

Measuring response of photosynthetic rate of *Sphagnum palustre* L. to water content: Importance of adjustment of the water content to the habitat condition

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SUMMARY

Sphagnum mosses play an important role in the carbon sink function of northern peatlands. *Sphagnum* photosynthesis is influenced by hydrological factors such as gravimetric water content (WC) and water table depth. However, there is no unified method for evaluating the photosynthetic rate under field conditions. In our study, *Sphagnum palustre* capitula collected from four regions in Japan were adjusted to equilibrium WC at three water levels, then changes in net photosynthetic rate (P_n) under saturated light were measured from equilibrium WC to dry condition. P_n was low at both high and low WC, indicating that there is an optimum WC at which P_n is maximised. The equilibrium WC varied with water level, resulting in different P_n values at equilibrium WC. Thus, to accurately estimate photosynthetic rate, the moisture status of the plants should be considered; and to understand the photosynthetic capacity of the habitat, WC should be adjusted to the equilibrium value corresponding to the water level in the habitat. The maximum P_n differed between collected regions, suggesting an influence of *Sphagnum* morphology or prior habitat conditions; however, the details remain unknown. Further studies on the correlation between P_n and *Sphagnum* morphology or environmental conditions in the source habitat are needed to understand and predict the potential response to climate change.

KEY WORDS: capitulum, equilibrium, net photosynthetic rate, *Sphagnum* morphology, water level

INTRODUCTION

Northern (mid- to high-latitude) peatlands store large amounts of soil carbon by accumulating dead plants as peat under cool and humid conditions. The total amount of soil carbon stored in northern peatlands accounts for one-third to one-fifth of the global soil carbon stock (Gorham 1991, Yu 2012). The environmental conditions of peatlands have attracted attention because environmental changes can affect the carbon pool. In these peatlands, *Sphagnum* mosses are widely distributed and contribute to the carbon sink function, as they comprise a sizeable proportion of the peat that makes up the carbon pool (Clymo & Hayward 1982). Moreover, they modify hydrochemistry producing acidic and oligotrophic water, which inhibits the decomposition of plant remains by microorganisms resulting in peat accumulation under cool and wet conditions. Therefore, the presence of *Sphagnum* moss species, which modify their habitat conditions as ‘ecosystem engineers’ (Clymo & Hayward 1982, van Breemen 1995, Granath *et al.* 2012), plays an important role in maintaining the carbon sink function of northern peatlands (Strack *et al.* 2006).

Global warming may negatively affect peatland ecosystems by changing the hydro-meteorological conditions. For example, it has been reported that *Sphagnum* forming high hummocks on peatlands in the Italian Alps dried out irreversibly during an extreme heatwave accompanied by severe drought (Bragazza 2008). Global warming increases the frequency of such extreme meteorological events (Jentsch & Beierkuhnlein 2008) and could eventually make it difficult for *Sphagnum* communities to survive, and thus further degrade the carbon sink function of peatlands. In addition, rising temperature accelerates peat decomposition (He *et al.* 2016, Wilson *et al.* 2016) and eventually increases ecosystem respiration (Samson *et al.* 2018), resulting in the acceleration of global warming (Gallego-Sala *et al.* 2018). Therefore, understanding the response of *Sphagnum* growth and photosynthesis to environmental changes is essential for predicting the response of peatland carbon budgets to climate change (Moore *et al.* 1998).

The photosynthetic rate of *Sphagnum* is strongly influenced by water availability (Silvola & Aaltonen 1984, Rydin & McDonald 1985, Silvola 1990, Williams & Flanagan 1996, van Gaalen *et al.* 2007,

Fukuta *et al.* 2012). *Sphagnum* mosses can store plenty of water inside their hyaline cells, which connect via pores with the so-called ‘external capillary system’ on the uneven outer surfaces of the shoots. Water is acquired through passive mechanisms such as capillary rise from groundwater and rainwater seepage (Yazaki *et al.* 2006). Because *Sphagnum* lacks stomata, it cannot control water loss by transpiration (Williams & Flanagan 1996). Also, water forms a layer on the surface of the plant body that controls CO₂ diffusion (Silvola 1990) and is directly involved in photosynthetic responses. Therefore, the hydrological environment in the habitat, which determines water availability, is important for survival of these mosses. In previous studies assessing water availability, gravimetric water content, hereafter referred to as ‘water content (WC)’, was used to express the amount of water in the *Sphagnum*, and the relationships between WC and photosynthetic rate were examined (Silvola & Aaltonen 1984, Rydin & McDonald 1985, Silvola 1990, Williams & Flanagan 1996, van Gaalen *et al.* 2007, Fukuta *et al.* 2012). The WC of living *Sphagnum* and intact peat is affected by distance from the water table (Clymo & Hayward 1982, Rydin 1985, Kellner & Halldin 2002, Yazaki *et al.* 2006). To understand the photosynthetic capacity of *Sphagnum* in the wild, it is necessary to quantify the photosynthesis performed by *Sphagnum* capitula when subjected to moisture conditions similar to those in their natural habitats. However, in previous studies on photosynthesis of *Sphagnum*, the capitula or shoot samples were not handled in such a manner. Many studies involved bringing samples to the laboratory and measuring the photosynthetic rate under a variety of moisture conditions, while others adjusted the WC to a defined level (Silvola 1990) or submerged the samples in water (Haraguchi & Yamada 2011). In other words, hitherto there has been no standard protocol for re-adjusting the WC of collected *Sphagnum* samples to reflect the habitat water level before measuring the photosynthetic rate.

The aim of our study was to establish a method for evaluating the relationship between WC at various water levels and photosynthetic rate of *Sphagnum*. We first created simple moisture-control equipment that can adjust the WC of *Sphagnum* capitula to equilibrium at different water levels. Next, we set up a closed chamber system that could continuously measure the photosynthetic rate under conditions of light saturation and controlled temperature. From relative humidity measurements inside the chamber we deduced that *Sphagnum* samples dried gradually during each series of measurements, and assessed the relationship between *Sphagnum* WC and

photosynthetic rate. These experiments were performed on samples that had been adjusted to different water levels. We tested *Sphagnum palustre* samples from several regions in Japan to investigate whether reactions of photosynthetic rate to capitulum wetness are widespread, and thus to gain insights about the photosynthetic characteristics of *Sphagnum*.

METHODS

Sample collection and pre-treatment

Sphagnum palustre L. is widely distributed in relatively eutrophic wetland environments with shallow water table (Daniels & Eddy 1985) in temperate zones, including warm-temperate central to southeastern Japan (Fukuta *et al.* 2012). In May 2018 we started to cultivate *Sphagnum palustre* originating from Iwate Prefecture in northern Japan that we purchased from a gardening store, as well as moss of the same species collected from the bank of a drainage channel at Bibai (43.32 °N, 141.81 °E, 16 m a.s.l.) in northernmost Japan (on Hokkaido Island). *Sphagnum palustre* was also collected from a reed-dominated (*Phragmites australis* (Cav.) Trin. ex Steud.) mire in Nanporo, Hokkaido (43.10 °N, 141.64 °E, 7 m a.s.l.) in northern Japan and from a *Sphagnum* cultivation field in Aso on Kyushu Island (32.89 °N, 131.00 °E, 492 m a.s.l.) in eastern Japan. The climatic, micrometeorological and hydro-chemical conditions are listed in Table 1.

The *Sphagnum palustre* from all sources was grown on the rooftop of a building in Kawasaki, central Japan (35.61 °N, 139.55 °E, 63 m a.s.l.). The climate is hot with humid summers and mild, dry winters. According to meteorological records obtained from the closest meteorological station (Fuchu, located 9.9 km to the north), the 1991–2020 annual mean air temperature was 15.4 °C and mean annual precipitation was 1599 mm (Table 1).

To allow the *Sphagnum* to grow under shaded conditions, the mosses originating from Iwate and Bibai were grown under cheesecloth (50 % shading) starting in May 2018 and those from Nanporo and Aso was grown under the eaves of the building (approximately 90 % shading) starting in August 2020. In both cases, the degree of shading was assessed by measuring the difference in photosynthetically active photon flux density (PPFD) above and below the shading material using a quantum sensor (LI190, LI-COR, USA).

The mosses were grown in water-filled plastic containers, on 5 cm thick floating polystyrene rafts with equally spaced holes of diameter 4 cm. Each



Table 1. Climate, micrometeorological and hydrochemical conditions at the sites from which *Sphagnum* was sampled and at the growing site on the rooftop of a building in Kawasaki. Shade is expressed as the quotient of PPFD incident on the *Sphagnum* and the global PPFD measured above the shading material. NA = no data available.

Collected region	Sampling or growing site	Mean air temperature ⁽¹⁾			Total precipitation ⁽¹⁾		Shade ⁽²⁾	Hydrochemical conditions ⁽²⁾		
		Annual 1991–2020 (°C)	January (lowest) 2018–2020 (°C)	August (highest) 2018–2020 (°C)	Annual 1991–2020 (mm)	Growing season (April–November) 2018–2020 (mm)		Water level (cm)	pH	EC ($\mu\text{S cm}^{-1}$)
Aso	<i>Sphagnum</i> cultivation field	13.2	2.8	24.8	3010	2369	0.0	-12	6.12	210.0
Iwate ⁽³⁾	Gardening store	10.6	1.2	25.1	1280	1031	NA	NA	NA	NA
Nanporo	Reed-dominated mire	7.3	-6.0	21.0	965	705	40 to 98	-20.0 to -26.5	5.29	207.0
Bibai	Shrub-shaded bank of a drainage channel	7.3	-6.7	21.2	1174	839	47	-8.3	5.37	47.3
Kawasaki	Rooftop of a building	15.4	4.7	28.3	1599	1324	50 and 90	-5 to -10	NA	NA

⁽¹⁾ Climate and meteorological data were obtained from the website of the Japanese Meteorological Agency (Japan Meteorological Agency 2022).

⁽²⁾ The shade and hydrochemical conditions were monitored from May to August 2022.

⁽³⁾ Meteorological data for Iwate were obtained from the nearest available Local Meteorological Observatory in Morioka, the capital of Iwate prefecture.

hole was filled with commercially available horticultural moss peat that had been being washed with deionised water (electrical conductivity (EC) less than $2.0 \mu\text{S cm}^{-1}$) until the EC of the pore water decreased to $< 50 \mu\text{S cm}^{-1}$. The entire surface of the raft was also covered with moss peat (approximately 1 cm thick) to create a foundation for growth of the collected *Sphagnum* (Hoshi 2017). Subsequently we placed shoots of *Sphagnum palustre* (including the capitula) on the rafts. The water level in the container was maintained through periodic irrigation with rainwater and deionised water.

The *Sphagnum* shoots from Aso, Iwate and Bibai were similar in size, whereas those from Nanporo were more slender with longer stem leaves (Figure 1). The shoots from Aso and Nanporo elongated by a few centimetres during the two months following planting whereas those from Iwate and Bibai did not elongate during the summer of the experimental year. Shoots were taken from the rafts for use in the experiments.

Moisture control

Moisture-control equipment made of PVC pipe (height 30 cm, inner diameter 7.5 cm) with holes (diameter approximately 0.5 cm) at 5 cm intervals on all sides was prepared. The base of the pipe was covered with (1 mm) cheesecloth mesh, then it was filled to the uppermost holes with washed horticultural peat and placed in a plastic container. Deionised water was added up to the target water level (5, 10 or 15 cm below the upper surface of the peat). The target water levels of -5 cm to -15 cm were chosen because *Sphagnum palustre* grows naturally in wet conditions (Daniels & Eddy 1985) with water level ranging from -2 cm to -20 cm throughout the growing season (Ohtake & Kashimura 1993). The positive correlation between water level and matric potential, which was measured using a tensiometer-connected handy manometer (102RP, Nidec Copal Electronics, Japan; Figure 2), confirmed the capillary water connection between water table and peat surface.

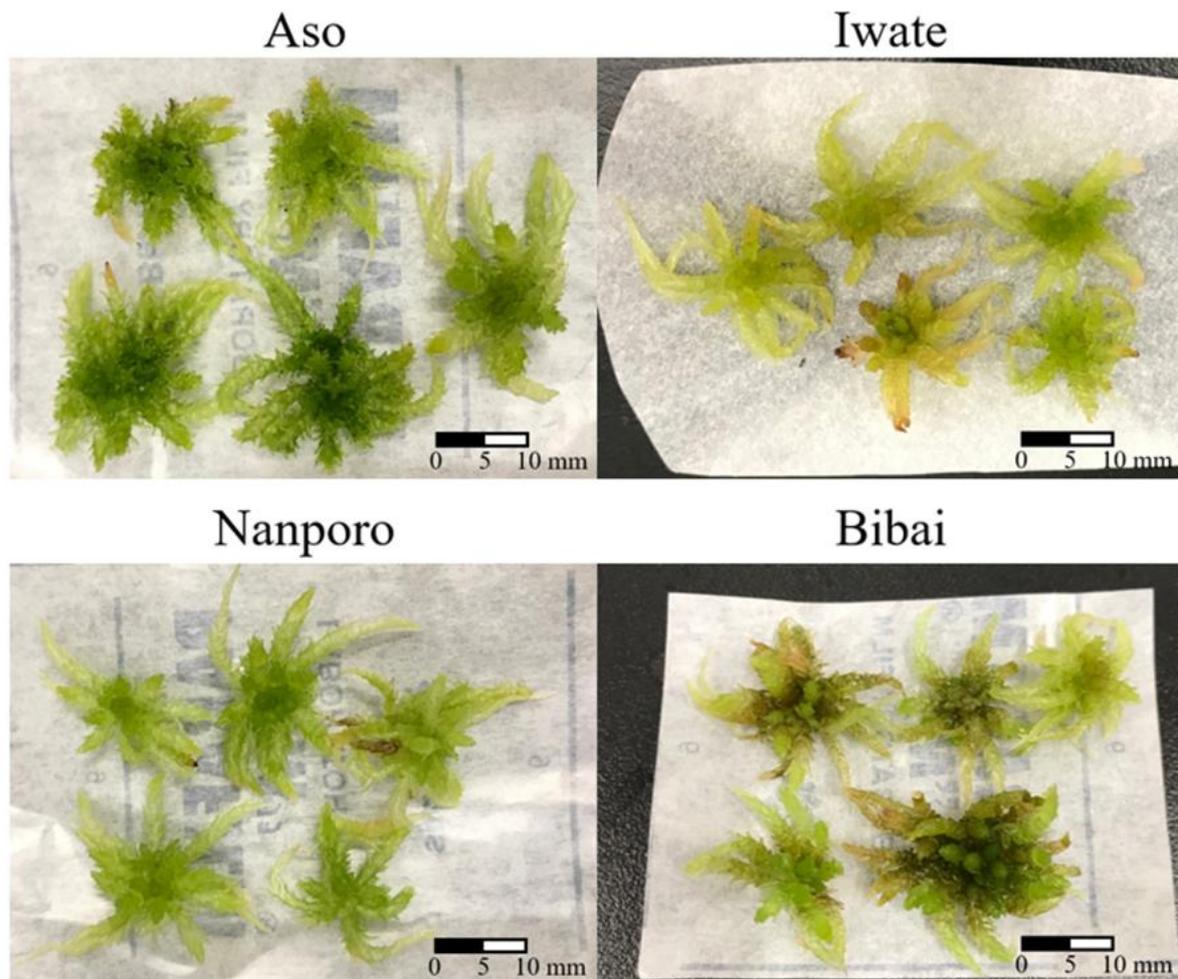


Figure 1. Typical-sized samples of *Sphagnum palustre* capitula from Aso, Iwate, Nanporo and Bibai.

Five capitula of Iwate *Sphagnum palustre* growing on a raft were cut from their stems 1 cm below the apex, submerged in deionised water for approximately 10 minutes, then placed in close contact with the peat surface in the moisture-control equipment. Thus, the water content of the capitula should approach the equilibrium level corresponding to the matric potential at the peat surface. To discover the time required for moisture conditions to reach equilibrium, we followed the relationship between gravimetric WC and the time elapsed since removing the *Sphagnum* capitula from the water. During this experiment we covered the upper end of the PVC pipe with a stainless steel lid to prevent evaporation of water from the capitula. The experiment was conducted at water levels of -5, -10 and -15 cm, and the capitula were weighed using an electronic balance (ATX124R, Shimadzu, Japan) at intervals of 15 minutes. Equilibrium was reached after 90 minutes (see the ‘Moisture control of *Sphagnum* capitula’ subsection in RESULTS). Therefore, we concluded that moisture control should be performed for 90 minutes.

For the subsequent experiments described below, the moisture content of the samples was adjusted using the following procedure. First, five typical-sized sample capitula were cut (approximately 1 cm below the shoot apex) from a raft. These were submerged in deionised water for approximately 10 minutes, then placed in close contact with the peat surface in the moisture-control equipment. During moisture control, a plastic lid was placed over the container to prevent water loss by evaporation. After 90 minutes, the moisture-controlled capitula

were removed from the equipment and placed on a water-impermeable film to avoid overlap with each other. They were weighed before the photosynthesis measurement.

Photosynthesis measurements

We measured the photosynthetic rate of *Sphagnum palustre* from August to October 2020. We prepared a photosynthesis measurement system consisting of a handmade transparent acrylic chamber (250 mL), an infrared gas analyser (IRGA: LI820, LI-COR, USA), solenoid valves (AG43-03-4, CKD, Japan), flow meters (RK1710, KOFLOC, Japan), a pump (CM-15-24, Enomoto Micro Pump Manufacturing Co. Ltd., Japan) and an air-gas (420 ppmv CO₂) cylinder. These were connected together using nylon tubing, as shown in Figure 3. This system had two alternative air flow paths, namely the circulation path and the air-gas supply path. The path could be switched periodically using solenoid valves controlled by a data logger (CR1000, Campbell Scientific Inc., USA). In the circulation path, the connections to the air-gas cylinder and evacuation were closed, the air was circulated using a pump at a flow rate of approximately 0.8 L min⁻¹, and the IRGA detected fluctuations in the CO₂ concentration in the chamber. In the air-gas supply path, the connections to the air-gas cylinder and evacuation were open, air-gas from the cylinder was introduced into the circuit at a flow rate of approximately 0.8 L min⁻¹ or higher, and the air in the chamber was thus replaced with air-gas from the cylinder. During measurements, the path was switched every 120 seconds and the duration of one measurement cycle was 240 seconds.

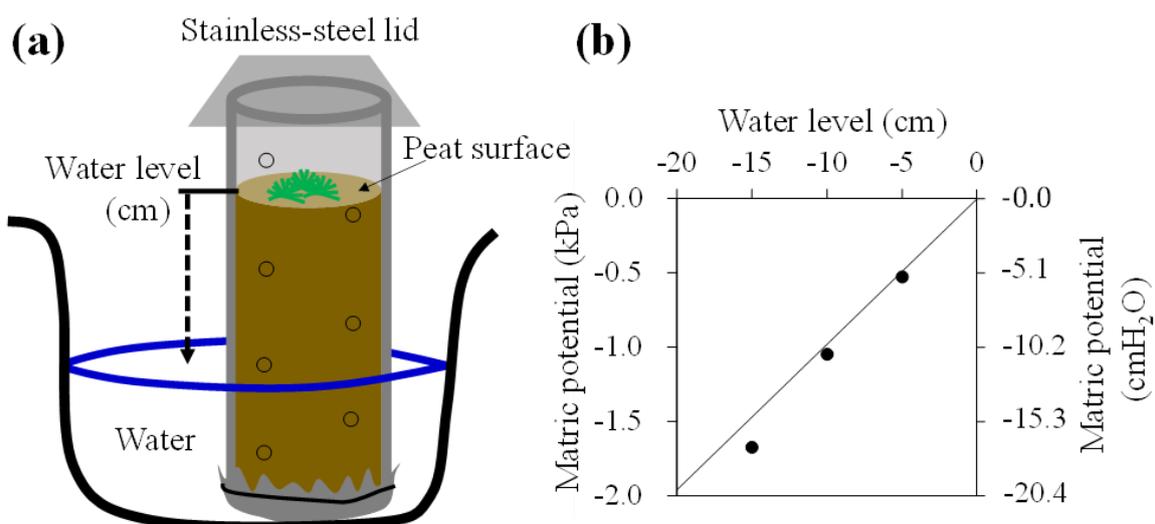


Figure 2. Moisture-control equipment: (a) schematic diagram of the equipment, and (b) the relationship between water level and equilibrium matric potential at the peat surface. The line in panel (b) shows how the equilibrium matric potential of soil water at the surface varies with water level.

The chamber was placed in an incubator. The air temperature in the chamber was maintained at a constant level by manipulating the temperature setting of the incubator, and was measured using a T-type thermocouple thermometer. Relative humidity and photosynthetic photon flux density (PPFD) inside the chamber were measured using, respectively, a hygrometer (HM1500LF, TE connectivity, USA) and a quantum sensor (SQ-110, Apogee, USA) which was calibrated using a high-precision quantum sensor (LI190, LI-COR, USA). An LED light (VEFA46WFJ, Altrader Co., Japan), which can change the PPFD from 0 to 500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ with a wavelength range of 380–800 nm was installed at a point 5 cm above the top of the chamber. Data were recorded by the data logger at 2-second time intervals.

Measurement of WC and photosynthetic rate

We measured the photosynthetic rate and *Sphagnum* WC simultaneously. During measurement, water evaporated from the *Sphagnum* samples and, in consequence, the photosynthetic rate decreased due to desiccation. *Sphagnum* WC was calculated from the changes in sample weight and air humidity during measurement. The total water loss from the samples was determined by weighing them before and after the chamber measurement. We could determine the

water loss associated with the circulation path (Figure 3) from the increase in water vapour concentration, but water loss via the air-gas supply path could not be measured. Therefore, we assumed that the rate of evaporation from the samples was the same in both paths and introduced a correction ratio to calculate the WC of the samples during measurement. The WC for each measurement cycle was calculated as follows:

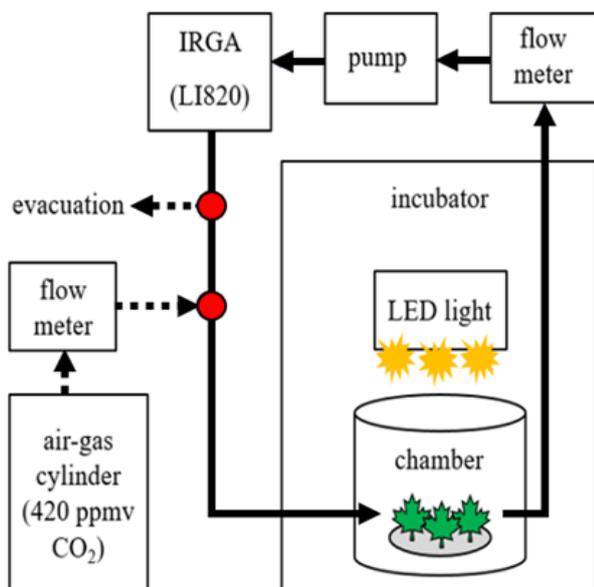
$$WC = \frac{(w_{start} - \sum E \times M_{cor})}{w_d} \times 100 \quad [1]$$

where w_{start} is the fresh weight (g) before the measurement, $\sum E$ is the cumulative evaporation (g) calculated as the cumulative increase in water vapour content during the measurement cycle, w_d is the dry weight (g) of the sample, and M_{cor} is a correction ratio for evaporative water loss calculated using Equation 2:

$$M_{cor} = \frac{(w_{start} - w_{end})}{\sum E_{total}} \quad [2]$$

where w_{end} is the fresh weight (g) after the measurement and $\sum E_{total}$ is the total evaporation calculated from the cumulative increase in water vapour content during all circulation path phases.

(a) Circulation path



(b) Air-gas supply path

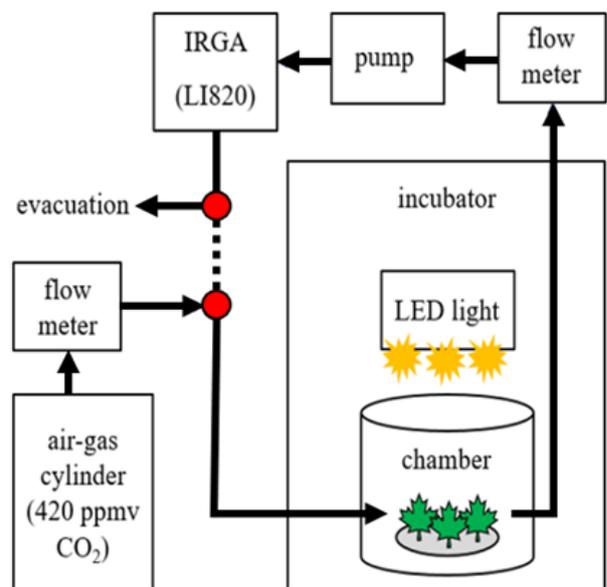


Figure 3. Photosynthesis measurement system. This system has two paths: (a) the circulation flow path to measure the CO₂ absorption/emission rate; and (b) the (dry) air-gas supply path to reset the CO₂ concentration. Solid arrows in the schematic diagrams denote the air flow during the respective paths. Red circles are solenoid valves, and the broken arrows and line denote connections in which gas does not flow.



During the photosynthesis measurements, the air temperature in the chamber was set to approximately 25 °C and the PPFD to 500 $\mu\text{mol m}^{-2} \text{s}^{-1}$, a value at which the photosynthetic rate of *Sphagnum palustre* is considered to reach a light-saturated condition (Maberly & Spence 1983, Harley *et al.* 1989). After the chamber temperature was stable at 25 ± 0.5 °C, moisture-controlled sample capitula were placed in the chamber and photosynthesis measurements were started. The photosynthetic rate changed with time and finally decreased because of sample desiccation. Measurement was stopped when the photosynthetic rate reached its minimum.

After the measurements were stopped, the samples were weighed using an electronic balance then oven-dried at 70 °C for at least 48 hours, after which the dry weights were measured.

Calculation of photosynthetic rate

The net photosynthetic rate per unit dry weight ($Pn_{original}$; $\text{mg CO}_2 \text{ g}^{-1} \text{ h}^{-1}$) was calculated from the rate of CO_2 concentration change during the last 70 s of the circulation path measurement using the following equation:

$$Pn_{original} = \frac{dc}{dt} \frac{PV}{RT} \frac{44}{w_d} \frac{60 \times 60}{1000} \quad [3]$$

where P is the atmospheric pressure (hPa), V is the volume of the chamber ($4.50 \times 10^{-4} \text{ m}^3$), R is the gas constant of the air ($8.314 \text{ Pa m}^3 \text{ mol}^{-1} \text{ K}^{-1}$), T is the air temperature in the chamber (K), and dc/dt is the rate of change of CO_2 concentration ($\mu\text{mol mol}^{-1} \text{ s}^{-1}$).

During measurement, the water vapour concentration changed remarkably because of evaporation from the samples. An increase in water vapour concentration could dilute the CO_2 in the chamber. Therefore, to eliminate the effect of dilution by water vapour (Matsuura *et al.* 2011), we also calculated the corrected net photosynthetic rate (Pn) using Equation 4:

$$Pn = \frac{dc}{dt} \frac{PV}{RT} (1 - f_w) \frac{44}{w_d} \frac{60 \times 60}{1000} \quad [4]$$

where f_w is the mole fraction of water vapour (mol mol^{-1}). Pn reached a maximum value at 'optimum WC', i.e. Pn was lower at both higher- and lower-than-optimum WC. The 'maximum Pn ' and the optimum WC were determined from the average of the top five Pn values and their WC values, respectively. The Pn and WC values used for analysis were obtained 20 or more minutes after starting the measurement, when the gas exchange of the samples

was considered to have stabilised. The values of Pn and WC after 20 minutes were denoted as ' $Pn_{eq,WL}$ ' and 'equilibrium WC' or ' WC_{eq} ', respectively. $Pn_{eq,WL}$ denotes the Pn at WC_{eq} .

Statistical analysis

To examine the effects of collected region and water level on Pn and WC, a two-way factorial ANOVA was performed for the maximum Pn and the optimum WC, as well as for the $Pn_{eq,WL}$ and WC_{eq} . In addition, to compare WC and Pn between collected regions, multiple comparisons were performed using the Tukey-Kramer method. Before these analyses, the photosynthesis and WC data (x) were transformed to $\log(x + 1)$. These tests were conducted using R (version 4.02) statistical analysis software.

RESULTS

Moisture control of *Sphagnum* capitula

After installation on the moisture-control equipment, the relative water content (g g^{-1} of initial value) of *Sphagnum palustre* capitula from Iwate decreased asymptotically with time (Figure 4) and approached equilibrium within 90 minutes at all water levels.

The WC_{eq} of the *Sphagnum* capitula, which is the equilibrium WC specific to the water level, differed between samples from different regions and between water levels (Figures 5 and 6a). The results of the two-way ANOVA indicated that both the collected region and water level affected WC_{eq} (Table 2). WC_{eq} was largest at the -5 cm, intermediate at the -10 cm and smallest at the -15 cm water level (Figure 6a). Under the same water level conditions, WC_{eq} was significantly ($p < 0.05$) higher in the Nanporo sample than in the samples from the other collected regions, at all water levels (Table A1 in the Appendix).

Response of net photosynthetic rate to wetness

At all water levels, the relationships between net photosynthetic rate (Pn) and WC for *Sphagnum* from all collected regions were convex (Figure 5), i.e. there was an optimum WC that maximised Pn . The results of the two-way ANOVA indicated that the maximum Pn differed among the collected regions (Figure 7b, Table 2). The maximum Pn was higher in the samples from Aso and Nanporo than in the samples from Iwate and Bibai at all water levels (Figure 7b, Table A1). The optimum WC ranged from 1141 ± 108 % (Aso, water level -15 cm) to 2045 ± 175 % (Nanporo, water level -5 cm). The two-way ANOVA also indicated that the optimum WC differed with respect to both collected region and water level (Figure 7a,

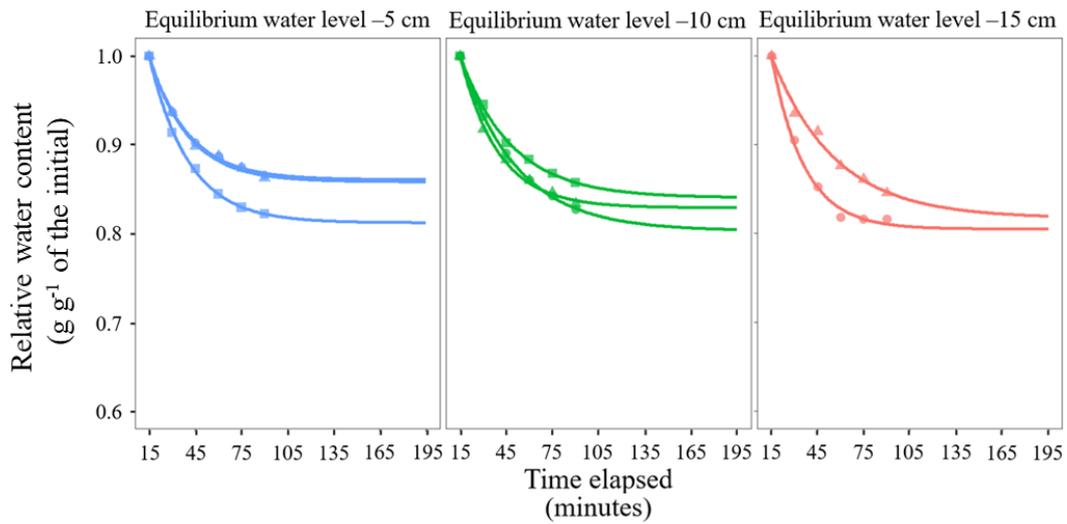


Figure 4. Time series of relative water content (g g^{-1} of the initial weight) of *Sphagnum palustre* (five capitula) from Iwate after installation in the moisture-control equipment ($n = 3$, but only 2 at -15 cm water level). Different symbols (closed circle, triangle, square) represent results for different samples.

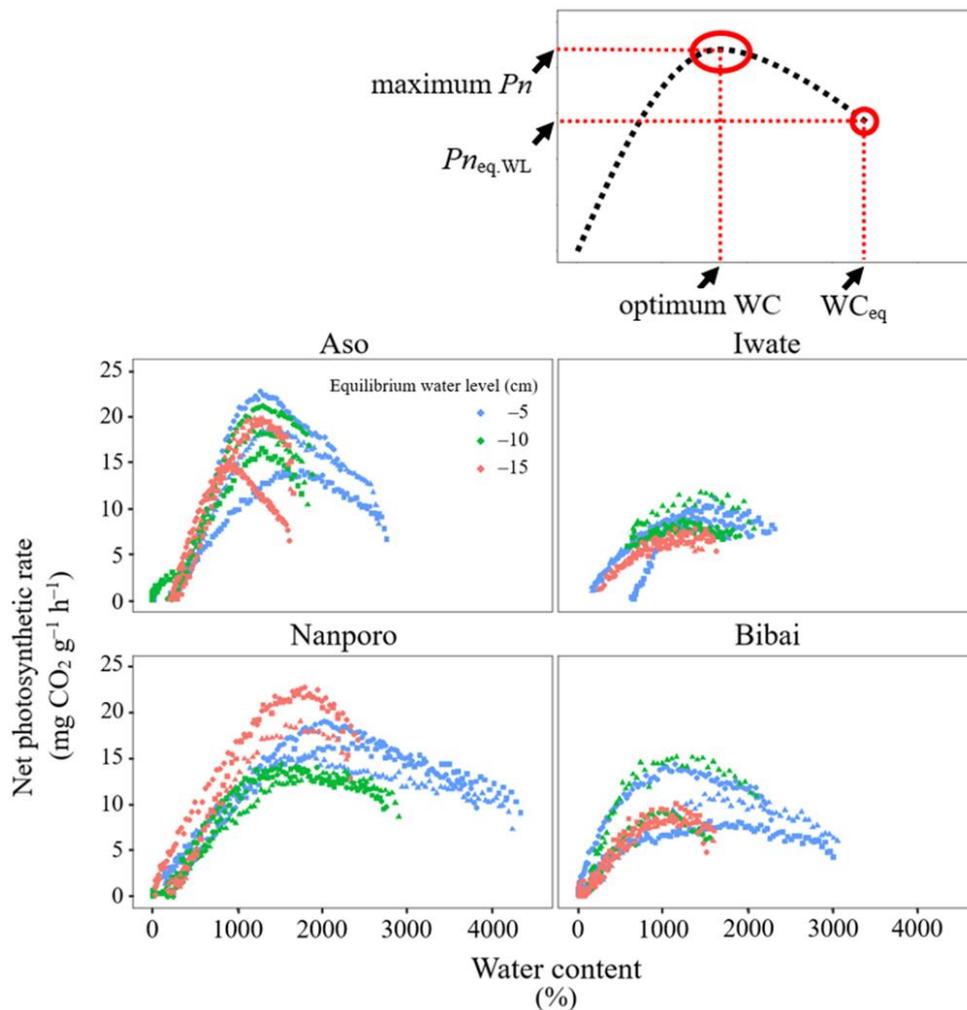


Figure 5. Relationships between gravimetric water content (WC) and net photosynthetic rate (P_n) of *Sphagnum palustre* from the various collected regions. P_n was measured at 25.0 ± 0.5 °C under saturated light (PPFD $500 \mu\text{mol m}^{-2} \text{s}^{-1}$). Different symbols (closed circle, triangle and square) represent results for different samples.

Table 2). The optimum WC was higher in the sample from Nanporo (1749–2045 %) than in the samples from the other regions (1141–1527 %) at all water levels (Figure 7a, Table A1).

Net photosynthetic rate at different water levels

In all experiments, WC_{eq} was higher than the optimum WC at all water levels (Figure 5). The difference between the optimum WC and WC_{eq} tended to be largest at the -5 cm, followed by the -10 cm and -15 cm water levels (Table A1). The

results of the two-way ANOVA indicated that $Pn_{eq,WL}$, which is the Pn corresponding to WC_{eq} for the water level, was influenced by the collected region (Figure 6b, Table 2). According to multiple comparisons, $Pn_{eq,WL}$ at the -10 cm and -15 cm water levels was higher in the samples from Aso and Nanporo than in those from Iwate and Bibai (Figure 6b, Table A1). The difference between the maximum Pn and $Pn_{eq,WL}$ tended to be larger at the -5 cm water level than at the -10 cm and -15 cm water levels (Table A1).

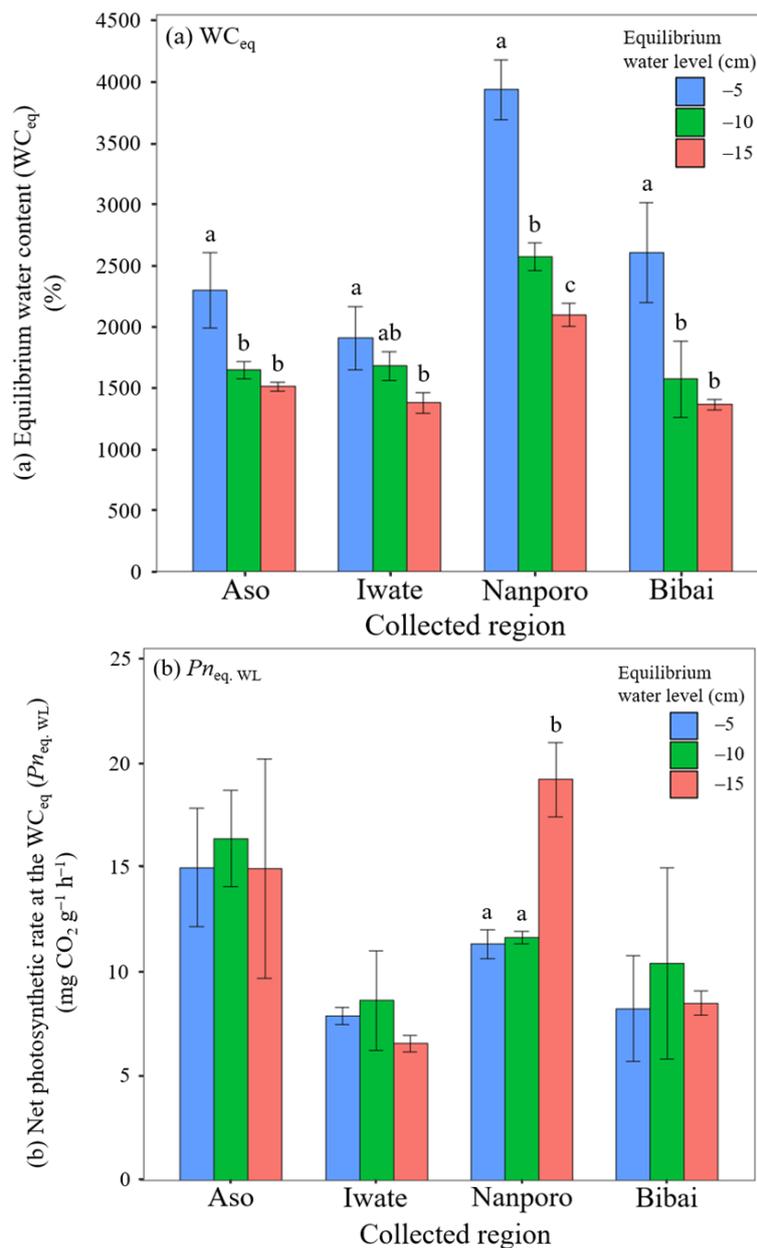


Figure 6. Comparison of (a) equilibrium water content for the water level (WC_{eq}) (%) and (b) net photosynthetic rate at WC_{eq} ($Pn_{eq,WL}$; $mg\ CO_2\ g^{-1}\ h^{-1}$) of *Sphagnum palustre* from Aso, Iwate, Nanporo, and Bibai. The error bars denote standard deviation ($n = 3$), except for the sample from Bibai at -10 cm ($n = 2$).

Table 2. Two-way factorial ANOVA showing the effects of water level and collected region on the optimum water content (optimum WC), maximum net photosynthetic rate (maximum P_n), equilibrium water content specific to the water level (WC_{eq}), and net photosynthetic rate at WC_{eq} ($P_{n_{eq,WL}}$) in the samples from Aso, Iwate, Nanporo and Bibai. Key: df = degrees of freedom; * significant at $p < 0.05$, ** significant at $p < 0.01$.

	df	Optimum WC		Maximum P_n		WC_{eq}		$P_{n_{eq,WL}}$	
		F value	p-value	F value	p-value	F value	p-value	F value	p-value
Water level	2	4.177	0.028 *	0.431	0.655	92.872	<0.01 **	1.412	0.264
Collected regions	3	12.699	<0.01 **	42.741	<0.01 **	56.218	<0.01 **	18.745	<0.01 **
Interaction	6	0.697	0.655	2.872	0.031 *	2.971	0.027 *	2.254	0.074

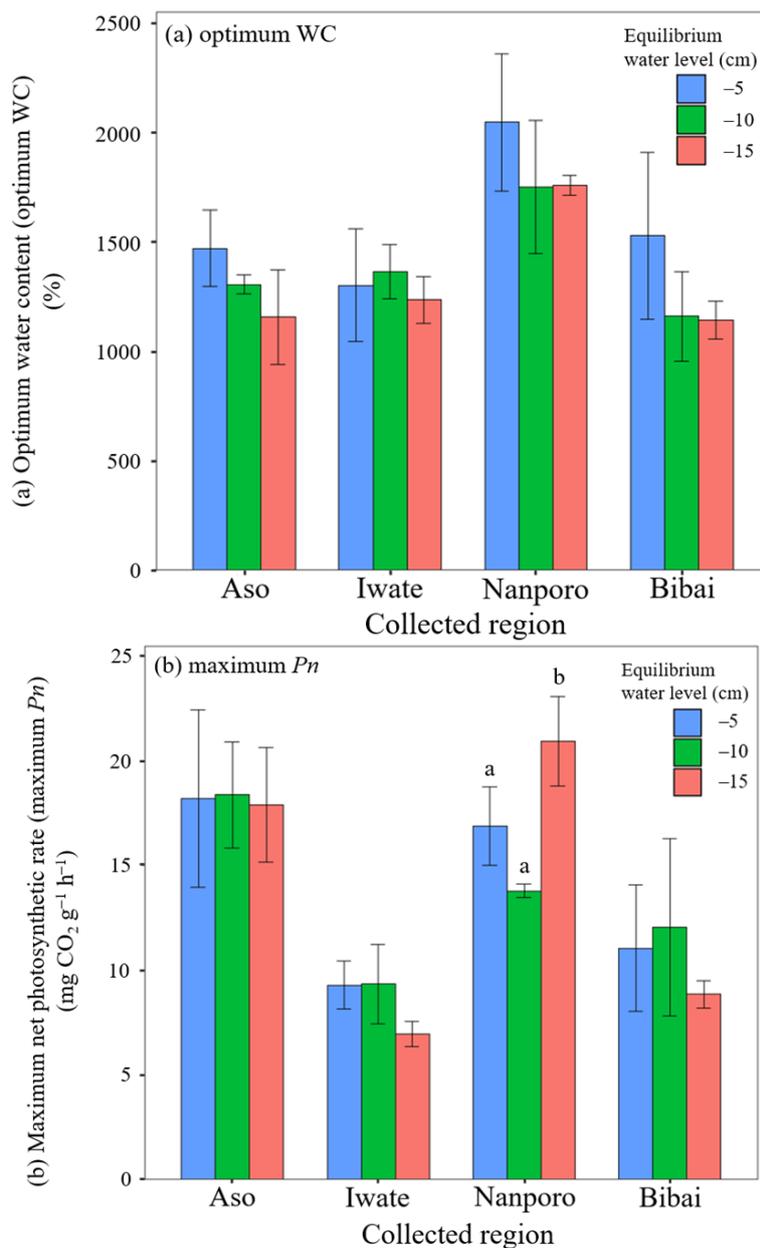


Figure 7. Comparison of (a) optimum water content (optimum WC) (%) and (b) maximum net photosynthetic rate (maximum P_n) (mg CO₂ g⁻¹ h⁻¹) of *Sphagnum palustre* from Aso, Iwate, Nanporo, and Bibai. The error bars denote standard deviation ($n = 3$), except for the sample from Bibai at -10 cm ($n = 2$).



DISCUSSION

Moisture conditions of *Sphagnum capitula*

Low WC_{eq} was observed in the *Sphagnum palustre* capitula subjected to low water level conditions (Figure 6a). The results, which were obtained on a species from temperate wetlands that has robust plant bodies (belonging to Section *Sphagnum*; Daniels & Eddy 1985), were consistent with those obtained by Rydin (1985) in studies using four species from boreal peatlands with small plant bodies (belonging to Sections *Acutifolia* and *Cuspidata*; Daniels & Eddy 1985). At low water levels, the low WC_{eq} is a consequence of the reduced matric potential at the peat surface (Hayward & Clymo 1982) in the moisture-control equipment. Although the results of our study were obtained under laboratory conditions, the results of field observations by Rydin (1985) corroborate that the WC of *Sphagnum capitula* decreases with falling water level (i.e. increasing distance from the capitula to the water table).

Response of photosynthetic rate to water content

When comparing photosynthetic capacity between habitats or growing environments, the thickness of the sample (i.e. whether or not it includes stems) may affect the photosynthetic rate. Many previous studies on *Sphagnum* photosynthetic capacity have used capitula that were sampled without stems, while others have used samples with stems (Table 3). Laing *et al.* (2014) showed that stems (without capitula) do not show a high photosynthetic rate in many *Sphagnum* species. Hence, we did not consider the contribution of stems to photosynthesis.

Our study showed that there is an optimum WC (range 1141–2045 %) that maximises P_n (Figure 5), which is consistent with many previous studies indicating an optimum WC of 600–1600 % (Table 3). Fukuta *et al.* (2012) reported that *Sphagnum palustre* from a wetland in warm-temperate southeastern Japan also had a maximum P_n of approximately 3.5 mg CO₂ g⁻¹ h⁻¹ with an optimum WC of 800–1600 % under saturated light conditions at 25 °C. The optimum WC tends to be higher in species belonging to Section *Sphagnum*, which have larger and more robust plant bodies than species of other Sections. This might be responsible for the high optimum WC. In the case of *Sphagnum* species from a boreal peatland, Silvola (1990) reported that *Sphagnum fuscum* (Section *Acutifolia*) from a raised bog in Finland reached the maximum photosynthetic rate at a WC of 600–800 % under saturated light at 21–23 °C, but the photosynthetic rate at a water level of -5 cm was 20–40 % of the maximum P_n . In addition, van Gaalen *et al.* (2007) reported that the P_n of

Sphagnum teres (Section *Squarrosum*) from a moderately rich fen in Alberta, Canada, reached a maximum WC of approximately 800 % under saturated light at 25 °C. The reason for the decrease in P_n at high moisture levels is the inhibition of CO₂ diffusion by a thick water layer formed on the plant surface, which acts as a resistance to CO₂ diffusion, resulting in reduced assimilation (Silvola 1990, Williams & Flanagan 1996, van Gaalen *et al.* 2007). The decline in P_n at low moisture levels is caused by depression of the overall metabolic process resulting in reduced assimilation (Silvola 1990, Williams & Flanagan 1996, van Gaalen *et al.* 2007).

The photosynthetic rate of the *Sphagnum* was highly responsive to water content (Figure 5). Thus, to compare the photosynthetic capacity (potential) of *Sphagnum* from different habitats, it is necessary to evaluate (maximum) P_n at the optimum WC. To evaluate *Sphagnum* traits including photosynthesis, Laing *et al.* (2014) measured and compared the maximum photosynthetic rate in *Sphagnum* species. The maximum P_n of *Sphagnum palustre* found in our study (6.94–20.9 mg CO₂ g⁻¹ h⁻¹; Figure 7b) was remarkably higher than values obtained by other authors in south-eastern Japan (3.00–4.00 mg CO₂ g⁻¹ h⁻¹; Haraguchi & Yamada 2011, Fukuta *et al.* 2012, Haraguchi 2015). This difference may be partly attributed to the difference in moisture adjustment before P_n measurement. For example, Haraguchi & Yamada (2011) measured P_n after submerging the samples in water 30 minutes after starting the measurement. The moisture conditions might have been different in the samples used in our study. To compare the photosynthetic capacity between habitats, we have to reproduce the WC of *Sphagnum* at equilibrium with the water table in the habitat.

Factors affecting photosynthetic capacity

Although variables related to the shape of the capitula were not measured, a high water-holding capacity (high equilibrium WC; Figure 6a) or high water retention (difference in equilibrium WC between the -5 cm and -10 cm water levels; Figure 6a) was observed in the capitula from the Nanporo site, which can be influenced by the capitulum shape. It has been reported that in addition to having large leaves, *Sphagnum papillosum* (Section *Sphagnum*) has a large water-holding capacity (Hayward & Clymo 1982). Green (1968) reported that *Sphagnum imbricatum* (also in Section *Sphagnum*) changed its morphology (branch length and number of branches per shoot) in response to water level. Thus, it is possible that the capitula of *Sphagnum palustre* sampled from Nanporo hold more water than capitula sampled from other regions; however, this is unclear

Table 3. Optimum water content for photosynthesis in *Sphagnum* species from our study and global literature.

Sample region	Section	Species	Tested sample	Optimum water content (%)	Source
Southwestern to northern Japan	<i>Sphagnum</i>	<i>Sphagnum palustre</i>	capitulum (1 cm)	1141–2045	Our study
Southwestern Japan	<i>Sphagnum</i>	<i>S. palustre</i>	upper 5 cm layer of <i>Sphagnum</i> colony	800–1600	Fukuta <i>et al.</i> (2012)
	<i>Sphagnum</i>	<i>S. magellanicum</i>		1015	
	<i>Sphagnum</i>	<i>S. papillosum</i>		1306	
	<i>Acutifolia</i>	<i>S. fuscum</i>		664	
	<i>Acutifolia</i>	<i>S. fuscum</i>		722	
Eastern central Sweden	<i>Acutifolia</i>	<i>S. fuscum</i>	capitulum (1 cm)	600	Laing <i>et al.</i> (2014)
	<i>Cuspidata</i>	<i>S. augustifolium</i>		837	
	<i>Cuspidata</i>	<i>S. augustifolium</i>		766	
	<i>Cuspidata</i>	<i>S. balticum</i>		1117	
	<i>Cuspidata</i>	<i>S. fallax</i>		1064	
Eastern Finland	<i>Acutifolia</i>	<i>S. fuscum</i>	1~1.5 cm from <i>Sphagnum</i> living surface layer	600–1000	Silvola & Aaltonen (1984)
	<i>Cuspidata</i>	<i>S. augustifolium</i>		900–1300	
Eastern Finland	<i>Acutifolia</i>	<i>S. fuscum</i>	1.5 cm from <i>Sphagnum</i> living surface layer	600–800	Silvola (1990)
	<i>Acutifolia</i>	<i>S. fuscum</i>		600–800	
Eastern Sweden	<i>Cuspidata</i>	<i>S. balticum</i>	capitula (the top centimetre of the plant)	600–800	Rydin & McDonald (1985)
	<i>Cuspidata</i>	<i>S. tenellum</i>		600–800	
Ontario, Canada	<i>Acutifolia</i>	unknown	5 cm stems of <i>Sphagnum</i>	700	Williams & Flanagan (1996)
Alberta, Canada	<i>Squarrosom</i>	<i>S. teres</i>	1 cm from <i>Sphagnum</i> living surface layer	900	van Gaalen <i>et al.</i> (2007)

because we did not evaluate capitulum morphology. Additionally, the histories of environmental conditions experienced during growth varied between the *Sphagnum* samples in our study.

Other authors have shown that the light-saturated photosynthetic rate of *Sphagnum* species is influenced by the availability of light in the habitat (Laing *et al.* 2014, Bengtsson *et al.* 2016). In the study by Laing *et al.* (2014), the photosynthetic capacity of four *Sphagnum* species was higher in a treed (light-limited) habitat than in an open habitat. The photosynthetic capacity may also differ amongst individuals (even of the same species) growing in different microclimates; Bengtsson *et al.* (2016) found that the maximum P_n was higher in heavily shaded habitats than in open habitats. In our study, the maximum P_n and $P_{n_{eq,WL}}$ of *Sphagnum palustre* capitula apparently differed between regions (Figures 6b, 7b). However, the natural habitat of the sample from Nanporo was light-limited (up to 98 % shading) during summer, and this sample showed high maximum P_n in our experiments. The samples from Iwate and Bibai experienced three hot summers and 50 % shading under cheesecloth for more than two years after sampling, whereas the samples from Aso and Nanporo were approximately 90 % shaded under eaves on the rooftop for only two months and did not experience hot summers. Thus, the results of our study are consistent with the findings of Bengtsson *et al.* (2016), and it is possible that the demonstrated differences in photosynthetic capacity amongst collected regions might be caused by the history of environmental conditions experienced before the experiments, both in the natural habitat and on the rooftop.

In the current study, several questions arose about the relationship between *Sphagnum* morphology and photosynthetic capacity or the effects of growing environmental history on photosynthetic capacity. Moreover, the inter-provenance variation of the photosynthetic capacity of *Sphagnum palustre* remains unknown. Further studies are needed to clarify the effects of *Sphagnum* morphology and the history of environmental conditions during growth on photosynthesis or interprovenance variation in photosynthetic capacity, in order to understand or predict the response to global climate change.

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AUTHOR CONTRIBUTIONS

The study was conducted by HI under the supervision of TY. YH was involved in the experimental planning, field sampling and environmental measurements in Aso. KY was involved in field monitoring and environmental measurements in Nanporo and planned the analyses of experimental data. The first draft of the manuscript was written by HI, and all authors commented on previous versions of the manuscript.

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Appendix

Table A1. Results of a Tukey-Kramer multiple comparison of optimum water content (optimum WC), maximum net photosynthetic rate (maximum Pn), equilibrium water content specific to the water level (WC_{eq}) and net photosynthetic rate at WC_{eq} ($Pn_{eq,WL}$) between samples of *Sphagnum palustre* originating from Aso, Iwate, Nanporo and Bibai. The same suffixed letter indicates no significant difference ($p > 0.05$) among collected regions.

Water level (cm)	Collected region	Optimum WC (%)	Maximum Pn (mg CO ₂ g ⁻¹ h ⁻¹)	WC_{eq} (%)	$Pn_{eq,WL}$ (mg CO ₂ g ⁻¹ h ⁻¹)
-5	Aso	1469 ± 258a	18.20 ± 4.25a	2430 ± 259b	10.80 ± 3.78
-5	Iwate	1300 ± 314a	9.28 ± 1.14b	1984 ± 243b	7.53 ± 0.42
-5	Nanporo	2045 ± 175b	16.89 ± 1.86a	3979 ± 291a	11.31 ± 0.70
-5	Bibai	1527 ± 381ab	11.05 ± 3.02ab	2644 ± 410b	7.64 ± 2.53
-10	Aso	1304 ± 123ab	18.23 ± 2.54a	1685 ± 116b	17.25 ± 2.31a
-10	Iwate	1363 ± 304ab	9.33 ± 1.90b	1813 ± 113b	8.23 ± 2.38b
-10	Nanporo	1749 ± 42a	13.79 ± 0.32ab	2621 ± 71a	11.61 ± 0.30ab
-10	Bibai	1159 ± 205b	12.06 ± 4.24ab	1625 ± 309b	10.03 ± 4.59ab
-15	Aso	1141 ± 108b	17.91 ± 2.76a	1538 ± 84b	14.66 ± 5.27ab
-15	Iwate	1263 ± 45b	7.00 ± 0.59b	1417 ± 92b	6.61 ± 0.39c
-15	Nanporo	1757 ± 216a	22.89 ± 2.15a	2153 ± 37a	19.18 ± 1.78a
-15	Bibai	1141 ± 86b	8.85 ± 0.66b	1403 ± 41b	8.29 ± 0.57bc