

A mesocosm approach to study the response of *Sphagnum* peatlands to hydrological changes: setup, optimisation and performance

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SUMMARY

Sphagnum-dominated peatlands are major carbon pools and sinks, but these functions are threatened by climate change. There is, therefore, a need to better understand how microclimatic changes (soil temperature, soil moisture and water table depth) are affecting their functioning. Experimental studies on *Sphagnum* peatlands conducted under precisely controlled (e.g. mesocosm) conditions are relatively rare, especially those aiming to understand the system as a whole. Furthermore, mesocosm designs are generally described only briefly in the literature. In this article we provide a comprehensive account of a mesocosm experiment designed to study the response of *Sphagnum* peatlands to water table manipulation. We describe our experimental setup (3 water levels \times 3 amplitudes of water table fluctuation \times 5 replicates); and explain how we built the mesocosms, the issues we faced and the solutions we chose to solve them. We provide a detailed description of the devices we conceived to manipulate the water level, including software codes and electronic diagrams (as supplementary material), and explain how to address data loss in such an experimental design. We show that it is possible to build a reliable and powerful experimental setup at moderate cost using standard technology. The aim of this article is to provide a useful resource for researchers wishing to design similar experiments in the future.

KEY WORDS: bog; ecohydrology; equipment; mesocosm experiment; water manipulation; water table depth

INTRODUCTION

Northern *Sphagnum* peatlands are a significant yet threatened carbon (C) pool. Although they represent 3–5 % of the worldwide land area (Froking & Roulet 2007), they hold one-third of the soil C pool (Turunen *et al.* 2002); and the 500 (\pm 100) gigatons of C (GtC) in northern peatlands (Yu 2012) is comparable to the atmospheric C pool (around 750 GtC). In peatlands, the key to C accumulation is low decomposition rate, which is driven primarily by the anaerobic conditions in water-saturated soil. Therefore, the C sink function of peatlands is likely to be highly sensitive to climate-induced variations in soil water content (Davidson & Janssens 2006, Bragazza *et al.* 2009). Studying how ongoing climate change and associated changes in soil water content affect the functioning of peatlands, and especially their C sequestration, is thus a research priority.

Sphagnum-dominated peatlands in the northern hemisphere are primarily situated in the boreal and subarctic zones, which are experiencing the largest climate changes, making the identification and quantification of potential feedbacks from these high-latitude ecosystems essential for future climate projections (IPCC 2014). One scenario is that rising temperature and drought will increase the

decomposition rate in *Sphagnum* peatlands and thus cause a release of C to the atmosphere, inducing a positive feedback to global warming (Belyea & Malmer 2004, Rydin & Jeglum 2006).

Although *Sphagnum* peatlands have been widely studied in recent decades, relatively few experimental studies have been performed. These were usually carried out in the field, and the majority of them investigated only a single response of the peatland rather than its functioning as a whole. For instance, some previous studies have focused on decomposition (Thormann *et al.* 2004); GHG exchange including, in some cases, the role of microbial communities in these exchanges (Morris *et al.* 2002, Rinnan *et al.* 2005, Haapala *et al.* 2011, Rahman *et al.* 2011, van Winden *et al.* 2012); DOC (Preston *et al.* 2011); or plant communities (Dieleman *et al.* 2015). Most existing mesocosm studies have, likewise, focused on a single response variable such as GHG (Blodau & Moore 2003, Tiiva *et al.* 2009, Faubert *et al.* 2010) and investigated only one explanatory variable. Few have assessed the effect of manipulating water table depth on *Sphagnum* peatlands. A notable exception is the mesocosms work by Bridgham and colleagues (Bridgham *et al.* 1999, Bridgham *et al.* 2008), in which both water level and temperature were

manipulated. However, in all cases, the equipment is only briefly described, making it difficult for other researchers to evaluate or exactly reproduce the setup.

We designed a mesocosm experiment to enable a comprehensive ecological study of the potential response of *Sphagnum* peatlands to climate change. Our climate change scenario is based on water table variation, expecting (in line with IPCC predictions) increasing precipitation and a more marked contrast between summer and winter precipitation patterns, with more frequent droughts and/or flooding events. The intention is to use the mesocosms to investigate the effects of simulated hydrological changes on the structure and functioning of *Sphagnum* peatlands at different levels such as microbial communities, water chemistry, decomposition and respiration.

The objective of this article is to provide a description of our experimental setup, the issues we had to cope with in developing it and the solutions chosen to overcome these issues, to help researchers designing or planning similar experiments in the future. As is typically the case, development of the experimental setup presented here was constrained by time and budget, and was iterative. We discuss its strengths and weaknesses as well as possible improvements.

METHODS

Experimental design

The mesocosms were built to assess the effects of water table variation on the ecological functioning of a pure *Sphagnum fallax* layer through time. We defined three average water table depths (AWTD) 4 cm, 15 cm and 25 cm below the top of the moss carpet, and for each we determined three amplitudes of fluctuation (± 2 cm, ± 7.5 cm, ± 12.5 cm) (Table 1). Each treatment was replicated five times. The 45

mesocosms were installed outdoors in two rows, of 22 and 23 mesocosms, respectively, in the botanic garden at Neuchâtel, Switzerland (46° 59' 59.95" N; 6° 56' 0.33" E; altitude 554 m) in late July 2012. The treatments were placed randomly along the two rows (Figure 1). A detailed account of installation is provided in the supplementary material.

Mesocosm functioning

Hardware

Each mesocosm consists of a polypropylene tank (type WIVA VAT4, 60 L) containing a perforated PVC tube. Inside the PVC tube is a peat core on top of which lies a *Sphagnum fallax* layer. The PVC tube is 45 cm long, has a diameter of 12 cm and is perforated with 5 mm holes, spaced approximately 5 cm apart both horizontally and vertically, all around the tube. The top of the *Sphagnum* layer was 5 cm below the upper rim of the tube at the beginning of the experiment. The tank was filled with water, and the water level was set and regulated according to one of the treatments described above.

Each mesocosm is defined by three conditions: Average Water Table Depth (AWTD), Maximum Water Table Depth (MaxWTD) and Minimum Water Table Depth (MinWTD), as shown in Figure 2. MaxWTD is defined by a 3.5 mm drainage hole drilled through the side of the tank. MinWTD is controlled by an automatic mechanical system (toilet flush device, Geberit Impuls380). If the water table drops below the minimum level, the valve of this device opens, allowing water to flow into the tank. As the water level rises it lifts the float of the device, and when MinWTD is reached, the valve closes.

The water for MinWTD was initially supplied by a Millipore filtration device (Elix 10 model), providing MilliQ water. However, because it was too sensitive to frost, this device was replaced by

Table 1. Details of the nine treatments. Water table depth (WTD) is measured in cm from the top of the *Sphagnum fallax* layer at the outset of the experiment.

Treatment code	HL	HM	HH	ML	MM	MH	LL	LM	LH
Average Water Table Depth (cm from top)	High (4 cm)			Medium (15 cm)			Low (25 cm)		
Fluctuation amplitude (code)	Low	Medium	High	Low	Medium	High	Low	Medium	High
Fluctuation amplitude (cm)	4	10	25	4	10	25	4	10	25
Max WTD (=overflow) (cm)	2	-1	-8.5	13	10	2.5	23	20	13
Min WTD (cm)	6	9	17	17	20	28	27	30	38



Figure 1. General view of the experimental site, with the 45 mesocosms installed in two rows. Each mesocosm consists of a blue tank, which houses a PVC tube containing a peat core covered by a *Sphagnum fallax* layer. Each tank has a Styrofoam board cover (with a hole) to prevent direct insolation of the wall of the PVC tube. The (black) lid of the tank is used to increase the amount of water supplied by rainfall. The whole experimental area is fitted with a net that filters out 50 % of the solar radiation; here, the north-facing part of the net has been removed for display purposes.

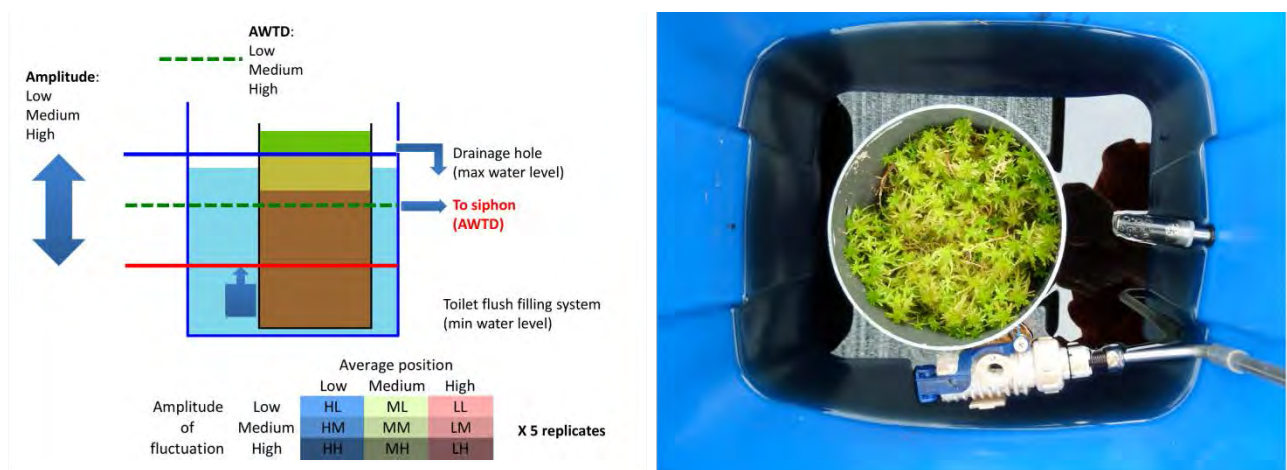


Figure 2. Cross-section diagram and photograph (taken from above) of one mesocosm. The minimum water level is set by the toilet flush device (white device in the photograph), which is mounted on a rail fixed onto the PVC tube. Water is supplied through the stainless steel pipe. The lower stainless steel pipe is connected to the siphon. A filter was added to avoid large particles entering and clogging the system.

a simpler filtration cartridge composed of three layers of sand, polyester wadding and activated charcoal. The cartridge is filled with deionised water and exits through a UV lamp for sterilisation. The water is supplied by gravity from a small garden shelter containing the cartridge with its deionised water supply, situated 4 m above the mesocosms. The rationale for using (sterile) deionised water is that it can be stored in tanks exposed to temperature variations without being colonised by bacteria or algae, and algal blooms in the pipes (which would lead to clogging) are avoided. It is justified on the basis that the quantity of supplied water necessary to maintain MinWTD is very small and, thus, has little impact on the chemical composition of the water in the mesocosm (most of this being meteoric water with less than 1 % supplied by the toilet flush system).

To ensure WTD homogeneity, all five replicate mesocosms for each medium- and high-amplitude treatment were connected together *via* silicone pipes plugged into a siphon. The replicate mesocosms for the stable treatment (with low amplitude of fluctuation) did not need to be connected together. The connection to the (mesocosm or siphon) tank was a 10 cm length of stainless steel pipe (0.5 cm diameter) plugged into the tank and sealed by a rubber O-ring. Silicone pipes were fitted onto the stainless steel pipes. To simulate horizontal water transfers, a system of pumps dynamically regulated by a microcontroller controlled lowering of the water level from MaxWTD to AWTD.

The mesocosms were shaded with a net that filtered out 50 % of solar radiation, to simulate the shading effect of vascular plants under natural conditions. The net also prevented damage by animals, mainly birds. To simulate the higher amount of rainfall occurring at the altitude from which the *Sphagnum* and peat were sampled (around 1000 m a.s.l.), we added the lid of each tank as a gutter, tilted at 45° with its lower edge placed over the top of the tank. This increased rainwater input by ~ 70 % estimated as follows:

$$S_{tot} = S_{tank} + S_{tank} \times \cos(45^\circ)$$

Thus,

$$S_{tot} = S_{tank} + \frac{S_{tank} \times \sqrt{2}}{2}$$

$$S_{tot} = S_{tank} \times \left(1 + \frac{\sqrt{2}}{2}\right) \approx 1.71 \times S_{tank} \quad [1]$$

where S_{tot} is the total rainfall input surface and S_{tank} is the rainfall input surface of the tank. The equation is

dimensionless.

Finally, a Styrofoam collar was placed around the outer edge of each PVC tube, to prevent direct insolation and unwanted heating on the side of the peat core.

With the exception of WTD, which is monitored every twelve hours, environmental variables are monitored hourly. The site was equipped with a Decagon PYR solar radiation sensor (pyranometer) placed under the net, along with a high-resolution raingauge (Decagon ECRN-100) and air moisture and temperature sensor (Decagon VP-3). In one replicate of each treatment, a Decagon E5-TM soil moisture sensor was inserted 5 cm below the top of the *Sphagnum* layer. The E5-TM sensors were calibrated with the default Decagon calibration equation (Topp equation) at the time of installation, but the raw data can be corrected *a posteriori* using a *Sphagnum*-specific calibration equation that was calculated for the *Sphagnum* mosses we used in the project. A HOBO 8K UA-001-08 temperature logger (± 0.53 °C accuracy) was placed at 15 cm depth in every mesocosm (i.e. at the base of the *Sphagnum* carpet, between old and new peat; see below).

Biological material

Peat cores were extracted from the Marais rouge sud (47° 00' 00.22" N; 006° 44' 57.01" E; altitude 999 m), a cutover bog in the Vallée des Ponts-de-Martel, Canton Neuchâtel, using a peat corer (Buttler *et al.* 1998). The peat cores were immediately inserted into the PVC tubes while still on the sampling site, so as to preserve the vertical structure of the peat. *Sphagnum fallax* was collected from the Creux de l'Épral peatland, Canton Jura (47° 12' 18.31" N; 006° 56' 05.83" E; altitude 990 m) using a home-made *Sphagnum* corer consisting of a 25 cm long cylindrical steel blade of the same diameter as the PVC tubes, which allowed sampling of the *Sphagnum* layer intact. All mesocosms were seeded at the beginning of the experiment with an extract of micro-organisms from pools, hummocks and lawns on the Cachot peatland (47° 0' 17.15" N; 006° 39' 52.21" E; altitude 1052m) so as to provide the full range of soil organisms potentially adapted to the different water table regimes. It would have been preferable to use full peat monoliths instead of harvesting peat and *Sphagnum* separately, but this was not possible in our case for reasons that are detailed in the Discussion.

WTD monitoring and WTD control

The key variable to monitor in our design is WTD, which is recorded every 12 hours by a water level

sensor installed in one mesocosm of each treatment. The water level sensor chosen (600 mm eTape™ Continuous Fluid Level Sensor produced by Milone Technologies) is a solid-state sensor with a resistive output that varies with the water level. The nine water level sensors are connected to an Arduino Uno R3 microcontroller board through a 16-Channel Analog/Digital Multiplexer/Demultiplexer breakout board (Sparkfun CD74HC4067). WTD is recorded on a SD card in csv (comma-separated values) format, through an Adafruit Data logging shield equipped with a SD card reader and a Real Time Clock (RTC) module. Originally, each water level sensor required individual calibration to transform input voltage into WTD; but Milone™ now produce a 0–5 V Linear Output Module that facilitates calibration. The electronic diagram and microcontroller code are provided in supplementary material.

Water table lowering, to simulate horizontal drainage through the acrotelm, is also controlled by an Arduino Uno R3 microcontroller. Each siphon incorporates a self-priming pump (DC pump by Trossen Robotics) and a water level float switch which is used to inform the microcontroller whether the WTD is above or below AWTd. Every hour, the microcontroller will check the water level for each treatment and run the appropriate pump for a given time if the water level is above the AWTd. The pump runtimes have been calibrated so that the water level will fall from MaxWTD to AWTd in one week without precipitation. Then, lowering of the water table below AWTd will be determined by evaporation. The three stable treatments (with low amplitude of fluctuation) are not connected to a siphon (as stated above) and have no active regulation of water table lowering. The electronic diagram and microcontroller code are provided in supplementary material.

For our design we chose to install electricity on the experimental site, rather than having autonomous devices. However, it would be reasonably easy to build similar devices powered by solar panels. To achieve this, we could use a LiPo Rider electronic card, whose purpose is to deliver a stable 5V power supply by pairing a solar panel and a battery. If the experiment was located close to running water (e.g. a stream), we could power it using 3.6 V Micro Hydro Generators, which are low-cost turbines that can convert water flows as low as 3 L min⁻¹ to electricity. Both of these devices (LiPo Rider and Micro Hydro Generator) cost less than 50 USD.

The frost-sensitive components of the system (toilet flush device, filtration device and pumps) are removed during the winter months.

RESULTS

Cost

The overall cost of our handcrafted mesocosm system (including sensors) was moderate, at less than 10,000 USD (Table 2). An estimate obtained from a local company for WTD monitoring alone was 34,500 Swiss francs (approximately 38,300 USD). We were able to build our data logger and water regulating devices ourselves because of the tremendous rise of interest in DIY electronics in the USA, which has brought to the market cheap yet efficient electronic components along with a huge quantity of documentation. The Arduino microcontroller board was released in 2005 and quickly became a benchmark. Because of its open-source nature and its highly competitive price (around 30 USD), many compatible boards and shields have been developed, enabling those without specialist training to perform various tasks (e.g. reading sensor inputs, controlling motors and valves) that only skilled engineers could undertake previously. Its programming simplicity makes it extremely handy to use. The microcontroller is programmed in C++, and thus accessible even to novice programmers. The Arduino board comes with a dedicated IDE (Integrated Development Environment) written in Java (compatible with any operating system) and can be configured *via* a USB port. In some recent applications the board is controlled from a smartphone (iOS or Android). A more complete account of the use of the Arduino board in environmental monitoring is given by Fisher & Gould (2012).

The time spent learning how to design the electronic part of the microcosm system should be taken into account when estimating its true cost. As our devices are very simple and the online documentation extremely abundant, it took approximately two full weeks to learn the Arduino and necessary basics of electronics, and two more full weeks to build and install the devices, making a total of 160 hours. This is largely compensated for by the fact that, once the water level sensors were in place, we no longer had to pay a technician to measure the water table depths (using a lasermeter) each day. The technician's workload was approximately five hours *per* week (260 hours annually) so, on an hour-for-hour basis, the 160 hours spent developing the electronic system were recovered in approximately eight months. A monetary value might be attached to the time invested in terms of the ~28,300 USD saving achieved on the commercial estimate for water level monitoring mentioned above.

Table 2. Cost (in Swiss francs, approximately = USD) of the hardware used for setting up the mesocosm experiment. The net cost, i.e. the cost of non-reusable, specifically purchased hardware amounts to approximately 30 % of the total cost of the experiment.

Item	Cost (CHF)	Specifically bought	Reusable
Tanks (WIVA VAT4, 60 L)	540	yes	barely
Pipes	200	yes	no
PVC tubes	200	yes	barely
WTD sensors (see sup. mat.)	450	yes	yes
WTD data logger (see sup. mat.)	100	yes	yes
WTD controller (see sup. mat.)	300	yes	yes
Toilet flush devices (Geberit)	1,800	yes	barely
Soil moisture sensors (9 x Decagon E5-TM @ 172.-)	1,548	no	yes
PYR sensor (Decagon)	238	no	yes
Rain gauge (Decagon ECRN-100)	307	no	yes
Decagon data loggers (3 x EM50 @ 458.-)	1,375	no	yes
HOBO data loggers (8K - UA-001-08)	1,800	no	yes
Various components (cables, power plugs, etc.)	200	yes	no
Filtration cartridge	150	yes	yes
Garden shelter	300	yes	yes
Total cost of all items	9,508		
Total items specifically bought		4,240	
Total reusable items			6,568

Environmental data

Dealing with missing data

Although the system was fairly reliable overall, several problems were encountered. First, the incorporation of several sensors increases the probability of a technical issue occurring in at least one of them. However, using sensors and loggers in quantity means that a global data shutdown, which would be dramatic from an experimental point of view, is highly unlikely. This problem aside, the sensors were reliable; over two years, only 4.19 % of the 1,239,540 expected data points were missing or erroneous. Problems occurred in the HOBO Logger 8K UA-001-08 temperature sensor, whose batteries sometimes failed due to the severe winter frosts of

2012–2013. Also, data from one E5-TM sensor were not reliably recorded because a damaged cable induced power drainage from one of the Decagon dataloggers, leading the batteries to empty too rapidly. Finally, the air moisture and temperature sensor did not tolerate the high temperatures of summer 2013, and yielded erroneous output data during this period.

Several strategies were used to fill data gaps introduced by defective sensors or data loggers, and are summarised in Table 3. The Amelia II R package (Honaker *et al.* 2011) is suitable for small data gaps and time series, and its built-in modified ‘expectation maximization importance sampling’ (EMis) algorithm, called EMB, was used to computationally infer scarce missing data entries. For longer data gaps

Table 3. Missing data and solutions used to remedy data gaps.

Sensor	Data loss (%)	Impacted sensors/ total	Data gap > 10 days	Solution
Soil moisture	3.65	2/9	yes	Generalised additive model based on the seven remaining sensors and valid data, plus rainfall, pyranometer, water level and air temperature data.
Soil temperature	3.65	2/9	yes	
Peat temperature	4.11	30/45	yes (3 sensors)	Amelia II.
Air temperature	13.70	1/1	yes	Generalised additive model based on valid data and temperature data from the nearest national weather station.
Relative humidity of air	13.70	1/1	yes	Generalised additive model based on valid data plus rainfall, pyranometer and air temperature data.
Raingauge	2.74	1/1	yes	Linear model based on valid data and precipitation data from the nearest national weather station.
Pyranometer	0.00	0/1	/	/
Water level sensor	4.11	45/45	no	Linear interpolation.
Overall	4.19	81/112		

(> 10 days), depending on the variable to be reconstructed, several tools are available including least square linear regression using the `lm` function from the R package (Chambers & Hastie 1991), autoregressive moving average (ARMA) (Gurland & Whittle 1954), and autoregressive integrated moving average (ARIMA) models or their seasonal equivalents SARMA and SARIMA. However, given the nature of our dataset, these classical models may not be the most accurate or the best suited for this purpose. For instance, to reconstruct missing air temperature data, we fitted a linear model expressing recorded air temperature as a response variable to the air temperature recorded at the nearest national weather station. We then computed the missing values using this model. Similarly, we reconstructed missing soil moisture data using a generalised additive model (GAM, a generalisation of the GLM)

by expressing soil moisture as a function of air temperature, water table depth, soil temperature, solar radiation and precipitation variables. The model performed well and gave a Root Mean Square Error (RMSE) of 0.076. Consequently, our setup allowed us to closely monitor the environments of the mesocosms, as shown in Figure 3. The model fitting is highly dependent on climatic conditions at the location of the experiment. The aim of such models is not to construct general equations for understanding climatic mechanisms, but rather to rely on correlation between climatic variables to infer missing data points. Therefore, a universal model that would be suitable for any research location around the world could not be developed in this way. An increasingly used and efficient method for inferring missing data is the artificial neural network (ANN) (Kuligowski & Barros 1998, Kajornrit *et al.* 2012).

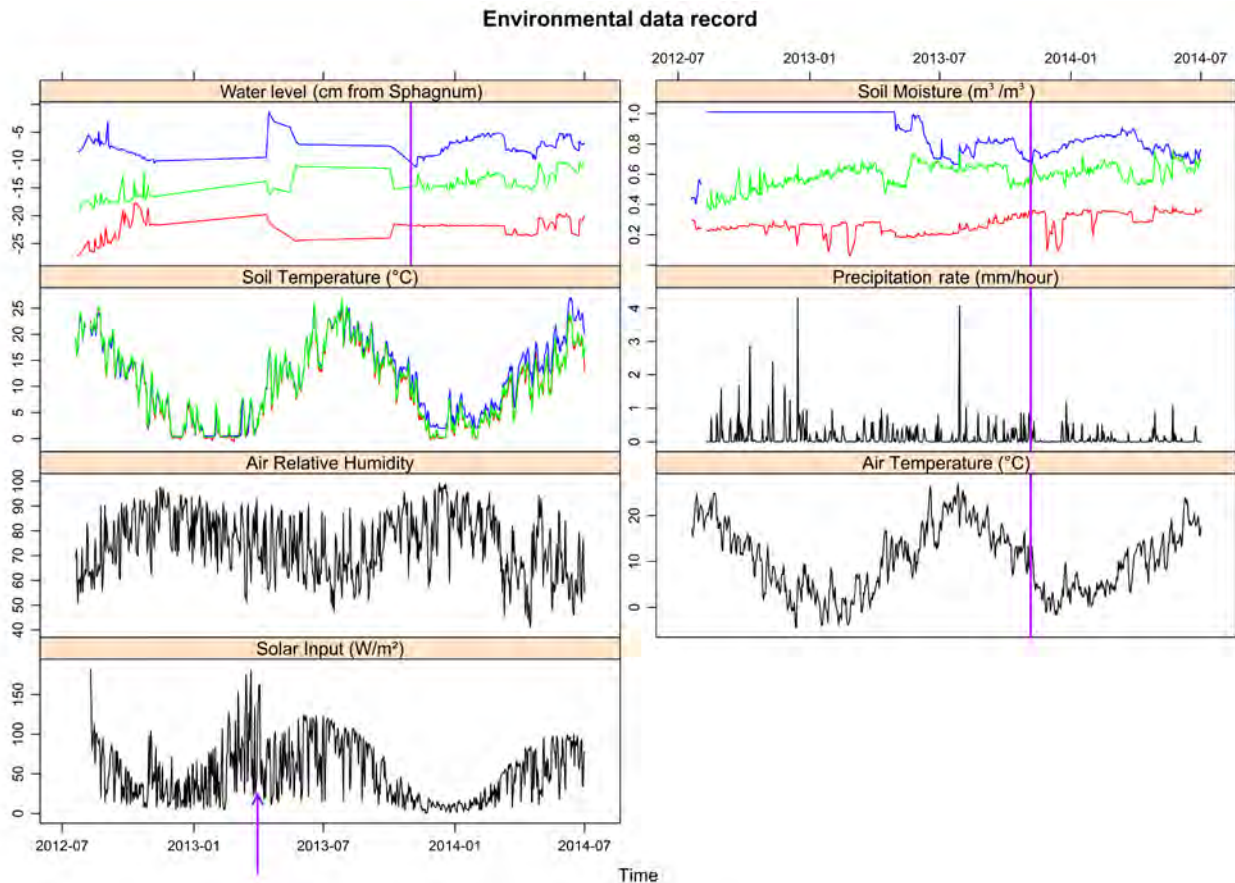


Figure 3. Environmental data recorded at the experimental site (black) and in three mesocosms: LM (red), MM (green) and HM (blue). The regulation of WTD dynamics was effective from November 2013 onwards (purple lines). It was removed during the freezing period and re-installed after the thaw. The significant reduction of solar input in spring 2013 (purple arrow) is due to re-installation of the net, which filters approximately 50 % of the solar input when in place but was removed during the previous winter to avoid damage by the heavy snowfalls. It was not removed during the following winter because there was virtually no snowfall.

The main strength of ANNs is that they can infer any mathematical function (universal approximation theorem (Hornik (1991))). The drawbacks are that they require large datasets and the network structure must be chosen carefully to avoid overfitting.

Homogeneity assessment and trials

One concern about our design was the potential presence of a row effect. Our 45 mesocosms were installed in two rows, the first oriented SSE and the second oriented NNW. We analysed the temperature data recorded by the HOBO data loggers inserted at the catotelm/acrotelm interface in each mesocosm. Over the first nine months there was no significant temperature difference between rows as determined by one-way ANOVA (nine-month mean temperature for each mesocosm, $p = 0.475$), confirming that the

location of each mesocosm had no impact on its average temperature. It is likely that the daily temperature range or other variables differed between the rows, but our goal here was not to analyse this in detail. Further analyses of the temperature dynamics among water level treatments are yet to be performed.

Because the WTD control mechanism required several trials and prototypes before it became effective, the three amplitudes of water level fluctuation were not applied until after one year of operation. Our first idea was to use slow leakage from medical drips to simulate horizontal water transfer. This was unsuccessful because peat particles released by the peat core clogged the drips. We then attempted to simulate natural infiltration by routing the water flowing in the pipes through pierced bottles filled with peat, but the water flowed through these devices

so rapidly that the water level could not rise above AWTD. The final design, which uses dynamically regulated pumps, is an apparently perfect solution. Interconnecting the replicates of each treatment allowed us to maintain homogeneity between them even in the eventuality of unpredicted events (leakage, clogging, *etc.*). For connecting the replicates, we recommend pipes of minimum diameter 5 mm to avoid clogging, and ideally of larger diameter to lower the resistivity of the pipes, which can induce small WTD differences among replicates of the same treatment. However, pipes of diameter > 3 cm should be used with care because of the increased risk of leaks at the O-ring. From July 2012 to September 2013, we observed significant differences between the three AWTD treatments (ANOVA, $p < 0.01$), even though the water level was approximately constant throughout. The sparse clogging induced some temporal disturbance that had no overall effect on the AWTD over time. From November 2013 to the present (April 2015), the nine treatments were well contrasted (shown in Fig. 3: November 2013 to August 2014) although we had to fix some leakage issues. Additionally, it seems that water level, as controlled by the float/pump/regulator system, fell slightly more slowly than initially planned and calibrated. Although it had no impact for our setup, this could be an issue for experiments in which water level drop has to be finely controlled ($\pm 1 \text{ mm h}^{-1}$ for instance). However, this can easily be fixed by modifying the microcontroller program to precisely adjust the settings.

DISCUSSION

Because it is crucial to understand the responses of peatlands to climate change in the context of global warming, it is important to develop protocols and methods for manipulating micro-climatic conditions in order to fill knowledge gaps and improve peatland-climate feedback models. Experimental field studies are essential for the assessment of actual ecosystem functioning. However, field manipulations are difficult and expensive to develop and typically allow testing of only small climatic changes. For instance, the field Open Top Chambers (OTC) design allows a temperature increase of 1–4 °C only (Huguet *et al.* 2013). Thus, there is a need for mesocosm studies in which many environmental variables can be controlled and, if necessary, highly contrasted.

Although our system allowed control and monitoring of WTD only, we could easily control other factors such as rainfall, solar input, wind, *etc.* using devices similar to those used here for WTD

regulation but installed in a growth chamber. The water level regulation device could easily be modified to control fans or heating resistors, and the pumps could be set up to simulate rainfall by pumping rainwater from a tank onto the mesocosms.

We showed that it is possible and relatively simple (with some practical skills) to build an efficient mesocosm experiment to assess the effect of water table depth on *Sphagnum* peatlands. Although efficient, our system is not perfect and could be improved in several ways. We made several mistakes and learned a lot during the construction and maintenance of our experiment. Perhaps the main limitation is the rather small diameter of the peat core, which introduces an edge effect. The advantage of this choice is that it requires less peat and is, therefore, less damaging to natural ecosystems (a critical issue in countries like Switzerland, where there are no vast expanses of peatland and most of the remaining peatlands are protected). The solution would be to fill the entire tank with peat and cover it with *Sphagnum*, and to use a small tube (e.g. 5–10 cm diameter) for WTD monitoring and manipulation. The counter-indication for such a design is that it would require much larger amounts of peat and *Sphagnum* and, consequently, involve more damage to the sites from which the material is collected. For instance, using the whole surface of the 60 L tanks would have required more than 7 m² of *Sphagnum* instead of the 0.6 m² necessary for our design. Because peatlands and *Sphagnum* are protected in Switzerland, we would not have been allowed to harvest such a large area of *Sphagnum*. The weight of the peat to be carried off the field site should also be taken into account. Assuming a peat density of $\sim 1.1 \text{ g cm}^{-3}$ (wet peat), we transported $\sim 230 \text{ kg}$ of peat to build the mesocosms. If we had used the 60L tanks filled up to 5/6 capacity, we would have needed to transport 2,245 kg of peat. The final choice is, therefore, a compromise.

Harvesting peat and *Sphagnum* separately rather than as single monoliths (as it is commonly done) was not the best theoretical scenario but was still the best practical choice. We needed peat whose chemical composition was similar to that of the natural peatland which hosted a parallel field experiment (i.e. Linje Mire in Poland). We found such peat in a cutover peatland where few mosses were growing. We also needed pure *Sphagnum fallax* carpets. We obtained authorisation for *Sphagnum* sampling at a peatland with patches of pure *Sphagnum fallax* carpet, but unfortunately the peat on this site was too minerotrophic for our purposes. As our study focused on relatively short-term changes in microbial communities found in the top three centimetres of the

Sphagnum layer with three strongly contrasting water level treatments, the effect of the artificial setup was probably lower than the effect of the water level itself. Moreover, the *Sphagnum* layers were 20 cm thick, allowing authentic hydrological behaviour in this part of the profile. For accurate estimations of respiration and decomposition, it would be preferable to use larger, single peat monoliths. Similarly, the fact that the peat cores were not insulated prevented simulation of the naturally-occurring temperature gradient in the cores, thus also affecting respiration processes. However, the impact of this isothermality of the peat core on the microbial communities inhabiting the top three centimetres of the *Sphagnum* layer is likely to be less than critical, as the temperature of the moss surface is mostly driven by atmospheric and water temperature.

Another limitation is that the contrasting treatments we used caused major changes in the system. Under wetter conditions the high growth rate of *Sphagnum* and limited soil respiration caused the mosses to grow ~5cm above the rims of the tubes, while in the low water table treatments *Sphagnum* growth was minimal, soil respiration was high and the surface of the peat subsided by ~10cm. Thus, after nine months there was a height difference of approximately 20 cm between the two most different treatments. In the first year we decided to level the *Sphagnum* layers back to their original positions by adding or removing peat from underneath the *Sphagnum* layer. However, this manipulation created disturbance in the mesocosms, especially in the *Sphagnum* structure, and should be avoided if the study aims primarily to assess peat decomposition. Again, this was less critical for our surface microbial studies. Nevertheless we decided not to reproduce this manipulation after the second growing season. This issue could be solved by installing the peat cores on jacks, which would be adjusted to maintain the top of the *Sphagnum* carpet at the same level in all treatments. If the entire tank had been filled with peat, then the water level could be adapted to the *Sphagnum* layer level by moving the siphon connecting the replicates up or down.

The cost and design of the monitoring setup could be greatly improved. So far, we have visited the experimental site to retrieve the data from the different data loggers. This is not an issue because our experimental site is easily and quickly accessible. For a less accessible experiment, or for maintenance issues, we would prefer to set up data logging on a webserver, through GPRS connection. It is possible to do this using commercial data loggers (e.g. Decagon), which are expensive, or to use any microcontroller board (such as Arduino, Tiva, or

Parallax propeller) associated with a GPRS shield. Although it would be possible to set up our own webserver from scratch, various platforms already exist, such as Nimbits (<http://www.nimbits.com>) and Xively (<https://xively.com/>) and can easily be used with home-made data loggers. The GPRS shield associated with an Arduino board can, for instance, be used with the Pushingbox service (<http://www.pushingbox.com/>) to send data to a Google spreadsheet, *via* simple POST requests.

Finally, building and maintaining such a system requires permanent awareness of weather conditions. Every system dealing with water is extremely sensitive to frost and the frost-sensitive parts have to be easy to remove in winter. In our setup, the pumps are installed on a rack that can be disassembled and stored during winter, as frost would permanently damage the pumps. Similarly, we had to disassemble the toilet flush devices. For this reason, it is crucial to carefully label every piece with the identity of the mesocosm to which it belongs, so that it will not be necessary to recalibrate the whole system in spring. To avoid surprises like sudden frost or potentially harmful events such as hail, we developed a Python script parsing the online daily weather forecast using the Wunderground Weather API website. This small script sends us emails if it detects frost, storm, hail or any given keyword. It can, for instance, be installed on a university server or a RaspberryPi, and launched periodically using Linux cron command scheduler. The code is provided in the supplementary material.

To conclude, mesocosm approaches are useful for studying global change impacts on ecosystems. Although often simple in their conception at first glance, they easily become challenging to optimise and maintain. We hope the description given here will be useful to other researchers planning comparable experiments. It may indeed be interesting to develop a standard mesocosm system that could then be used in multisite studies. In such a context, cost and robustness will be critical, as well as optimised and automated data transfer to allow project co-ordinators to follow the progress of the whole experiment in real time.

ACKNOWLEDGEMENTS

This project was supported by grant no. PSPB-013/2010 from Switzerland through the Swiss Contribution to the enlarged European Union and additional funds from the University of Neuchâtel. We thank Andy Baird and an anonymous reviewer for their constructive criticisms of this article.

REFERENCES

- Belyea, L.R. & Malmer, N. (2004) Carbon sequestration in peatland: patterns and mechanisms of response to climate change. *Global Change Biology*, 10, 1043–1052.
- Blodau, C. & Moore, T.R. (2003) Experimental response of peatland carbon dynamics to a water table fluctuation. *Aquatic Sciences - Research Across Boundaries*, 65, 47–62.
- Bragazza, L., Buttler, A., Siegenthaler, A. & Mitchell, E.A.D. (2009) Plant litter decomposition and nutrient release in peatlands. In: Baird AJ, Belyea LR, Comas X, Reeve AS, Slater LD, editors. *Geophysical Monograph Series. Vol. 184*. Washington, D. C.: American Geophysical Union. pp. 99–110.
- Bridgman, S.D., Pastor, J., Dewey, B., Weltzin, J.F. & Updegraff, K. (2008) Rapid carbon response of peatlands to climate change. *Ecology*, 89, 3041–3048.
- Bridgman, S.D., Pastor, J., Updegraff, K., Malterer, T.J., Johnson, K., Harth, C. & Chen, J. (1999) Ecosystem control over temperature and energy flux in northern peatlands. *Ecological Applications*, 9, 1345–1358.
- Buttler, A., Grosvernier, P. & Matthey, Y. (1998) A new sampler for extracting undisturbed surface peat cores for growth pot experiments. *New Phytologist*, 140, 355–360.
- Chambers, J.M. & Hastie, T.J. (1991) *Statistical Models in S*. New Edition, Chapman and Hall/CRC, 624 pp.
- Davidson, E.A. & Janssens, I.A. (2006) Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. *Nature*, 440, 165–173.
- Dieleman, C.M., Branfireun, B.A., McLaughlin, J.W. & Lindo, Z. (2015) Climate change drives a shift in peatland ecosystem plant community: Implications for ecosystem function and stability. *Global Change Biology*, 21, 388–395.
- Faubert, P., Tiiva, P., Rinnan, Å., Rätty, S., Holopainen, J.K., Holopainen, T. & Rinnan, R. (2010) Effect of vegetation removal and water table drawdown on the non-methane biogenic volatile organic compound emissions in boreal peatland microcosms. *Atmospheric Environment*, 44, 4432–4439.
- Fisher, D.K. & Gould, P.J. (2012) Open-source hardware is a low-cost alternative for scientific instrumentation and research. *Modern Instrumentation*, 01, 8–20.
- Froking, S. & Roulet, N.T. (2007) Holocene radiative forcing impact of northern peatland carbon accumulation and methane emissions. *Global Change Biology*, 13, 1079–1088.
- Gurland, J. & Whittle, P. (1954) Hypothesis testing in time series analysis. *Journal of the American Statistical Association*, 49, 197–200.
- Haapala, J.K., Mörsky, S.K., Rinnan, R., Saarnio, S., Martikainen, P.J., Holopainen, T. & Silvola, J. (2011) Long-term effects of ozone on CO₂ exchange in peatland microcosms. *Atmospheric Environment*, 45, 4002–4007.
- Honaker, J., King, G. & Blackwell, M. (2011) Amelia II: A program for missing data. *Journal of Statistical Software*, 45(7), 1–47.
- Hornik, K. (1991) Approximation capabilities of multilayer feedforward networks. *Neural Networks*, 4, 251–257.
- Huguet, A., Fosse, C., Laggoun-Défarge, F., Delarue, F. & Derenne, S. (2013) Effects of a short-term experimental microclimate warming on the abundance and distribution of branched GDGTs in a French peatland. *Geochimica et Cosmochimica Acta*, 105, 294–315.
- IPCC (2014) *Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change* [Core Writing Team, R.K. Pachauri and L.A. Meyer (eds.)]. IPCC, Geneva, Switzerland, 151 pp. Online at: http://www.ipcc.ch/pdf/assessment-report/ar5/syr/SYR_AR5_FINAL_full.pdf
- Kajornrit, J., Wong, K.W. & Fung, C.C. (2012) Estimation of missing precipitation records using modular artificial neural networks. In: Huang T., Zeng, Z., Li, C., & Leung, C.S., (eds.) *Neural Information Processing*, 7666, 52–59.
- Kuligowski, R.J. & Barros, A.P. (1998) Using artificial neural networks to estimate missing rainfall data. *Journal of the American Water Resources Association*, 34, 1437–1447.
- Morris, S.A., Radajewski, S., Willison, T.W. & Murrell, J.C. (2002) Identification of the functionally active methanotroph population in a peat soil microcosm by stable-isotope probing. *Applied Environmental Microbiology*, 68, 1446–1453.
- Preston, M.D., Eimers, M.C. & Watmough, S.A. (2011) Effect of moisture and temperature variation on DOC release from a peatland: Conflicting results from laboratory, field and historical data analysis. *Science of the Total Environment*, 409, 1235–1242.
- Rahman, M.T., Crombie, A., Moussard, H., Chen, Y. & Murrell, J.C. (2011) Acetate repression of methane oxidation by supplemental *Methylocella silvestris* in a peat soil microcosm. *Applied and*

- Environmental Microbiology*, 77, 4234–4236.
- Rinnan, R., Rinnan, Å., Holopainen, T., Holopainen, J.K. & Pasanen, P. (2005) Emission of non-methane volatile organic compounds (VOCs) from boreal peatland microcosms—effects of ozone exposure. *Atmospheric Environment* 39, 921–930.
- Rydin, H. & Jeglum, J.K. (2006) *The Biology of Peatlands*. Oxford University Press, 360 pp.
- Thormann, M.N., Bayley, S.E. & Currah, R.S. (2004) Microcosm tests of the effects of temperature and microbial species number on the decomposition of *Carex aquatilis* and *Sphagnum fuscum* litter from southern boreal peatlands. *Canadian Journal of Microbiology*, 50, 793–802.
- Tiiva, P., Faubert, P., Rätty, S., Holopainen, J.K., Holopainen, T. & Rinnan, R. (2009) Contribution of vegetation and water table on isoprene emission from boreal peatland microcosms. *Atmospheric Environment*, 43, 5469–5475.
- Turunen, J., Tomppo, E., Tolonen, K. & Reinikainen, A. (2002) Estimating carbon accumulation rates of undrained mires in Finland—application to boreal and subarctic regions. *The Holocene*, 12, 69–80.
- van Winden, J.F., Reichart, G.-J., McNamara, N.P., Benthien, A. & Damsté, J.S.S. (2012) Temperature-induced increase in methane release from peat bogs: a mesocosm experiment. *PLoS ONE*, 7(6), e39614.
- Yu, Z.C. (2012) Northern peatland carbon stocks and dynamics: a review. *Biogeosciences*, 9, 4071–4085.
- Submitted 15 Apr 2015, revision 04 Oct 2015
Editor: Olivia Bragg
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