.6034	43.7477
		2	1.4117	10.11116	1	-33.7638	36.5872
		4	-43.4147*	10.11116	**0.016**	-78.5903	-8.2392
	4	1	51.9869*	10.11116	0.005	16.8114	87.1624
		2	44.8264*	10.11116	0.013	9.6509	80.002
		3	43.4147*	10.11116	0.016	8.2392	78.5903

Table 3. 4-Way ANOVA of the log-transformed data with three factors (time, species, site) showing significant effects of all factors as well as an interaction between species and site.

Tests of Between-Subjects Effects					
Dependent Variable:Log					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	5.794[a]	31	0.187	8.74	0
Intercept	61.232	1	61.232	2863.381	0
Site	0.195	1	0.195	9.102	**0.004**
Species	0.18	1	0.18	8.423	**0.005**
Time	0.995	3	0.332	15.513	**0**
Concentration	2.634	1	2.634	123.185	**0**
Site * Species	0.051	1	0.051	2.404	0.126
Site * Time	0.183	3	0.061	2.859	**0.044**
Site * Concentration	0.003	1	0.003	0.139	0.71
Species * Time	0.01	3	0.003	0.16	0.923
Species * Concentration	0.014	1	0.014	0.676	0.414
Time * Concentration	1.144	3	0.381	17.836	**0**
Site * Species * Time	0.1	3	0.033	1.558	0.208
Site * Species * Concentration	0.003	1	0.003	0.13	0.72
Site * Time * Concentration	0.156	3	0.052	2.432	0.073
Species * Time * Concentration	0.02	3	0.007	0.305	0.821
Site * Species * Time * Concentration	0.104	3	0.035	1.628	0.192
Error	1.369	64	0.021		

Table 4. 3-Way ANOVA of the log-transformed data at the low concentration with three factors (time, species, site) showing significant effects of all factors as well as an interaction between species and site.

Tests of Between-Subjects Effects[b]					
Dependent Variable:Log					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.292[a]	15	0.019	3.172	0.003
Intercept	19.233	1	19.233	3136.276	0
Site	0.075	1	0.075	12.188	**0.001**
Species	0.046	1	0.046	7.543	**0.01**
Time	0.085	3	0.028	4.622	**0.009**
Site * Species	0.039	1	0.039	6.366	**0.017**
Site * Time	0.017	3	0.006	0.921	0.442
Species * Time	0.001	3	0	0.07	0.976
Site * Species * Time	0.028	3	0.009	1.549	0.221
Error	0.196	32	0.006		

Table 5. Bonferroni post hoc analysis for differences between the potential CH_4 oxidation of *Sphagnum* species by treatment at the low concentration.

Bonferroni						
					95% Confidence Interval	
(I) NSite	(J) NSite	Mean Difference (I-J)	Std. Error	Sig.	Lower Bound	Upper Bound
1	2	-.1191*	0.03525	**0.009**	-0.2165	-0.0217
	3	-.1410*	0.03525	**0.001**	-0.2384	-0.0436
	4	-.1360*	0.03525	**0.002**	-0.2333	-0.0386
2	1	.1191*	0.03525	0.009	0.0217	0.2165
	3	-0.0219	0.03525	1	-0.1193	0.0755
	4	-0.0168	0.03525	1	-0.1142	0.0805
3	1	.1410*	0.03525	0.001	0.0436	0.2384
	2	0.0219	0.03525	1	-0.0755	0.1193
	4	0.005	0.03525	1	-0.0923	0.1024
4	1	.1360*	0.03525	0.002	0.0386	0.2333
	2	0.0168	0.03525	1	-0.0805	0.1142
	3	-0.005	0.03525	1	-0.1024	0.0923

Table 6. 3-Way ANOVA of the log-transformed data at the high concentration with three factors (time, species, site) showing significant effects of time as well as an interaction between site and time.

Dependent Variable:Log					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	2.868[a]	15	0.191	5.218	0
Intercept	44.634	1	44.634	1218.275	0
Site	0.123	1	0.123	3.354	0.076
Species	0.148	1	0.148	4.049	0.053
Time	2.054	3	0.685	18.692	**0**
Site * Species	0.015	1	0.015	0.413	0.525
Site * Time	0.322	3	0.107	2.934	**0.048**
Species * Time	0.029	3	0.01	0.26	0.854
Site * Species * Time	0.176	3	0.059	1.601	0.209
Error	1.172	32	0.037		

Table 7. Bonferroni post hoc analysis for differences between the potential CH_4 oxidation of *Sphagnum* species by treatment at the high concentration

Bonferroni						
(I) NSite	(J) NSite	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	-0.1467	0.11924	1	-0.4761	0.1827
	3	-0.2124	0.11924	0.491	-0.5418	0.1171
	4	-0.1367	0.11924	1	-0.4662	0.1927
2	1	0.1467	0.11924	1	-0.1827	0.4761
	3	-0.0657	0.11924	1	-0.3951	0.2638
	4	0.01	0.11924	1	-0.3195	0.3394
3	1	0.2124	0.11924	0.491	-0.1171	0.5418
	2	0.0657	0.11924	1	-0.2638	0.3951
	4	0.0757	0.11924	1	-0.2538	0.4051
4	1	0.1367	0.11924	1	-0.1927	0.4662
	2	-0.01	0.11924	1	-0.3394	0.3195
	3	-0.0757	0.11924	1	-0.4051	0.2538

Table 8a. Regression analysis of the relationship between potential CH_4 oxidation and water table in the hummock site at the low concentration.

ANOVA[b,c]						
Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	0.692	1	0.692	1.121	.301[a]
	Residual	13.577	22	0.617		
	Total	14.269	23			

Table 8b. Regression analysis of the relationship between potential CH_4 oxidation and water table in the hollow site at the low concentration.

ANOVA[b,c]						
Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	1.225	1	1.225	0.705	.410[a]
	Residual	38.238	22	1.738		
	Total	39.462	23			

Table 8c. Regression analysis of the relationship between potential CH_4 oxidation and water table in the hummock site at the high concentration.

ANOVA[b,c]						
Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	708.599	1	708.599	48.512	.000[a]
	Residual	321.345	22	14.607		
	Total	1029.945	23			

Table 8d. Regression analysis of the relationship between potential CH_4 oxidation and water table in the hollow site at the high concentration.

ANOVA[b,c]						
Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	46.082	1	46.082	1.185	.288[a]
	Residual	855.607	22	38.891		
	Total	901.689	23			

Table 9a. Regression analysis of the relationship between potential CH_4 oxidation and 10 cm peat temperature in the hummock site at the low concentration.

ANOVA[b,c]						
Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	0.087	1	0.087	0.135	.716[a]
	Residual	14.182	22	0.645		
	Total	14.269	23			

Table 9b. Regression analysis of the relationship between potential CH_4 oxidation and 10 cm peat temperature in the hollow site at the low concentration.

ANOVA[b,c]						
Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	0.367	1	0.367	0.207	.654[a]
	Residual	39.095	22	1.777		
	Total	39.462	23			

Table 9c. Regression analysis of the relationship between potential CH_4 oxidation and 10 cm peat temperature in the hummock site at the high concentration.

ANOVA[b,c]						
Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	1.412	1	1.412	0.03	.864[a]
	Residual	1028.533	22	46.751		
	Total	1029.945	23			

Table 9d. Regression analysis of the relationship between potential CH_4 oxidation and 10 cm peat temperature in the hollow site at the high concentration.

ANOVA[b,c]						
Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	2.246	1	2.246	0.055	.817[a]
	Residual	899.443	22	40.884		
	Total	901.689	23			

Table 10a. Regression analysis of the relationship between potential CH_4 oxidation and water content in the hummock site at the low concentration.

ANOVA[b,c]						
Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	2.968	1	2.968	5.778	.025[a]
	Residual	11.301	22	0.514		
	Total	14.269	23			

Table 10b. Regression analysis of the relationship between potential CH_4 oxidation and water content in the hollow site at the low concentration.

ANOVA[b,c]						
Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	0.641	1	0.641	0.363	.553[a]
	Residual	38.822	22	1.765		
	Total	39.462	23			

Table 10c. Regression analysis of the relationship between potential CH_4 oxidation and water content in the hummock site at the high concentration.

ANOVA[b,c]						
Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	0.578	1	0.578	0.012	.913[a]
	Residual	1029.367	22	46.789		
	Total	1029.945	23			

Table 10d. Regression analysis of the relationship between potential CH_4 oxidation and water content in the hollow site at the high concentration.

ANOVA[b,c]						
Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	31.397	1	31.397	0.794	.383[a]
	Residual	870.292	22	39.559		
	Total	901.689	23			

APPENDIX

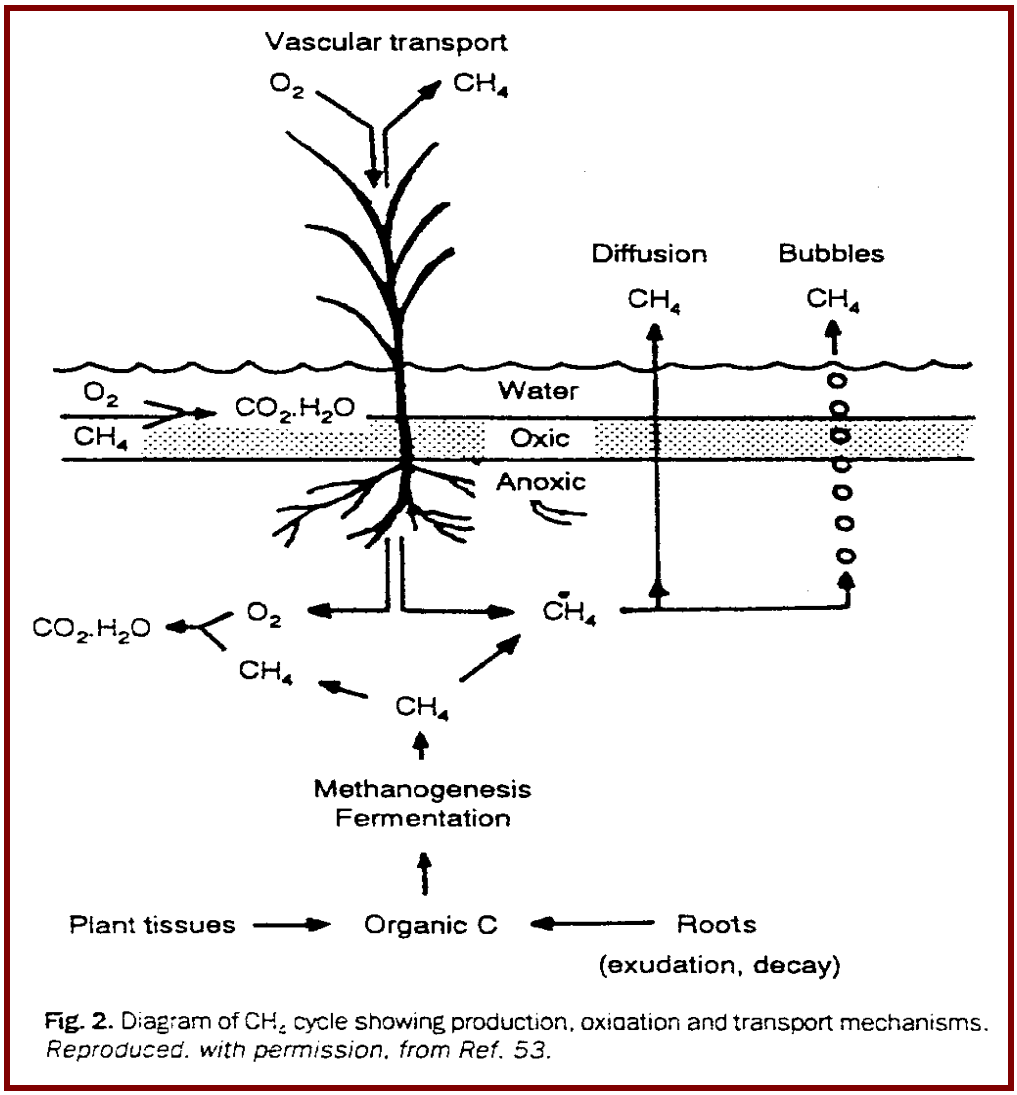
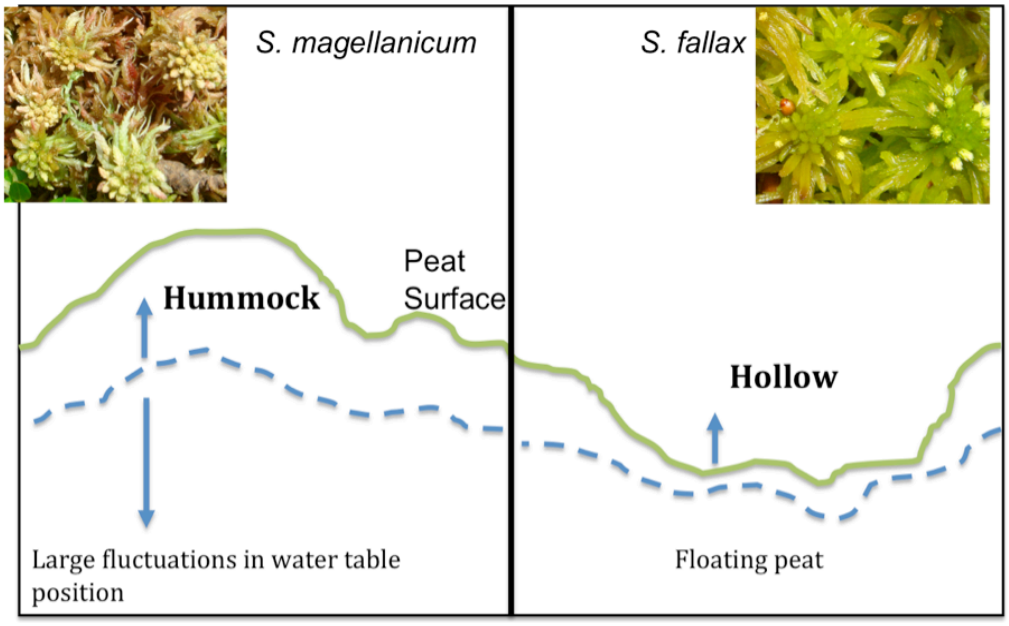


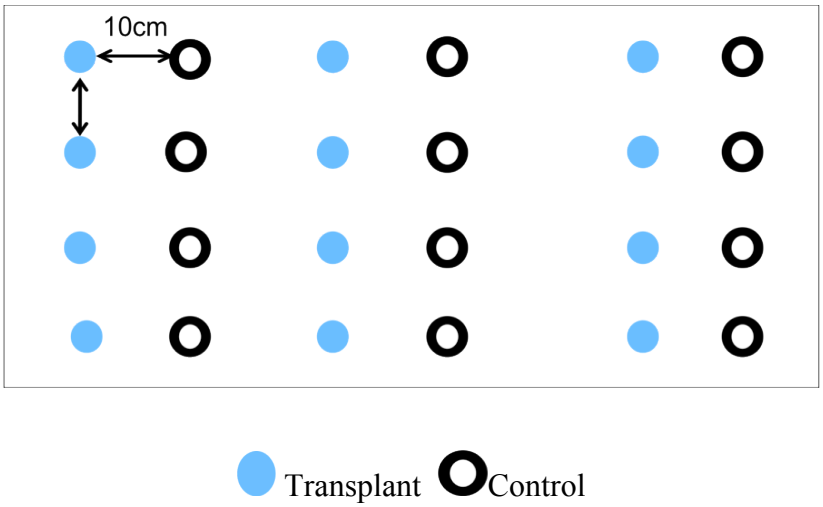
Fig. 2. Diagram of CH_4 cycle showing production, oxidation and transport mechanisms. *Reproduced. with permission, from Ref. 53.*

Appendix 1. Diagram of CH_4 production, oxidation, and transport from Bubier and Moore (1994).

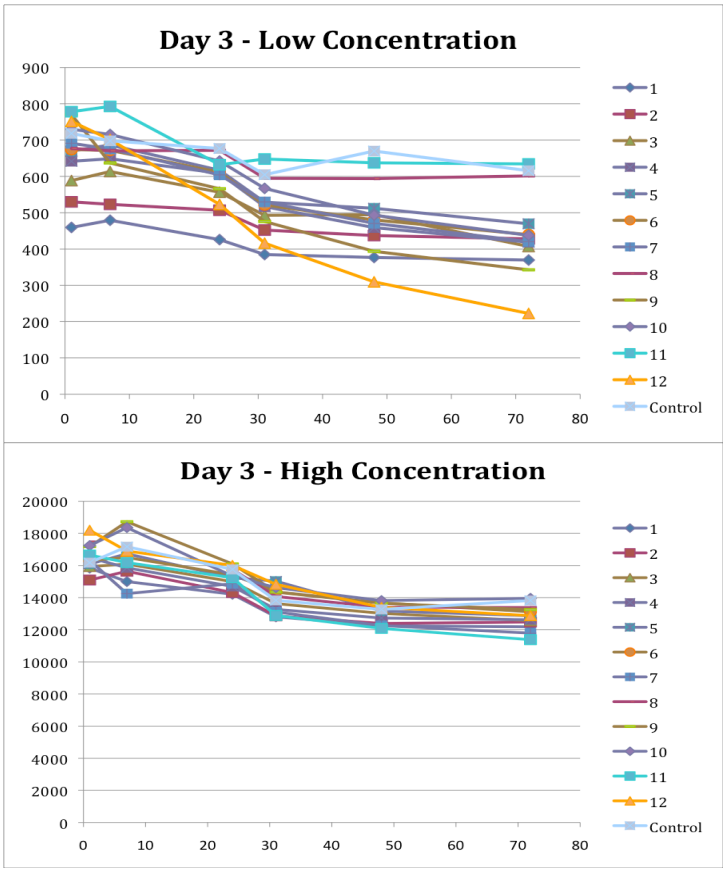
Appendix 2. Map of Sallie's Fen with hummock and hollow sites indicated by white stars.

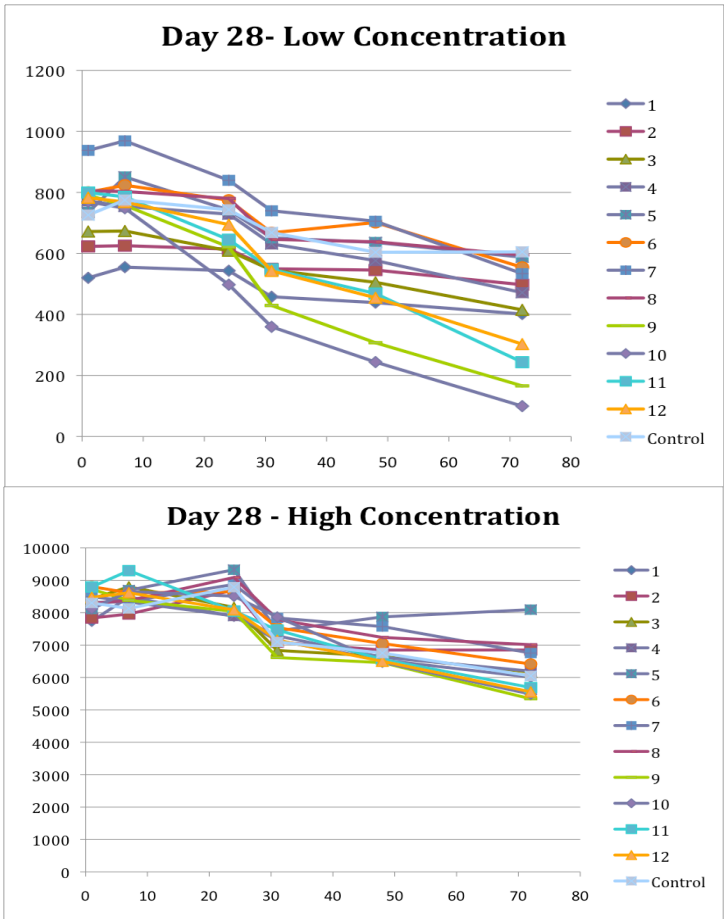


Appendix 3. Diagram of the relationship between the water table position in hummock and hollow features in peatlands.



Appendix 4. Experimental design representing the layout of the transplanted mosses in each site.


Day 3 - Low Concentration
900
800
700
600
500
400
300
200
100
0
0
10
20
30
40
50
60
70
80
1
2
3
4
5
6
7
8
9
10
11
12
Control
Day 3 - High Concentration
20000
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
0
10
20
30
40
50
60
70
80
1
2
3
4
5
6
7
8
9
10
11
12
Control



Appendix 5. Examples of change in CH_4 ppm graphs used to calculate fluxes

Potential Oxidation						
Concentration	Day 3	Day 7	Day 14	Day 28	Site	Treatment
Low	0.10872672	-0.321405736	0.288257955	0.563186284	1	c
	0.96542891	2.327187534	1.217192053	2.294657579	1	t
	0.328608133	0.747168338	0.464854777	1.180502056	1	c
	1.069054648	2.047042777	1.019931578	1.123210651	1	t
	0.57533858	0.320333817	0.687379211	0.313535295	1	c
	1.172573009	1.087184564	1.127303502	3.081401783	1	t
	1.107762972	2.25115693	2.379547331	5.523940033	2	c
	1.17816451	2.472587427	0.698775792	3.910118555	2	t
	2.437318765	1.005915362	0.753713022	0.834470583	2	c
	0.983994574	1.554258512	0.867122469	1.291723206	2	t
	0.885458812	2.424330748	1.220703232	1.561307983	2	c
	0.242512695	4.015516875	1.06323134	3.727831595	2	t
High	15.3148569	9.728754332	13.83225831	8.135976643	1	c
	14.7808519	12.83005448	8.752550299	3.359322363	1	t
	10.63182338	7.122400096	3.797649127	-1.903206387	1	c
	19.5757266	2.86334689	6.156142501	2.398127675	1	t
	21.76437742	5.354742834	4.93556975	0.586722815	1	c
	15.07740491	3.272747074	9.774347785	12.3803928	1	t
	24.38336643	0.888419332	14.67827131	16.64269079	2	c
	11.28144091	8.337197493	2.734609965	10.83229556	2	t
	14.3200824	9.99038372	9.174061649	5.299156059	2	c
	16.53532352	3.311802233	1.475143513	6.303016117	2	t
	17.23219054	10.00996387	5.72862196	6.796544696	2	c
	11.11099036	6.233430734	8.772195339	14.37633166	2	t

Appendix 6. Raw data of potential CH_4 oxidation by day, site, treatment and concentration