

# *Sphagnum* growth in floating cultures: Effect of planting design

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## SUMMARY

To establish rapid and stable *Sphagnum* growth, capitulum culture of a selected strain of *S. palustre* was carried out using a floating culture method. Four planting treatments were tested at mountain and urban sites in Kumamoto Prefecture on Kyushu Island, south-west Japan. Capitula were planted in colonies of different sizes on 30 cm square floating rafts, but with strict control of the number (75–77) of capitula *per* raft. The initial cover of live green *Sphagnum* ranged from 15 to 20 %. Growth of the colonies was followed throughout the growing season (April to November) of 2008. After three months, green coverage rates reached 40–50 % in all planting treatments. At the end of the growing season, the highest *Sphagnum* cover (almost 90 % at the urban site) was recorded in the planting treatment with eleven re-introduced colonies of seven capitula ('11×7cap'), while the highest capitulum number and biomass (dry weight) gain occurred in the '4×19cap' planting treatment. Average stem elongation ranged from 5 cm to 7 cm in the '77×1cap' and '4×19cap' planting treatments, respectively, indicating that the larger sized colony grew longer stems. However, contrary to expectation, the '4×19cap' planting treatment - which had the largest colony size - did not deliver the highest number of newly formed side shoots.

**KEY WORDS:** capitulum, Japan, 'palustre meeno', *Sphagnum palustre*, subtropical

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## INTRODUCTION

*Sphagnum* is the most economically valuable genus of mosses (Caron & Rochefort 2013). It is commonly used worldwide, in dried and/or decomposed condition, for various purposes in commercial horticulture (Gaudig *et al.* 2017). To satisfy part of this demand, huge amounts of *Sphagnum* are harvested from natural habitats in New Zealand, Chile, China and other countries, and exported to consuming countries including Japan (Díaz & Silva 2012).

Approximately 35 *Sphagnum* species are known to occur naturally in Japan (Iwatsuki 2001). All of them are in danger of imminent extinction because wetlands with *Sphagnum* habitats are being lost or degraded year by year. *Sphagnum palustre* is the most widely distributed of the Japanese species. Suzuki (1956) investigated its distribution quite precisely, recording *S. palustre* at 466 locations across the four main islands of Japan: 42 on Hokkaido (the most northerly island), 346 on Honshu (the largest island), 28 on Shikoku (the smallest island) and 30 on Kyushu (the most southerly island). Despite its widespread occurrence, *S. palustre* is currently designated as an endangered species due to over-harvesting. Because *Sphagnum* distribution is not currently monitored in Japan, it is unclear how many populations remain in the country today.

Generally, *Sphagnum* carpets recover only slowly (at best) after field harvesting, and cultivation of *Sphagnum* is difficult. Many of the sites that have been harvested are vegetationless and remain dry for a long time, even in the growing season (Tuittila *et al.* 2003). Thus, human activities promoting the regeneration of mire ecosystems are rather important.

Recently, some intensive research on *Sphagnum* propagation and recolonisation has been performed. The re-introduction of *Sphagnum* fragments with the capitulum (apical part of the plant body) still attached has emerged as a promising method for reinstating *Sphagnum* carpets on cut-over peatland in Canada (Rochefort *et al.* 2003, Díaz & Silva 2012). On the other hand, a novel *Sphagnum* culture technique has been developed in Japan (Takeda & Shimura 2006). This technique, which uses dried *Sphagnum* moss held in a situation that allows it to be in contact with water in a water-pooling unit, consistently achieves rapid *ex-situ* establishment of *Sphagnum* carpets on small floating rafts, and is expected to contribute significantly to mire restoration in various situations.

The general goal of the study reported here was to develop *Sphagnum* cultures in the field, on the basis that making cultured *Sphagnum* available in Japan should stop illegal collections of wild *Sphagnum* and thus reduce the degradation of natural bog sites. More specifically, the aim was to establish a stable *Sphagnum* carpet rapidly from capitula of a selected

strain of *S. palustre*, using the Japanese ‘floating raft’ culture technique. The effects of different planting treatments on *Sphagnum* growth and carpet establishment were tested at mountain and urban locations in Kumamoto Prefecture on Kyushu Island in south-west Japan.

## METHODS

### Study sites and weather conditions

The experimental sites were located at Minamiaso-mura, Aso-gun (32° 53' 10.0" N, 130° 59' 39.5" E) on Mount Aso (MA experimental site) and at Musashi-zuka (32° 51' 17.2" N 130° 46' 10.1" E) in Kumamoto City, Kumamoto prefecture, Japan (KC experimental site), respectively (Figure 1).

The Minamiaso-mura area has a mountain-type climate with high rainfall. Meteorological records for this area indicate that mean annual rainfall is 2337 mm and mean annual temperature is 17.4 °C. In contrast, Kumamoto City experiences an urban-type climate with a heat-island effect. Meteorological records for the city indicate an annual rainfall of 1651 mm and a mean annual temperature of 21.5 °C (Figure 2). During the year of this study (2008), the warm season started in early April and the cold season started at the end of November at both sites,

but there was a temperature difference of about 5 °C between Mount Aso and Kumamoto City throughout the year (Figure 2). The mean seasonal (April–November 2008) temperature at the experimental site on Mount Aso was 17.7 °C and in Kumamoto City it was 21.8 °C, both values being only slightly higher than the respective 1981–2010 long-term averages (17.4 °C and 21.5 °C). However, mean temperature for the month of July 2008 was higher than the 1981–2010 monthly average (Figure 2). Cumulative precipitation for April–November was clearly higher than the 1981–2010 long-term average both on Mount Aso (2813 mm *versus* 2337 mm) and in Kumamoto City (1977 mm *versus* 1651 mm). The peak seasonal precipitation in June–July 2008 was also higher than the 1981–2010 long-term average at both sites (Figure 2). Daytime (10:00 am–2:00 pm) light intensity values measured on fine days ranged from 10,000 to 20,000 lux.

### Plant material

The large species *S. palustre* was chosen as the experimental material (a) because it is quite valuable for the commercial market (like *S. magellanicum* and *S. cristatum*) and (b) because it is widely distributed from the north to the south of Japan. A vigorously growing strain of *S. palustre* was selected on the basis

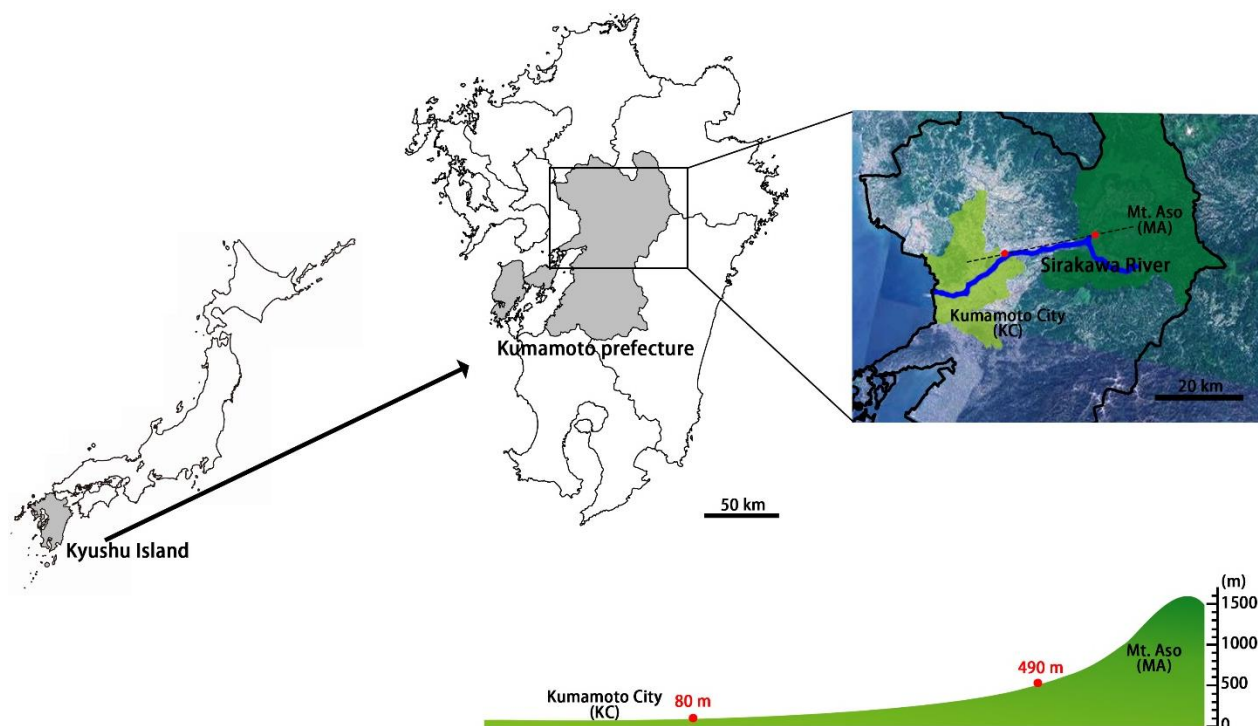


Figure 1. Locations and altitudes of the study sites at Mount Aso (Aso Campus of Tokai University) and Kumamoto City (Musashi-zuka), Kumamoto Prefecture, Japan. Source of the map: Digital map (Kashmir) of Geographical Survey Institute Japan.

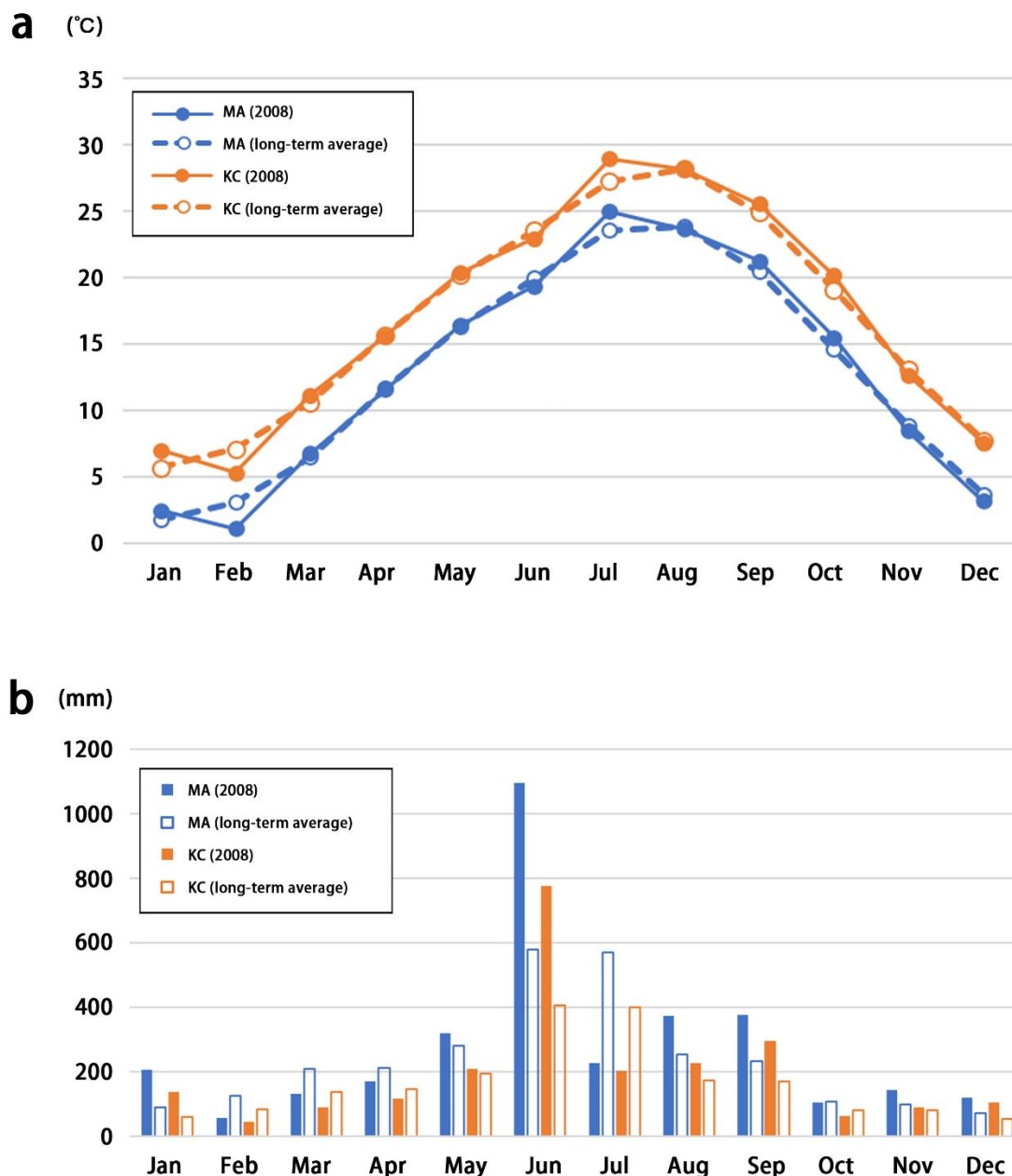


Figure 2. Progress of (a) monthly mean air temperature in 2008 (solid line with solid circles) and long-term average monthly air temperature (dotted line with open circles), and (b) monthly total precipitation in 2008 (shaded column) and long-term average monthly total precipitation (unshaded column) at the MA (Mount Aso, blue) and KC (Kumamoto City, red) experimental sites. The reference period for the long-term averages is 1981–2010. Source: weather service of Japan Meteorological Agency.

of its performance in an open-air greening culture test conducted during high summer on a rooftop in Tokyo. This strain was registered in the Japan Patent Office as ‘palustre meeno’ (trademark registration no. 5775251, former strain name was ‘TMC’). All of the plants used here were purchased from Moss Catch System (Tokyo) Company Limited and cultivated in the Laboratory of Plant Environment Science, Department of Plant Science, School of Agriculture, Tokai University.

### Planting treatments

Each culture was established on a floating raft consisting of a 30 × 30 cm polystyrene block (10 cm thick) which floated in an open-topped watertight box of dimensions 48 cm (length) × 36 cm (width) × 11.5 cm (height) containing water 5 cm deep. The chemical properties of the irrigation water at both study sites are shown in Table 1. Fifty grams of dried *Sphagnum* moss (the Chilean commercial product) was applied to the upper surface of each raft (forming

Table 1. Comparison of water quality at the mountain (MA) and urban (KC) experimental sites. ND = not detected. Units are mg L<sup>-1</sup> unless otherwise indicated.

	experimental site	
	MA	KC
pH	7.0	6.3
EC (mS m <sup>-1</sup> )	16	17
Cl <sup>-</sup>	5.6	5.9
NO <sub>2</sub> <sup>-</sup>	ND	ND
NO <sub>3</sub> <sup>-</sup>	2.6	2.9
SO <sub>4</sub> <sup>2-</sup>	29.8	45.0
Na <sup>+</sup>	7.5	9.8
NH <sub>4</sub> <sup>+</sup>	>1	>1
K <sup>+</sup>	3.3	3.7
Mg <sup>2+</sup>	4.0	5.7
Ca <sup>2+</sup>	10.5	15.0

a layer one centimetre thick when rewetted), and 76 (±1) living *Sphagnum* ‘capitula’ (the tops of *Sphagnum* moss plants with compact clusters of branches) were planted on this, singly (77×1cap planting treatment), in 25 groups of three (25×3cap planting treatment), eleven groups of seven (11×7cap planting treatment) or four groups of 19 (4×19cap planting treatment) (Table 2, Figures 3 and 4). The four planting treatments were each replicated three times and tested within a complete randomised experimental design at both experimental sites. The preparation of cultures began in early March (2008), just after the end of the frosty season, and they had all been laid out on outdoor concrete floors at the study sites by the middle of the same month.

### Measurements

Live moss cover was measured at monthly intervals from early April to the end of November (2008). The plants were then ‘harvested’ by carefully picking them up from the raft, one by one, using forceps; and number of capitula, stem length, number of side shoots and dry weight were measured. Statistical calculations were performed in R 3.1.2 (R Development Core Team 2014). Mean values are given as ±SD. Differences between samples were assessed using Tukey-tests and one-way ANOVA.

Table 2. Colony sizes and capitulum numbers for the four *Sphagnum* planting treatments.

planting treatment	metric		
	capitula per colony	colonies per raft	capitula per raft
77×1cap	1	77	77
25×3cap	3	25	75
11×7cap	7	11	77
4×19cap	19	4	76

### RESULTS

The *Sphagnum* growing season lasted from April until November at both experimental sites. During this time the *Sphagnum* colonies grew rapidly and developed into full *Sphagnum* carpets in a rather similar fashion for all planting treatments (Figure 5). The initial live green *Sphagnum* cover values in April were 15–20 % (Figure 6). In early July, three months after planting, the live cover of colonies for all planting treatments at both experimental sites reached similar values (40–50 %), except for the ‘11×7cap’ planting treatment at KC (62 %). Because many capitula in smaller colonies (‘77×1cap’ and ‘25×3cap’) became chlorotic in September, significant differences in live *Sphagnum* cover appeared in the ‘77×1cap’ planting treatment at KC ( $p < 0.05$ ) but not at MA (Figures 5 and 6). At the end of the growing season, the highest *Sphagnum* cover was observed in the ‘11×7cap’ planting treatments, especially at KC (nearly 90 %) (Table 3, Figure 6). Of the three replications of the ‘4×19cap’ planting treatment at MA, one replica was removed from the analysis for the period September–November because the colonies suffered animal damage. Cover in the two undamaged ‘4×19cap’ planting treatments remained quite stable with low standard deviation, and similar to that in the ‘11×7cap’ planting treatments.

Comparing results for the different planting treatments between the two experimental sites, we found that the densities of *Sphagnum* capitula at MA were higher than at KC, except in the ‘11×7cap’ planting treatment (Table 3, Figure 7a). The highest density of *Sphagnum* capitula occurred in the ‘4×19cap’ planting treatment at MA (Table 3, Figure 7a). At MA, the mean length increments of *Sphagnum* stems during the entire growing season were 5.1, 5.2, 5.9 and 6.6 cm for the ‘77×1cap’,



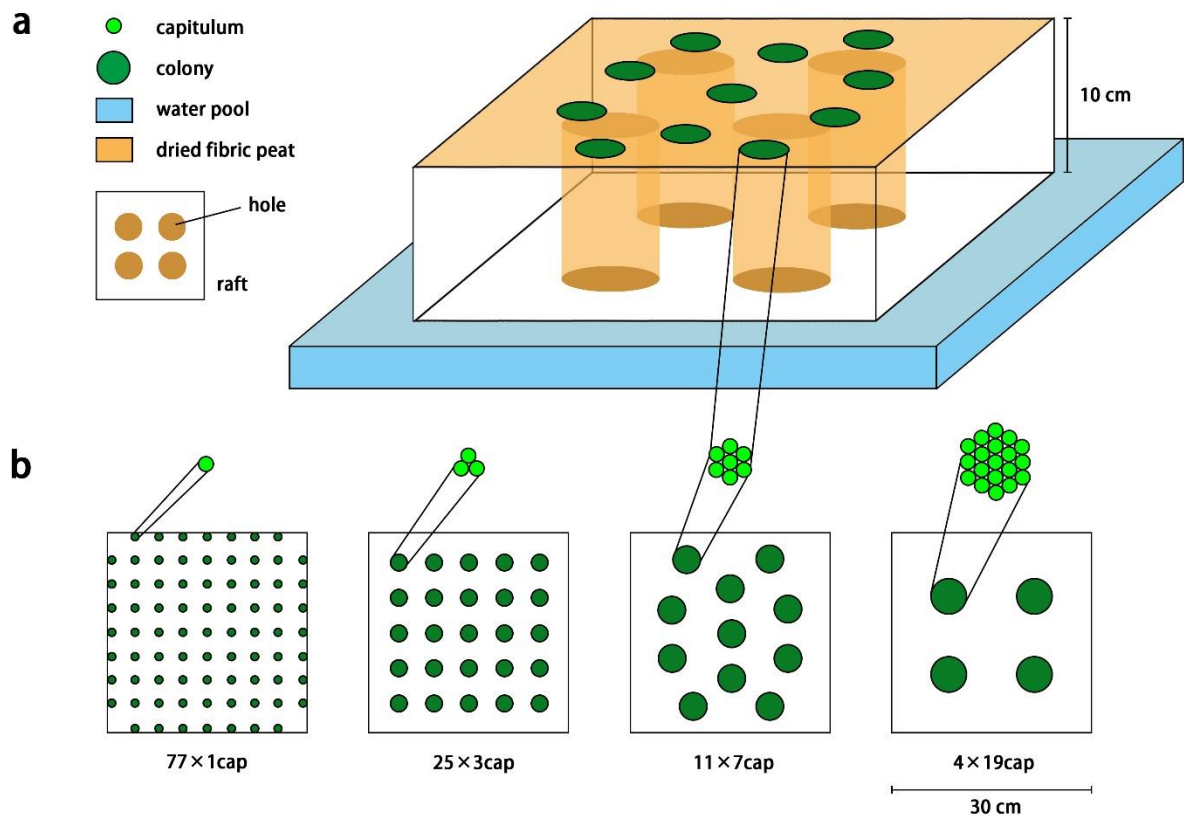


Figure 3. Diagrams showing the arrangement of floating polystyrene raft and *Sphagnum* colonies (a) and the structure and arrangement of colonies in the ‘77×1cap’, ‘25×3cap’, ‘11×7cap’ and ‘4×19cap’ planting treatments, which consisted of 77, 25, 11 and 4 colonies of 1, 3, 7 and 19 capitula, respectively (b).

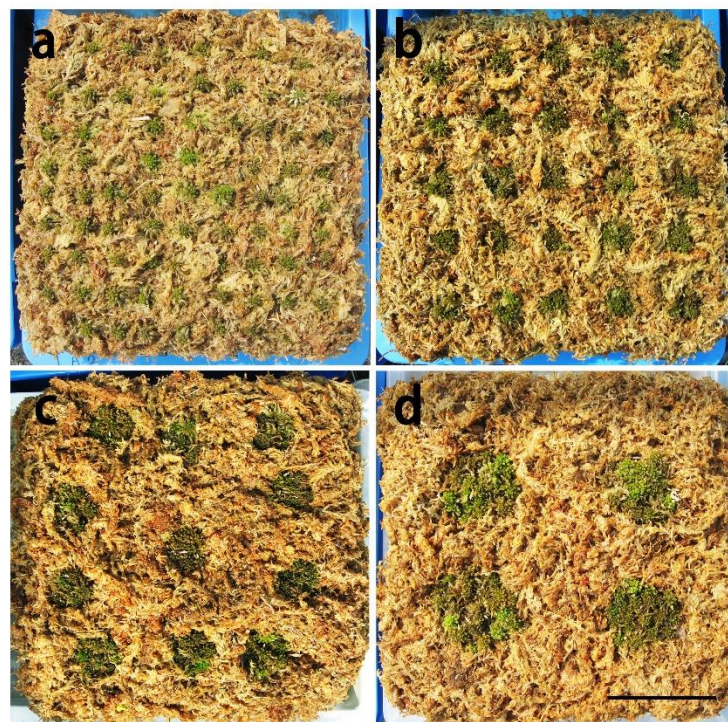


Figure 4. Example cultures with different sizes and numbers of *Sphagnum* colonies formed by planting live capitula, in the planting treatments ‘77×1cap’(a), ‘25×3cap’(b), ‘11×7cap’ (c) and ‘4×19cap’ (d). Scale bar = 10 cm.



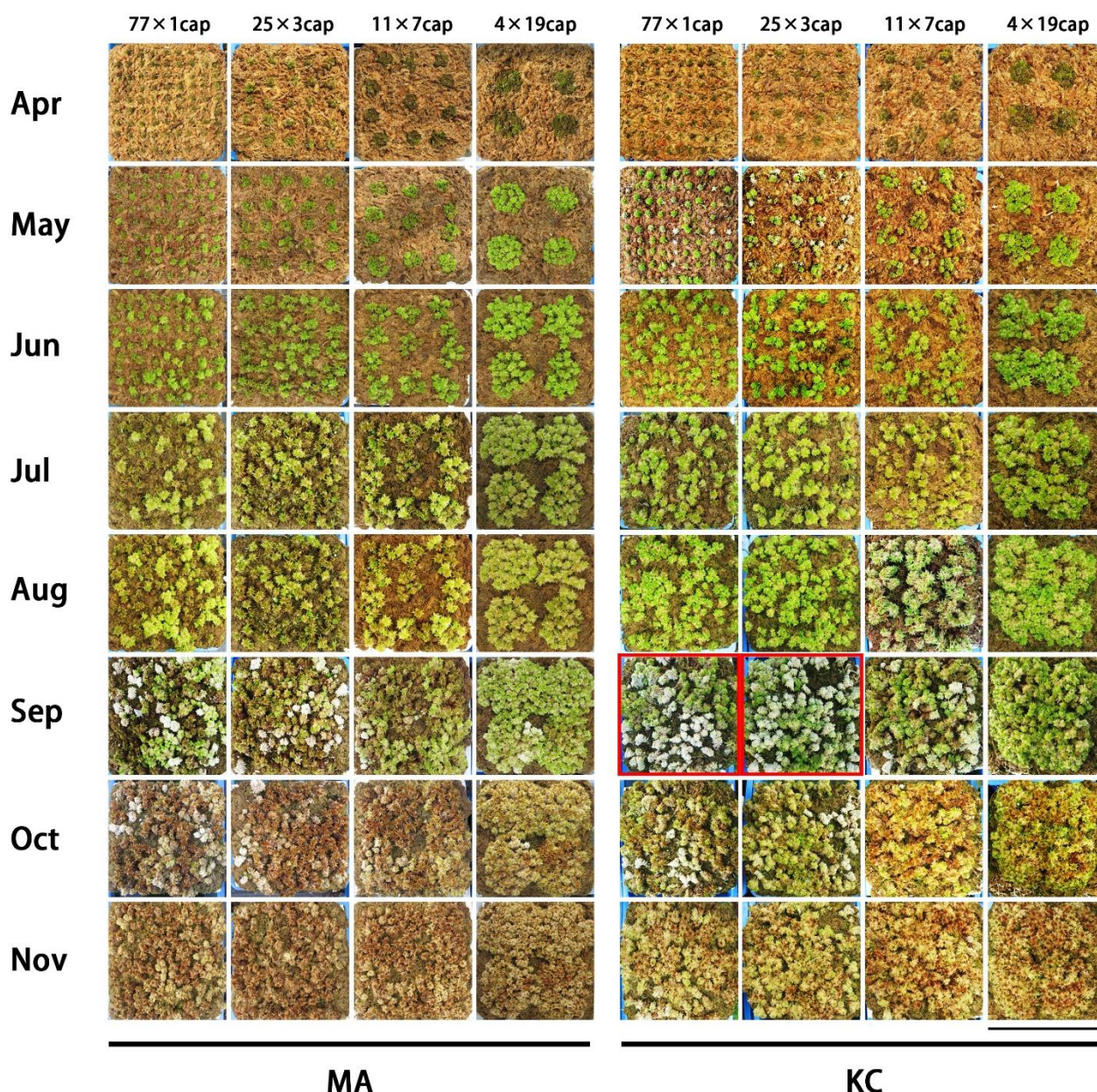


Figure 5. The progress of ‘greening’ of *Sphagnum* cultures established using the four planting treatments ‘77×1cap’, ‘25×3cap’, ‘11×7cap’ and ‘4×19cap’ at the MA (Mount Aso) and KC (Kumamoto City) experimental sites during the seven-month growing season of 2008. Many capitula in smaller colonies (‘77×1cap’ and ‘25×3cap’) became chlorotic in September, especially at KC (red outlines). Scale bar = 30 cm.

‘25×3cap’, ‘11×7cap’ and ‘4×19cap’ planting treatments, respectively; the corresponding mean length increments at KC were 5.8, 6.2, 6.6 and 6.9 cm (Table 3, Figure 7b). At both experimental sites, the largest colonies (‘4×19cap’ planting treatment) had the greatest mean stem length. A significant difference was observed between the ‘4×19cap’ planting treatment at KC and the ‘77×1cap’ planting treatment at MA ( $p < 0.1$ ) (Figure 7b). The greatest production of side shoots occurred in the ‘25×3cap’ planting treatment at KC ( $p < 0.05$ ) for which, despite the unstable value with high standard deviation, the

number of side shoots was more than three times the average value for the ‘77×1cap’ planting treatment (Figure 7c). The highest *Sphagnum* biomass (dry weight) was obtained in the ‘4×19cap’ planting treatment at MA (Figure 7d). Overall, the most stable results for stem elongation and biomass production were obtained in the ‘4×19cap’ planting treatment, with each of four colonies having 19 capitula and quite similar growth at the two experimental sites, although the *Sphagnum* also grew well in the ‘77×1cap’ and ‘25×3cap’ planting treatments at both sites.

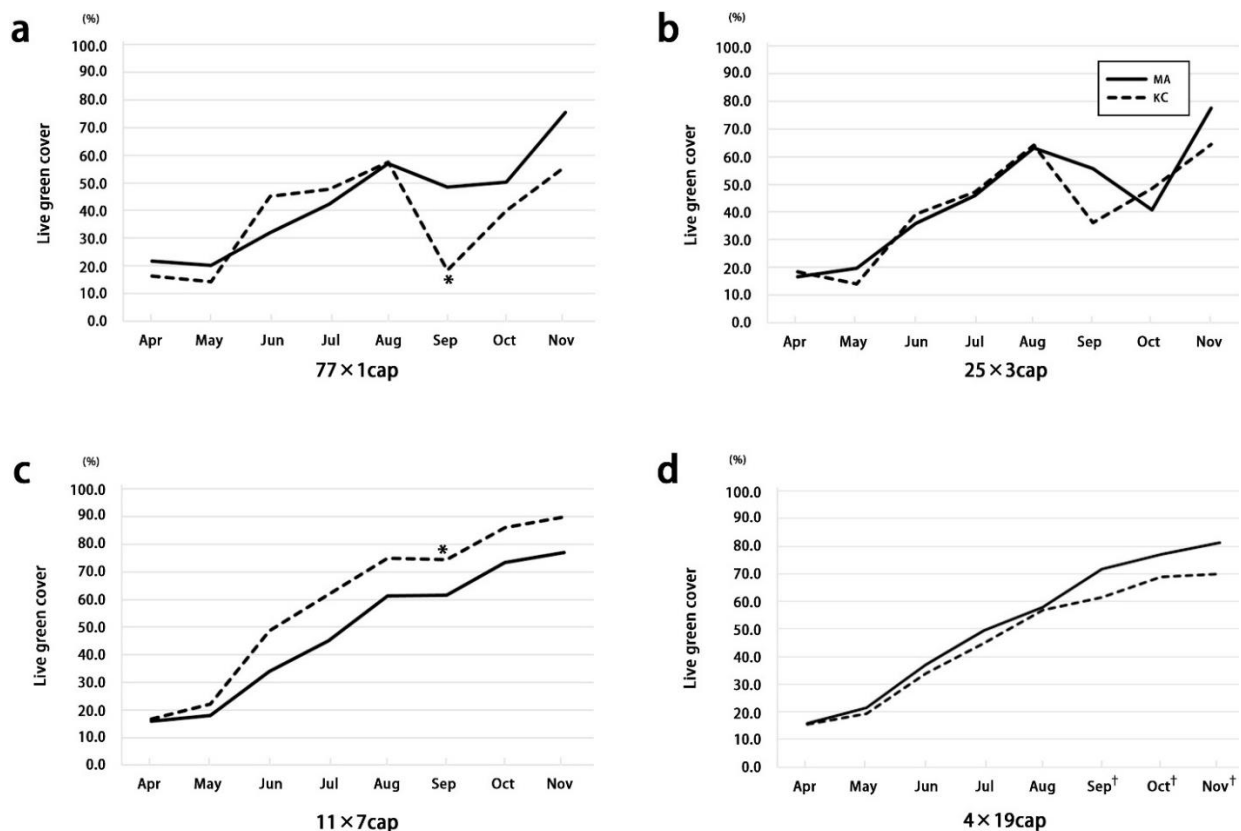


Figure 6. Monthly progress of live green *Sphagnum* cover (%; mean value for three replicas) for the four planting treatments ‘77×1cap’(a), ‘25×3cap’(b), ‘11×7cap’(c) and ‘4×19cap’(d) at the MA (Mount Aso, solid line) and KC (Kumamoto City, dotted line) experimental sites during the 7-month growing season of 2008. The data for months (September to November) marked with daggers (†) at MA do not include the cover values for one replica because the colonies suffered animal damage. \* denotes a significant difference (one-way ANOVA test) at  $p < 0.05$ .

Table 3. Measurements of *Sphagnum* colony attributes (mean  $\pm$  SD for three replicas) at the end of the first growing season (seven months, April to November 2008) for the Mount Aso (MA) and Kumamoto City (KC) experimental sites. Only two replicas of the planting treatment marked with a dagger (†) were included in the analysis because the third one suffered animal damage.

site	planting treatment	live green <i>Sphagnum</i> cover (%)	number of capitula	stem length increment (mm)	number of side shoots	dry weight (g)
MA	77×1cap	75.5 $\pm$ 20.4	249.0 $\pm$ 27.5	50.5 $\pm$ 4.7	102.0 $\pm$ 34.5	34.3 $\pm$ 6.7
	25×3cap	77.5 $\pm$ 8.8	219.0 $\pm$ 20.5	51.9 $\pm$ 6.5	69.7 $\pm$ 24.8	31.3 $\pm$ 0.8
	11×7cap	77.0 $\pm$ 6.0	235.0 $\pm$ 65.5	58.9 $\pm$ 10.1	137.7 $\pm$ 57.4	34.3 $\pm$ 4.6
	4×19cap†	81.3 $\pm$ 1.8	280.0 $\pm$ 29.7	65.7 $\pm$ 3.6	137.0 $\pm$ 15.6	40.1 $\pm$ 7.0
KC	77×1cap	56.0 $\pm$ 17.9	181.3 $\pm$ 16.6	58.0 $\pm$ 6.5	78.7 $\pm$ 21.5	32.4 $\pm$ 10.4
	25×3cap	64.5 $\pm$ 26.7	207.3 $\pm$ 67.7	62.1 $\pm$ 8.6	252.7 $\pm$ 109.9	31.1 $\pm$ 5.6
	11×7cap	89.8 $\pm$ 7.9	238.0 $\pm$ 58.5	65.9 $\pm$ 6.6	125.7 $\pm$ 27.7	34.6 $\pm$ 2.1
	4×19cap	70.0 $\pm$ 6.8	235.3 $\pm$ 70.9	69.1 $\pm$ 8.2	100.0 $\pm$ 22.1	33.5 $\pm$ 5.1



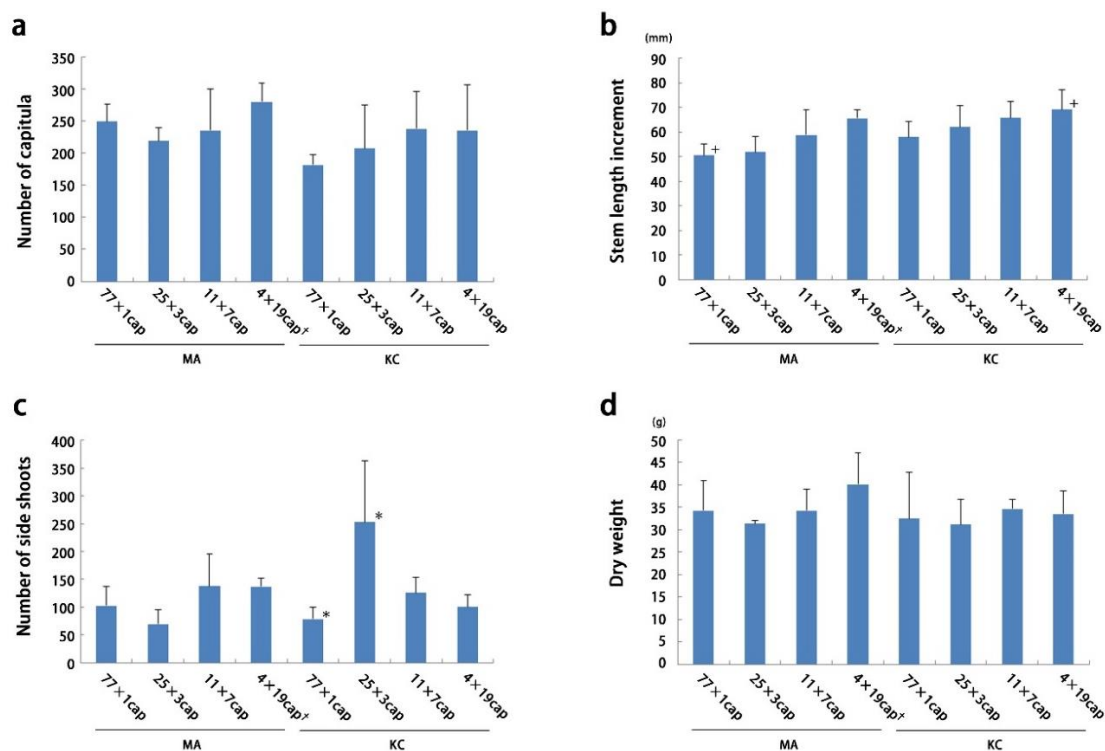


Figure 7. Mean values of number of capitula (a), stem length increment (b), number of side shoots (c) and dry weight (d) for sets of  $76 \pm 1$  *Sphagnum palustre* capitula grown in floating culture at the MA (Mount Aso) and KC (Kumamoto City) experimental sites using four different planting treatments (horizontal axis) replicated three times, at the end of the 2008 growing season (April to November). One replica of the ‘4×19cap’ planting treatment at MA, marked with daggers (†), was removed from the calculations of mean values because the colonies suffered animal damage. Vertical bars indicate SD of mean. Plus (+) and asterisk (\*) indicate significant differences (one-way ANOVA test) at  $p < 0.1$  and  $p < 0.05$ , respectively.

## DISCUSSION

### The growth of cultured *Sphagnum*

Wild *Sphagnum* has a northerly distribution in Japan, with most of the native species occurring in the northern part of the country and only a few on the southernmost island of Kyusyu, where they are confined to highly protected areas. Because of the high demand for *Sphagnum* arising from its uses in horticulture and gardening, there is a need to cultivate the moss fibres in order to eliminate the established practice of harvesting from the wild. In this study we used a novel approach to *Sphagnum* cultivation (Figure 8) which proved efficient in developing an almost complete moss carpet within one growing season of seven months. Having demonstrated the feasibility of growing *Sphagnum* on floating structures, we will next assess the performance of this approach against other cultivation methods.

*Sphagnum palustre* is an exceptional species whose distribution within Japan stretches from the subarctic to the subtropical zone. Even so, it was expected that a suitable *Sphagnum* culture method for the Kyusyu area would need to incorporate a means

of combating overheating in summer. This concern proved to be unfounded. As at other locations, the only criterion for successful *Sphagnum* growth proved to be that the height of the capitula above the water table should be maintained at a suitable (small) value (the optimum separation may be species dependent). Growing *Sphagnum* on a floating structure effectively delivers the stable water table condition that has been identified as a key factor to optimise when cultivating *Sphagnum* (Pouliot *et al.* 2015).

The cultivation of *Sphagnum* is generally difficult, requiring expert techniques and knowledge. Researchers in several countries have previously investigated the growth of cultivated *Sphagnum*. In Finland, annual elongation of *S. magellanicum* was reported to be 2–3 cm compared to 5–7 cm in our cultured *S. palustre*, which has a life form similar to that of *S. magellanicum* (Silvan *et al.* 2017). In Germany, Gaudig *et al.* (2017) reported that the thickness of a lawn dominated by *S. papillosum* increased by 1.9 cm *per year* (19 cm after ten years). The rapid and steady elongation rate of *S. palustre* by more than 5 cm, even in the first growing season, indicates that the floating cultivation approach is



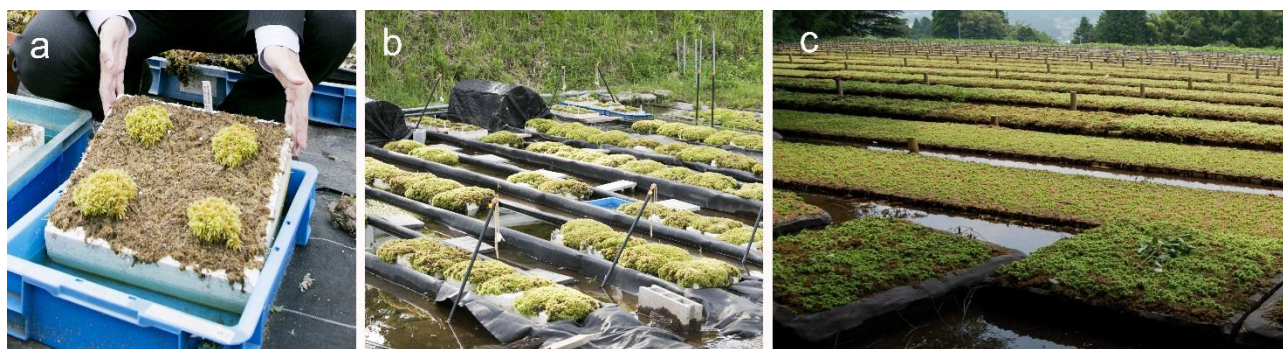


Figure 8. *Sphagnum* culture of (a) floating raft, (b) experimental site and (c) large scale performance on rice field at MA (Mount Aso).

efficient; although the rapid growth of *S. palustre* observed in this study probably results from a combination of the culture method and warmer temperatures during the growing season in South Japan compared to boreal countries. On the other hand, similarly rapid elongation (maximum of ~ 5 cm in one growing season) has been seen in *S. teres* grown in PVP pipe culture on a natural rich fen in Sweden (Granath *et al.* 2010). Enormous potential for further improvement is indicated by the observation that *S. palustre* growing under natural conditions on Kolkheti Mire in Georgia (41–42 °N) can increase in length by up to 30 cm yr<sup>-1</sup> (Krebs *et al.* 2016).

In our experiment, average stem elongation was 5 cm in the ‘77×1cap’ planting treatment and 7 cm in the ‘4×19cap’ planting treatment, indicating that larger colonies produce longer stems. New side shoots were observed when harvesting after one growing season. However, contrary to expectation, the ‘4×19cap’ planting treatment, which had the largest colony size, did not produce a large number of side shoots. Although the reason for this is not clear, some previous research indicates the possible involvement of environmental factors. Díaz & Silva (2012) suggest that the appearance of new shoots on *Sphagnum* stems can be regulated by factors such as water availability, temperature or light. Rochefort *et al.* (2002) found that, for *S. magellanicum*, the number of new shoots was greater in flooded than in non-flooded treatments under cold conditions but there were no differences under warm conditions. Nelson *et al.* (1998) investigated the effect of different light levels on *Sphagnum* growth under controlled conditions, and found that many side shoots were generated from stems without capitula. Clymo & Duckett (1986) found that light availability was the determinant for the appearance of new shoots. In the case reported here, the observed difference in formation of new side shoots between colonies of different sizes could be due to a combined light and temperature effect, the capitula of a small colony

experiencing higher availability of light than those of a large colony but a difference in number of side shoots resulting only at the warmer site (KC).

The next phase of this research will utilise the elongated parts of the cultured *Sphagnum* plants to produce new shoots during the next growing season, after capitulum cutting. *Sphagnum* regeneration at levels below the tops of the plants has also been previously investigated. Campeau & Rochefort (1996) found that more *S. magellanicum* capitula were generated in the 0–20 cm layer than at greater depths. For some *Sphagnum* species, Rochefort *et al.* (2003) observed that regeneration potential declined in fragments taken from 6 cm below the capitula, and that *S. magellanicum* fragments remained viable up to at least 10 cm depth. In a similar experiment, Díaz & Silva (2012) observed that the largest number of new shoots arose from stems at depths between 0 and 3 cm below the capitula.

### Potential applications

Kumamoto City does not have any natural *Sphagnum* mires. This study has demonstrated that *Sphagnum* cultivation can be established in completely new (urban or non-peatland) areas, even in the subtropical zone of Japan, and provides a strong indication that effective harvesting of cultivated *Sphagnum* could be achieved on inundated sites in this bioclimatic region. Japan already has inundation systems on many rice fields, which are flat and thus able to retain inflowing water. In recent years, Japan’s rice acreage reduction policy has resulted in the increasing abandonment of rice fields. This logically leads to a proposal that unused paddy fields could now acquire new value as locations where the production of *Sphagnum* biomass would be practical (Figure 8c). More scientific studies and amendments to management policies are needed to improve large-scale moss culture in Japan.

Mires and peat extraction sites have almost disappeared from Japan, which means that much of the country’s wetland biodiversity has been lost and

the collection of all species of wild *Sphagnum* is strictly controlled by law. Therefore, ‘*de novo*’ *Sphagnum* culture (from ‘ground zero’) is essential for plant protection and economic self-sufficiency in Japan, as in other countries with scarce *Sphagnum* resources. The combination of the *Sphagnum* strain ‘palustre meeno’ and the newly established culture method employed in this research has emerged as a system that is capable of generating *Sphagnum* biomass at high rates, even in the metropolitan ‘heat islands’ of Japan. The *Sphagnum* culture technique employed in this study also could be applied in the peatland restoration efforts of many other countries around the world where naturally collected reintroduction material is rarely available.

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