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The recovery of *Sphagnum capillifolium* following exposure to temperatures of simulated moorland fires: a glasshouse experiment

Keywords: blanket bog, bryophytes, burning**,** fire, heat stress, peatland, plant physiology

Abstract

Background. In the UK, government legislation allows the use of prescribed fire in peatlands for land management purposes. The use of fire, however, remains controversial, partly because of a distinct lack of data on the response of key peatland species to fire. *Sphagnum* species are key components of peatland ecosystems, yet a fundamental knowledge gap in the debate is the response of *Sphagnum* species to fire. *Aims*. To determine if a widespread species (Sphagnum capillifolium) has the ability to recover from exposure to high temperatures, analogous to those recorded in managed peatland fires. *Methods*. Samples of *S. capillifolium* were exposed to a range of temperature treatments. Recovery was monitored using chlorophyll fluorescence, CO_2 exchange and physical damage and new growth assessed. *Results*. We found that the degree of recovery of *S. capillifolium* was related to the temperature treatment, post-treatment environmental conditions and pre-treatment stem moisture content. The slowest recovery was found when samples were heated to 400 ^{o}C for 30 seconds. *Conclusions*. Our results demonstrate that *S. capillifolium* has the ability to recover following exposure to the temperatures experienced in prescribed fire, provided that at least some living material remains. Our results suggest that prescribed burning in the spring may allow for a quicker recovery than autumnal fires.

Introduction

Prescribed fire is a key management tool used on peatlands in Britain, to promote the regrowth of *Calluna vulgaris* (L.) Hull (*Calluna* hereafter) and grasses for grazing by game birds and livestock. There is considerable debate over the impact of fire on peatlands, and in particular, on the impacts on species of ecological and conservation importance, such as *Sphagnum* species. The debate surrounding prescribed fire remains contentious, partly due to the polarised views of the protagonists, but also because of the lack of evidence for the effects of fire on taxa including *Sphagnum* (Davies et al. 2016). Much of the data comes from studies on wildfires, which may be much more severe (sensu Keeley 2009) than prescribed fires, and result in greater depth of burn and exposure of bare peat (Benscoter 2006; Maltby et al. 1990). The effects of prescribed fires may be qualitatively quite different, and it was this that we aimed to investigate here. *Sphagnum* mosses are key peat-forming species and store large quantities of carbon (Rydin and Jeglum 2006). Their capacity for holding water and locking up nutrients, together with their recalcitrant litter, allows them to survive in, and maintain, the nutrient-poor and acidic peatland environment (Clymo and Hayward 1982; Jones et al. 1994; Kuhry et al. 1993; Rydin and Jeglum 2006). As key components of peatlands, understanding the response of the *Sphagnum* species to land management and the environment is of fundamental importance to peatland conservation.

In England, burning on blanket bog is only allowed as part of a pre-approved plan for conservation and restoration in a defined season (Anon 2007), and Wales has a similar set of regulations (Anon 2008). In Scotland, burning can only be legally carried out during a defined season, and only where *Calluna* constitutes more than 75% of the vegetation cover (Anon 2011); these guidelines are currently under review. Understanding the response of *Sphagnum* is a crucial aspect of these guidelines and needs to be based on evidence if the debate on the use of fire is to progress (see Davies et al. 2016). To date, little research has looked at the direct effect of fire on the *Sphagnum*. Observations suggest that the impact on the *Sphagnum* may depend on vegetation and environmental characteristics that influence temperature at the moss surface and the penetration depth and duration of high temperatures.

The typical adiabatic flame temperature of wood burning in air is 1980 $^{\circ}$C (Griffiths and Barnard 1995), so very high temperatures can be reached in the vegetation canopy during the passage of a fire. At the moss surface, temperatures can reach up to 600 $^{\circ}$C for relatively short periods (<30 seconds) (Davies 2005; Hamilton 2000), but typically, the moss layer is not exposed to such high temperatures (see Hobbs and Gimingham 1984). The limited available data suggest that the temperature at 2 cm below the moss surface rarely exceeds 50 °C (Davies 2005), although, Harris et al. (2011) recorded maximum temperatures of approximately 600 ºC 1 cm above the ground level in *Calluna* moorland. High surface temperatures can potentially affect *Sphagnum* growth through cell damage in the uppermost capitulum, the site of the majority of photosynthetic activity (Rydin and Jeglum 2006). However, fire may damage only the upper sections of stems, allowing re-growth from side shoots (Rydin and Jeglum 2006). This has been observed in the field in at least some circumstances (c.f. Clymo and Duckett 1986; Hamilton 2000).

The depth and duration of high temperatures will depend on the amount, composition and distribution of fuel above the moss layer, the moisture content and bulk density of vegetation and moss layer, and meteorological conditions (e.g. see Harris et al. 2011; Santana and Marrs 2014, 2016). A high density of above-ground fuel will prolong the residence time of the fire, causing greater heating and evaporation, and may allow the fire to penetrate the peat (Ashton et al. 2007; Davies et al. 2013). 'Hot spots' have been observed in *Calluna* fires in the moss layer immediately around the woody stems of *Calluna* (Davies 2005; Hamilton 2000). Conversely, high *Sphagnum* moisture contents could result in reduced temperatures and depth of penetration, as thermal energy would be dissipated by evaporation. A wetter *Sphagnum* layer may also have quicker recovery following the drying effect of the fire. However, given that fuel loads and moisture contents differ considerably among (and within) fires (Legg et al. 2010), the thermal impact of fire on *Sphagnum* will also vary. Here, we used a representative range of maximum temperatures, fire residence times and moisture contents to assess the effects of high temperature on photosynthesis and recovery in *Sphagnum capillifolium* (Ehrh.) Hedw *sensu lato*.

S. capillifolium is one of the most common species in *Calluna*-dominated heaths managed by fire (Rodwell 1991). From previous research (Glime 2007) and the well-known effects of temperature on enzymes, we expected that the photosynthetic capacity of *S. capillifolium* would be reduced following exposure to high temperature, with greater reduction at higher temperatures and longer residency times; and that a critical temperature and residency time would exist beyond which *S. capillifolium* could no longer recover.

Materials and methods

Experimental design

Sphagnum capillifolium was exposed experimentally to a high temperature over a short duration, reproducing the temperature dynamics in the range recorded in *Calluna* fires (Davies 2005). *S. capillifolium* was collected from Whim Moss, Penicuik, south-east Scotland (NT203532), an ombrotrophic blanket bog classified as M19 *Calluna vulgaris*-*Eriophorum vaginatum* National Vegetation Classification (NVC, Rodwell 1991) blanket mire. The site lies at 280 m a.s.l., with mean temperature of 8.6 ºC (Sheppard et al. 2013). Clumps of *S. capillifolium* (6 cm deep, 5 cm diameter) were collected a maximum of two days prior to the start of each run of the experiment from four separate hummocks (so enough material could be gathered), and placed into 5-cm diameter fibre pots (Grow It, Spalding, UK). Clumps were kept as intact as possible, ensuring the number of stems in each pot was representative of natural stem densities; the number of stems per pot varied between 42 and 83. For each run, 96 pots of *S. capillifolium* were placed within a tray containing a bed of *S. capillifolium* cuttings, to help maintain near natural moisture conditions with regularly watering in a glasshouse that was programmed to track external air temperatures (+/- 2 ºC). The tray was divided into four blocks so any variation in conditions across the tray could be reflected in the statistical models. Each pot was individually watered with distilled water, using a syringe, to maintain *S. capillifolium* moisture content to around 90% (moisture content is expressed throughout as mass of water / initial fresh mass of moss x 100, as this could be measured gravimetrically non-destructively over the whole course of the experiment; initially, the moss was near to saturation). A pilot study ensured that *S. capillifolium* samples could remain healthy under these conditions as indicated by chlorophyll florescence (see Taylor 2015).

The experiment was run three times in spring, autumn and winter, with one of three different temperature treatments (see below) randomly assigned to each of the 96 pots per run (Table 1). Pots were randomly assigned to one of four measurement procedures: chlorophyll fluorescence, CO_2 exchange, growth measurements or moisture content analysis, as both the chlorophyll fluorescence and moisture content analysis were destructive. Although the winter experiment in February 2013 was initiated only a month earlier in the seasonal cycle compared to the spring experiment (in March 2012), the prevailing weather conditions were quite different. In the two weeks prior to the winter experiment, the locally-measured air temperature averaged 1.4 ^{o}C, and was below freezing for much of the time. In the two weeks prior to the spring experiment, the air temperature averaged 7.4 ^{o}C, and the plants were physiologically active. Hence, we think these experiments approximate the typically contrasting conditions in these seasons, even though the timing in terms of the seasonal cycle was not large.

The temperature treatments were carried out by placing each pot in a perforated steel chamber, heated from above by a butane-propane gas burner (Parasene Weed Wand 550, Parasene, UK). The flame was held in place for the desired length of time once the surface of the pot reached the desired maximum temperature. The perforated steel chamber diffused the direct heat from the flame, so that temperatures could be better controlled at the moss surface. Temperature was logged (CR21X, Campbell Scientific, Utah, USA) every 2 s using k-type twisted thermocouples at, 2-cm and 5-cm depth. The temperature treatments were: 100 (100 ^{o}C for 3 s); 400 (400 ^{o}C for 3 s); 400+ (400 ^{o}C for 30 s); and 400+D (400 ^{o}C for 30s and air drying the *Sphagnum* for three days prior to treatment). Control pots were treated the same except that no heating was applied. For post-treatment recovery, the pots were maintained at stem moisture content of around 90%. The mean, minimum and maximum values of air temperature and photosynthetic photon flux density (PPFD) were also measured.

Chlorophyll fluorescence

Chlorophyll fluorescence was used to measure plant stress (Krause and Weis 1991; Maxwell and Johnson 2000), based on previous evaluations on *Sphagnum* (e.g. Hájek and Beckett 2008; Manninen et al. 2011; van Gaalen et al. 2007). The technique works on the principle that the ratio between variable florescence (F_v) and maximal florescence (F_m) approximates the maximum quantum yield of PSII, ranging between 0.75 and 0.84 in healthy mosses (e.g. Bates et al. 2013; Green et al. 1998; Hájek and Beckett 2008; Manninen et al. 2011; Proctor 2003; van Gaalen et al. 2007), with lower values indicating stress (Maxwell and Johnson 2000).

Chlorophyll fluorescence measurements were made using a Continuous Excitation Chlorophyll Fluorimeter (HandyPEA, Hansatech Instruments Ltd, UK) on the capitulum of one stem from each pot on 8 days beginning on the first day of temperature exposure and up to 100 days after exposure. Each capitulum was dark-adapted for 20 min, prior to measurements at a PPFD of 1500 µmol m^{-2} s^{-1}. During the autumn run chlorophyll fluorescence was assessed 100 days after treatment at 5-mm intervals down the stem.

CO_2 exchange

Gas exchange measurements were made five times on eight pots per treatment from day 3 to 99, using an infra-red gas analyser (LI-6400XT, Li-Cor, Lincoln, NE, USA) in an open gas exchange system, with a sample chamber designed to measure whole pots of *Sphagnum*. Each pot of *Sphagnum* was carefully transferred into an inert plastic pot of the same size as the gas exchange measurements. Air from the sample chamber was circulated through a column of silica gel to remove excess water vapour, such that the humidity of incoming and outgoing air from the sample chamber was similar. The Licor LI-6400XT was set to control the system flow rate (500 µmol air s^{-1}), chamber air temperature (20 °C), incoming CO_2

concentration (400 µmol mol^{-1}), and PPFD (0 or 2000 µmol photons m^{2} s^{-1} using a 6400-18 RGB light source, Licor, Lincoln, NE, USA). CO_2 concentrations were logged at 10 sintervals and averaged once stable, typically over 5 to 10 min. Because of the difficulties in quantifying leaf area, photosynthesis and respiration were expressed as µmol CO_2 (g dry mass)$^{-1}$ s^{-1}, correcting all CO_2 mixing ratios to a dry air basis. The dry mass of *Sphagnum* was calculated by oven-drying samples at the end of each run of the experiment (day 100) at 70 °C for 5 days before weighing. No respiration measurements were made during the spring run and measurements were made on only three occasions during the first half of the autumn run, as pots were infected with mould which covered some or all of the surface of the *Sphagnum*. No other runs of the experiment were affected by mould.

Physical damage and new growth

The depth of physical damage and bleaching (loss of pigment) was measured down the stem from the capitulum. Reduced structural integrity was estimated by gently running a finger across the surface of the pot showing breakage in brittle stems from pots assigned to the whole pot gas exchange and new growth.

The number and dry mass of new auxiliary stems was measured at the end of each run of the experiment and the length of the new stems was measured in a subset of samples. New stems oven-dried at 70 °C for 5 days, weighed and new growth calculated as the ratio of dry biomass (new growth plus original sample) to the original dry biomass to take into account the difference in the number of stems between pots.

Statistical analyses

Linear mixed-effects models were used for analysing chlorophyll fluorescence, photosynthesis and respiration data, accounting for the repeated measurement design. Initial models were composed of all fixed and random effect terms (Table 2). In subsequent models, non-significant fixed effect terms were dropped one by one (using AIC) to derive a model with the smallest AIC that consisted only significant fixed effect terms, as indicated by Wald tests. Statistics were carried out using R (v R i386 3.0.1) (R Core Team 2013) with mixed effects modelling computed using the package lme4 (Bates et al. 2009).

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Results

Chlorophyll florescence

Control plants had an *Fv/Fm* ratio closest to 0.7 but had distinct low periods during spring and winter (Figure 1a). In spring and autumn, the control plants had higher *Fv/Fm* ratios than plants from temperature treatments. The linear mixed effects model showed that both the fixed terms of Day and Treatment were significant as well as the interaction between Day and Treatment. Between-pot variability was found to be the largest random effect (Table S1, Figure S2). Physiological damage was confined to the upper portion of stems, where the *Fv/Fm* ratio was reduced in the top 20 mm of stems in the 400 and 400+ treatments compared to the control (Figure 2).

CO_2 exchange

Net CO_2 exchange under full light (2000 µmol m^2 s^{-1}), $A_{\max}$, varied considerably between runs and treatments, ranging from 84±13 to 252±26 µmol g^{-1} (dry weight) day^{-1} with highest values in control pots during autumn (Figure 1b). $A_{\max}$ in temperature-treated pots was only noticeably lower during the first half of the spring and autumn runs. In general, the respiration rate was less variable between treatments than $A_{\max}$ (Figure 1c) during both autumn and winter. Respiration rate also varied less between runs, ranging from -105±33 to -13±22 µmol g-^{1} (dry weight) day^{-1} in autumn and -103±11 to 17±12 µmol g-^{1} (dry weight) day^{-1} in winter.

The linear mixed effects models for $A_{\max}$, respiration and fluorescence showed that the Day and Treatment fixed-effect terms were significant (Table S2). In contrast, Day was not found to be significant in models of respiration rate (Table S3) with Hummock and Block the best random effects terms to explain the variance beyond the Treatment effect (Figure S3).

Common to both the models of $A_{\max}$ and respiration was that the random effects explained little of the within-treatment variance.

Physical damage and new growth

In control pots, bleaching was largely absent, only occurring for short periods on one or two stems per pot after particularly warm and dry conditions. Most stems in high-temperature treatments showed some bleaching (Figure 3) of the upper parts and capitulum, with the 100 ^{o}C treatment showing the least amount of bleaching (Figure 4), and bleaching being more pronounced a few days after heat treatment.

Depth of physiological damage was confined to the upper portion of stems; the *Fv/Fm* ratio was reduced in the 400 and 400+ treatments in the top 20 mm of stems (Figure 2). In the 400 ^{o}C treatment, the extent of damage increased with residence time. The greatest depth of damage occurred in the 400+D treatment (Figure 4). No damage was found down stems in control pots (Figures 2 and 4). A loss of structural integrity of the capitula was found in all 400+ treatments in autumn and winter, but was not seen at all in the control treatment (Figure 5).

New growth during the duration of the experiment arose in new, smaller and more elongated auxiliary stems in all treatments after 100 days in spring and winter (Figure 6). No new growth was found in any of the pots in autumn. Two distinct zones of growth were apparent in both spring and winter runs with new stems growing from upper side innovations and base innovations which grew from the lowest portion of the original stem. In both spring and winter, most new growth occurred in pots which had been subjected to the higher temperature treatments as opposed to control pots. Significantly more new growth was seen in spring in both control and temperature treatment pots than in winter.

The distance down the stem where new side innovations grew was correlated to the depth of bleaching, as the new side innovations grew from unbleached areas below the bleached stem (Figure 7).

Discussion

Our results show that the photosynthetic capacity of *S. capillifolium* was reduced following exposure to high temperatures, and that higher temperatures and longer residency times caused more physical damage. However, we found that *S. capillifolium* has the capacity to recover its photosynthetic capacity by producing new auxiliary growth.

Photosynthetic capacity and CO_2 exchange

The photosynthetic capacity, (chlorophyll fluorescence and CO_2 assimilation) of the upper sections of *S. capillifolium* was found to vary considerably between temperature treatments, and between each run of the experiment carried out in the different seasons. The highest *Fv/Fm* ratio closest to healthy plants ratio (around 0.75 Demmig and Bjorkman 1987), was found in control pots. Treated plants showed a general increase in *Fv/Fm* after an initial drop following high temperature treatment, but with quicker recovery to healthy *Fv/Fm* ratios in treatments with lower maximum surface temperatures and residency times. The ascending order of severity, indicated by the reduction in photosynthetic capacity and damage (bleaching) sustained, of the treatments can be summarised as control <100 <400<400+ (with the increased temperature residency time) <400+D (greatest damage caused when *S. capillifolium* was dried prior to temperature treatment.).

Other than the controls, the least reduction in photosynthetic capacity was seen in pots exposed to 100 °C, suggesting that this treatment did not cause severe damage. A similar effect was seen for bleaching (results not shown). Pots treated with a maximum surface temperature of 400°C showed the greatest reduction in *Fv/Fm*. Little difference was detected in *Fv/Fm* among 400 °C treatments where residence time and pre-treatment moisture content were varied. This suggests that the maximum temperature reached at the surface of the *Sphagnum* layer may be a sufficient indicator of the short-term impact on photosynthetic capacity. As shown by here, damage to plant cells brought about by fire, such as protein denaturation or lipid mobility (Levitt 1972) can be brought about by exposure to surface temperatures of around 400 °C for just 3 s in *S. capillifolium* at a pre-treatment moisture content of around 90%.

Another important observation was that the *Fv/Fm* varied both within a run and between runs, suggesting that both short-term changes in environment and seasonality are important. This was demonstrated by the control pots, which did not show the steady increase in *Fv/Fm* over time as seen in temperature-treated pots, but considerable variation between sample days. Stem moisture content was found to account for the most within treatment and sample day variation with lowest *Fv/Fm* in control pots corresponding to lower stem moisture content and a particularity warm period during spring. During the winter run of the experiment, it was also found that the lowest *Fv/Fm* found in control pots occurred after a period of a few days when the Sphagnum had frozen.

An optimum stem moisture content for photosynthesis has been shown in *Sphagnum,* with declining rates of CO_2 assimilation coupled with a reduction in stem moisture content (Clymo 1973; Johansson and Linder 1980; Strack et al. 2009; Titus et al. 1983; Williams and Flanagan 1996). The moisture content needed for maximum photosynthesis varies between species (Clymo 1973; Strack et al. 2009; Williams and Flanagan 1996) and seasonally (Johansson and Linder 1980; Titus et al. 1983). Specifically, the *Fv/Fm* measured using chlorophyll fluorescence has been shown to decline with reduced stem moisture content in *Sphagnum* (van Gaalen et al. 2007). *Sphagnum* has been shown to tolerate desiccation to a critical moisture threshold (Schouwenaars and Gosen 2007) when reached net photosynthesis ceases (Schipperges and Rydin 1998). This suggests that the drying experienced in control pots during this study was survivable and did not drop below this threshold. In the 400+D treatment, *Sphagnum* were dried to a moisture content of 80% prior to treatments, and they remained consistently drier, up to 88 days than other treatments. This could be caused by the water transport and holding capacity of the *Sphagnum* being compromised by exposure to high temperatures. This suggests that high temperatures caused by fire may make *Sphagnum* vulnerable to long-term damage brought about by drought, by increasing the likelihood of drying below the critical threshold. Therefore, post-fire conditions may be important and short-term environmental changes may have long-term influences on productivity in *Sphagnum* (Backéus 1988; McNeil and Waddington 2003).

$A_{\max}$ largely reflected the treatment effects on *Fv/Fm* with the exception of the lack of treatment effect on $A_{\max}$ in the winter run. During spring and autumn, the control pots showed higher $A_{\max}$ than the 400 ^{o}C temperature treatments with some degree of recovery shown in the temperature pots in spring. Recovery of the temperature treatments was not seen in either winter or autumn, but this could be because measurements were not continued for the duration of the autumn run because of mould contamination. There was no significant difference in $A_{\max}$ in the 100 ^{o}C treatment, suggesting that higher temperatures had a more detrimental effect on photosynthesis, which was further supported by the *Fv/Fm* observations. There was no clear treatment effect on respiration, despite it still being a significant term in the mixed effects model. This is likely to be due to differences in respiration rates observed on day 1 between the control pots and temperature treated pots in autumn.

There was considerable variation between sampling days particularly in winter A_{max} but no detectable difference between treatments. During both autumn and winter, A_{max} in temperature-treated pots followed the same temporal pattern of A_{max} of control pots. This suggests that A_{max} in all pots was determined by other factors beyond the temperature treatments.

Sphagnum has been found to exhibit strong seasonal variation in productivity, with short-day photoperiods (Gerdol 1995; Li and Glime 1991) and low temperatures associated with up to a five-fold reduction in growth (Gerdol et al. 1998). The findings here support this seasonality with the lack of CO_2 assimilation during the winter run of the experiment. However, the lower stem moisture content experienced throughout the winter run could also account for low A_{max} in the control pots. This suggest that the implications for prescribed burning may be that if photosynthesis and growth rates are lower during the colder and shorter days of winter, then rates of recovery could be much slower following fires which have taken place from October to February that burns which happen at from March to April. Seasonality and timing of fire is therefore an important consideration when reducing the impact on *Sphagnum* is a goal.

In real fires in the field, the deposition of ash on to the moss layer may have detrimental effects on photosynthesis, but very little is known about this. Future work using laboratory-based simulated fires could usefully separate the effects of ash deposition from the effects of high temperature, and examine any interaction effects.

New growth

New growth in side and base innovations were only found in spring and autumn. Low light levels in the winter run of the experiment, could account for the lack of new growth observed. An additional control on growth is night-time temperature and *S. capillifolium* has been demonstrated to have a five-fold increase in growth at a night-time temperature of 15 °C compared to 5 °C (Gerdol et al. 1998). The temperatures recorded during autumn declined from around day 50, making them lower than those in spring, so these low temperatures could also contribute to the lack of growth observed.

It is important to highlight the need to take into account post-burn conditions when assessing *Sphagnum* recovery especially when assessing fire severity. For example, management burns occurring in the spring may show faster rates of *Sphagnum* recovery due to the more favourable growing conditions than those found in winter. Thus, post-burn recovery may have as much to do with season than with the fire itself as post-fire conditions, most notably the moisture status of the *Sphagnum* layer and height of the water table may retard or promote growth (Robroek et al. 2007; Rochefort et al. 2002). This would make it necessary to include post-burn environmental variables in methods that assess fire severity.

Our observation regarding regeneration was that side innovations appeared to be very similar to those described by Clymo and Duckett (1986) who suspected that the ability of *Sphagnum* to produce new shoots was a widespread and important mechanism to overcome disturbance (see also Hamilton 2000; Rochefort et al. 2002). Regeneration and production of new innovations has been observed in the field (Burch 2009; Hamilton 2000),where patches of *Sphagnum* produced new green capitula on the surface and capitula regained colouration after bleaching, following a fire. This suggests that the side innovations observed here were not just a product of experimental conditions.

The most severe temperature treatment (400+D) used here was intended to be fatal to *S. capillifolium* but was still found to result in new growth. This suggests even higher surface temperatures and longer temperature residency times are needed to kill S. *capillifolium*. Clymo and Duckett (1986) demonstrated *Sphagnum* growth 30 cm below the surface, suggesting that high temperatures would have to penetrate very deep within the *Sphagnum* layer to prevent any regeneration. However, at this depth, light may limit regeneration after fire, and complete consumption would prevent any regeneration. Nevertheless, partial consumption may allow sufficient light for side or base innovations to proliferate and hence allow recovery.

Conclusions

The aim of this research was to determine the short-term responses of *Sphagnum capillifolium* to fire. We found that the rate of photosynthesis was reduced by exposure to high surface temperatures. High temperature also increased the extent of bleaching and capitulum loss. Importantly though, within the range of surface temperatures and residence times used here, no critical threshold was found to cause widespread death of *S. capillifolium*. Even in the treatment specifically designed to be lethal, new auxiliary stem growth was found. Our results provide evidence that *S. capillifolium* has the ability to recover from the high temperatures experienced in typical prescribed fires, provided that at least some living material remains. The experiment also suggests seasonal effects are important to *S. capillifolium* recovery, and that recovery may be conditional on the fire timing. Although these results demonstrate that *S. capillifolium* has the ability to survive a fire event, it is important to consider these results within the context of management burning regimes. Lee et al. (2013) for instance have demonstrated that, although *Sphagnum* may survive a fire, long-term frequent burning (every 10 years) can reduce the propagule bank within the peat. This could reduce the capacity for recovery from fire events which wholly consume the *Sphagnum* layer. Future research into the impact of the types of fires simulated here, which are supported by the current best practice guidance and legislation, should include other *Sphagnum* species, particularly those from differing micro-habitats, to establish if the findings are generalizable to other *Sphagnum* spp. and identify micro-habitats or species which may be most vulnerable.

References

Anon, 2007. The Heather and Grass Burning Code. Department for Environment, Food and Rural Affairs, London.

Anon, 2008. The Heather and Grass etc. Burning (Wales) Regulations 2008 Welsh Assembly Government. Welsh Statutory Instruments 2008 No. 1081 (W.115).

Anon, 2011. The Muirburn Code. Scottish Executive, Edinburgh.

Ashton, C., Rein, G., Dios, J., Torero, J.L., Legg, C., Davies, M., Gray, A., 2007. Experiments and Observation of Peat Smouldering Fires. International Meeting of Fire Effects on Soil Properties.

Backéus, I., 1988. Weather Variables as Predictors of Sphagnum Growth on a Bog. Holarctic Ecology 11, 146-150.

Bates, D., Maechler, M., Bolker, B., 2013. lme4: Linear mixed-effects models using S4, In R package version.

Bates, J.W., Wibbelmann, M.H., Proctor, M.C.F., 2009. Salinity responses of halophytic and non-halophytic bryophytes determined by chlorophyll fluorometry. Journal of Bryology 31, 11-19.

Benscoter, B.W., 2006. Post-fire bryophyte establishment in a continental bog. Journal of Vegetation Science 17, 647-652.

Burch, J., 2009. The regeneration of bryophytes after the burning of dry heat and wet heath moorland on the North York Moors. Natural England Commissioned Report NECR011., Sheffield.

Clymo, R.S., 1973. Growth of Sphagnum - Some Effects of Environment. Journal of Ecology 61, 849-869.

Clymo, R.S., Duckett, J.G., 1986. Regeneration of Sphagnum. New Phytologist 102, 589-614.

Clymo, R.S., Hayward, P.M., 1982. The ecology of *Sphagnum*, In Bryphoyte Ecology. ed. A.J.E. Smith, pp. 229-290. Chapman and Hall, London.

Davies, G.M., 2005. Fire behaviour and impact on heather moorland. . The University of Edinburgh, Edinburgh.

Davies, G.M., Gray, A., Rein, G., Legg, C.J., 2013. Peat consumption and carbon loss due to smouldering wildfire in a temperate peatland. Forest Ecology and Management 308, 169-177.

Davies, G.M., Kettridge, N., Stoof, C.R., Gray, A., Ascoli, D., Fernandes, P.M., Marrs, R., Allen, K.A., Doerr, S.H., Clay, G.D., McMorrow, J., Vandvik, V., 2016. The role of fire in UK peatland and moorland management: the need for informed, unbiased debate. Philosophical Transactions of the Royal Society B: Biological Sciences 371.

Demmig, B., Bjorkman, O., 1987. Comparison of the Effect of Excessive Light on Chlorophyll Fluorescence (77k) and Photon Yield of O-2 Evolution in Leaves of Higher-Plants. Planta 171, 171-184.

Gerdol, R., 1995. The Growth Dynamics of Sphagnum Based on Field-Measurements in a Temperate Bog and on Laboratory Cultures. Journal of Ecology 83, 431-437.

Gerdol, R., Bonora, A., Marchesini, R., Gualandri, R., Pancaldi, S., 1998. Growth response of Sphagnum capillifolium to nighttime temperature and nutrient level: mechanisms and implications for global change. Arctic and Alpine Research, 388-395.

Glime, J.M., 2007. Bryophyte Ecology. Volume 1. Physiological Ecology. Ebook sponsored by Michigan Technological University and the International Association of Bryologists.

Green, T., Schroeter, B., Kappen, L., Seppelt, R., Maseyk, K., 1998. An assessment of the relationship between chlorophyll a fluorescence and CO 2 gas exchange from field measurements on a moss and lichen. Planta 206, 611-618.

Griffiths, J.F., Barnard, J.A., 1995. Flame and combustion. CRC Press.

Hájek, T., Beckett, R.P., 2008. Effect of water content components on desiccation and recovery in Sphagnum mosses. Annals of botany 101, 165-173.

Hamilton, A., 2000. The characteristics and effects of management fire on blanket bog vegetation in north-west Scotland., In Institute of Ecology and Resource Management. University of Edinburgh, Edinburgh.

Harris, M., Allen, K., Le Duc, M., Eyre, G., Marrs, R., 2011. Prescribed fire characteristics and biomass reduction on upland moorland. Aspects of Applied Biology, 171-177.

Hobbs, R., Gimingham, C., 1984. Studies on fire in Scottish heathland communities: I. Fire characteristics. The Journal of Ecology, 223-240.

Johansson, L.G., Linder, S., 1980. Photosynthesis of Sphagnum in Different Microhabitats on a Subarctic Mire. Ecological Bulletins, 181-190.

Jones, C.G., Lawton, J.H., Shachak, M., 1994. Organisms as ecosystem engineers, In Ecosystem management. pp. 130-147. Springer.

Keeley, J.E., 2009. Fire intensity, fire severity and burn severity: a brief review and suggested usage. International Journal of Wildland Fire 18, 116-126.

Krause, G., Weis, E., 1991. Chlorophyll fluorescence and photosynthesis: the basics. Annual review of plant biology 42, 313-349.

Kuhry, P., Nicholson, B.J., Gignac, L.D., Vitt, D.H., Bayley, S.E., 1993. Development of Sphagnum-dominated peatlands in boreal continental Canada. Canadian Journal of Botany 71, 10-22.

Lee, H., Alday, J.G., Rosenburgh, A., Harris, M., McAllister, H., Marrs, R.H., 2013. Change in propagule banks during prescribed burning: A tale of two contrasting moorlands. Biological Conservation 165, 187-197.

Legg, C., Davies, G.M., Gray, A., 2010. Comment on: Burning management and carbon sequestration of upland heather moorland in the UK. Soil Research 48, 100-103.

Levitt, J., 1972. Responses of plants to environmental stresses, . Academic Press., New York.

Li, Y., Glime, J.M., 1991. Growth response of two Sphagnum species to photoperiod. Canadian Journal of Botany 69, 2643-2646.

Maltby, E., Legg, C., Proctor, M., 1990. The ecology of severe moorland fire on the North York Moors: effects of the 1976 fires, and subsequent surface and vegetation development. The Journal of Ecology, 490-518.

Manninen, S., Woods, C., Leith, I., Sheppard, L., 2011. Physiological and morphological effects of long-term ammonium or nitrate deposition on the green and red (shade and open grown) Sphagnum capillifolium. Environmental and Experimental Botany 72, 140-148.

Maxwell, K., Johnson, G.N., 2000. Chlorophyll fluorescence—a practical guide. Journal of experimental botany 51, 659-668.

McNeil, P., Waddington, J., 2003. Moisture controls on Sphagnum growth and CO2 exchange on a cutover bog. Journal of Applied Ecology 40, 354-367.

Proctor, M.C., 2003. Experiments on the effect of different intensities of desiccation on bryophyte survival, using chlorophyll fluorescence as an index of recovery. Journal of Bryology 25, 201-210.

R, Core Team 2013. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Robroek, B.J., Limpens, J., Breeuwer, A., Schouten, M.G., 2007. Effects of water level and temperature on performance of four Sphagnum mosses. Plant Ecology 190, 97-107.

Rochefort, L., Campeau, S., Bugnon, J.-L., 2002. Does prolonged flooding prevent or enhance regeneration and growth of Sphagnum? Aquatic Botany 74, 327-341.

Rodwell, J.S., 1991. British Plant Communities Volume 2: Mires and Heaths. Cambridge University Press, Cambridge.

Rydin, H., Jeglum, J.K., 2006. The biology of peatlands. Oxford university press.

Santana, V.M., Marrs, R.H., 2014. Flammability properties of British heathland and moorland vegetation: models for predicting fire ignition. Journal of environmental management 139, 88-96.

Santana, V.M., Marrs, R.H., 2016. Models for predicting fire ignition probability in graminoids from boreo–temperate moorland ecosystems. International Journal of Wildland Fire 25, 679-684.

Schipperges, B., Rydin, H., 1998. Response of photosynthesis of Sphagnum species from contrasting microhabitats to tissue water content and repeated desiccation. New Phytologist 140, 677-684.

Schouwenaars, J., Gosen, A., 2007. The sensitivity of Sphagnum to surface layer conditions in a re-wetted bog: a simulation study of water stress. Mires and Peat 2, np-np.

Sheppard, L., Leith, I., Leeson, S., Dijk, N.v., Field, C., Levy, P., 2013. Fate of N in a peatland, Whim bog: immobilisation in the vegetation and peat, leakage into pore water and losses as N 2 O depend on the form of N. Biogeosciences 10, 149-160.

Shetler, G., Turetsky, M.R., Kane, E., Kasischke, E., 2008. Sphagnum mosses limit total carbon consumption during fire in Alaskan black spruce forests. Canadian Journal of Forest Research 38, 2328-2336.

Strack, M., Waddington, J., Lucchese, M., Cagampan, J., 2009. Moisture controls on CO2 exchange in a Sphagnum-dominated peatland: results from an extreme drought field experiment. Ecohydrology 2, 454-461.

Taylor, E.S., 2015. Impact of fire on blanket bogs: implications for vegetation and the carbon cycle. The University of Edinburgh, Edinburgh.

Titus, J.E., Wagner, D.J., Stephens, M.D., 1983. Contrasting water relations of photosynthesis for two Sphagnum mosses. Ecology 64, 1109-1115.

van Gaalen, K.E., Flanagan, L.B., Peddle, D.R., 2007. Photosynthesis, chlorophyll fluorescence and spectral reflectance in Sphagnum moss at varying water contents. Oecologia 153, 19-28.

Williams, T.G., Flanagan, L.B., 1996. Effect of changes in water content on photosynthesis, transpiration and discrimination against 13CO2 and C18O16O in Pleurozium and Sphagnum. Oecologia 108, 38-46.

Table 1 Temperature treatments, designed to simulate conditions recorded in *Calluna vulgaris* fires, used on samples of *Sphagnum capillifolium* for three runs of an experiment to determine its capacity for recovery. Burn Season refers to the time of year the pots were exposed to each temperature treatment. All pots were observed and recovery measurements made for a total of 100 days after being exposed to each temperature treatment which is termed Observation Period in the table. n = 32 per treatment per run of the experiment. *400+D indicates where the treatment was carried out on pots of *Scapillifolium* subjected to three days of drying prior to the treatment. As some treatments were repeated in different runs total pots varied by treatment; control (n=96), 100 (n=32), 400 (n=64), 400+ (n=64), 400+D (n=32).

Run	**Simulated Burn Season**	**Observation Period**	**Treatment Name**	**Maximum Surface Temp (°C)**	**Maximum Temperature Residence Time at Surface (s)**	**Mean *Sphagnum* Moisture Content when burnt (% wet weight basis)**
1	Spring	Mar‘12 – Jun’12	Control	Ambient (~9°C)	-	
			100	100	3s at max surface temp	89.5
			400	400	3s at max surface temp	89.6
2	Autumn	Oct’12 – Jan’13	Control	Ambient (~16°C)	-	
			400	400	3s at max surface temp	89.6
			400+	400	30s between 350 & 450°C	92.5
3	Winter	Feb’13 – May’13	Control	Ambient (~3°C)	-	
			400+	400	30s between 350 & 450°C	92.5
			400+D*	400	30s between 350 & 450°C	80.6

Table 2 Fixed and random effects terms used in mixed effects modelling of the repeated measures of chlorophyll fluorescence and CO_2 exchange of *Sphagnum capillifolium* samples exposed to temperature treatments designed to simulate conditions recorded in *Calluna vulgaris* fires.*Moisture content term only used in chlorophyll fluorescence model as stems were harvested for moisture content analysis only on days fluorescence measurements were made.

Model term	**Abbreviation**	**Description**
Fixed effects		
Treatment	Treat	Treatment applied to each pot
Day		The day measurement was made (between 1 and 100 per run). Treated as fixed effect as measurements made on same day each run of the experiment
Random effects (accounting for variance within Treatment + Day fixed effect)		
Block		Block (1 to 4) within the tray pots were kept in
Run		Run of the experiment (1 to 3), synonymous with "Burn Season"
Hummock	Hum	Variance explained by the hummock from which potted *S. capillifolium* sample was taken (4 per run, 12 different hummocks in total)
Run:Hummock	Run:Hum	Hummock nested within run specifies variance between hummocks within the same run of the experiment (accounts for different hummocks used in each run)
Moisture Content*	MC	Moisture content of samples taken concurrently with fluorescence measurements
Pot		Random pot to pot variance
Run:Pot		Pot nested within run specifies variance between pots within the same run (accounts for different pots used in each run)

Figure Captions

Figure 1: (a) *Fv/Fm* ratio of *Sphagnum capillifolium* stems subjected to temperature treatments designed to simulate conditions recorded in *Calluna vulgaris* fires. The experiment was repeated in three seasons; spring, autumn and winter (n=8 per treatment per sampling time). Treatments were: control, no temperature treatment; 400 surface exposed to 400oC for 3 seconds; 400+, surface exposed to temperatures between 350 and 450oC for 30 seconds; and 400+D, where the moss sample was dried prior to exposure to surface temperatures between 350 and 450oC for 30 seconds. Points show mean *Fv/Fm* ±SEM bars. **(b)** A_{max} in each treatment group (described above) during each run. Points show mean ±SEM bars. Positive values show CO2 uptake (indicating photosynthesis). **(c)** Respiration of pots in each treatment group in autumn and winter. Points show mean ±SEM bars. Respiration is expressed as a negative quantity in our sign convention. No data were available from the spring experiment.

Figure 2: *Fv/Fm* ratio of *Sphagnum capillifolium* stems subjected to temperature treatments designed to simulate conditions recorded in *Calluna vulgaris* fires. The *Fv/Fm* ratio is shown in relation to distance down the stem, starting from the capitulum. Data are from a sub-sample of pots in the autumn experiment (n=6 per treatment). Treatments were either a control without temperature treatment (C), exposed to 400 °C for 3 seconds (400), or exposed between 350 and 450 °C for 30 seconds (400+).

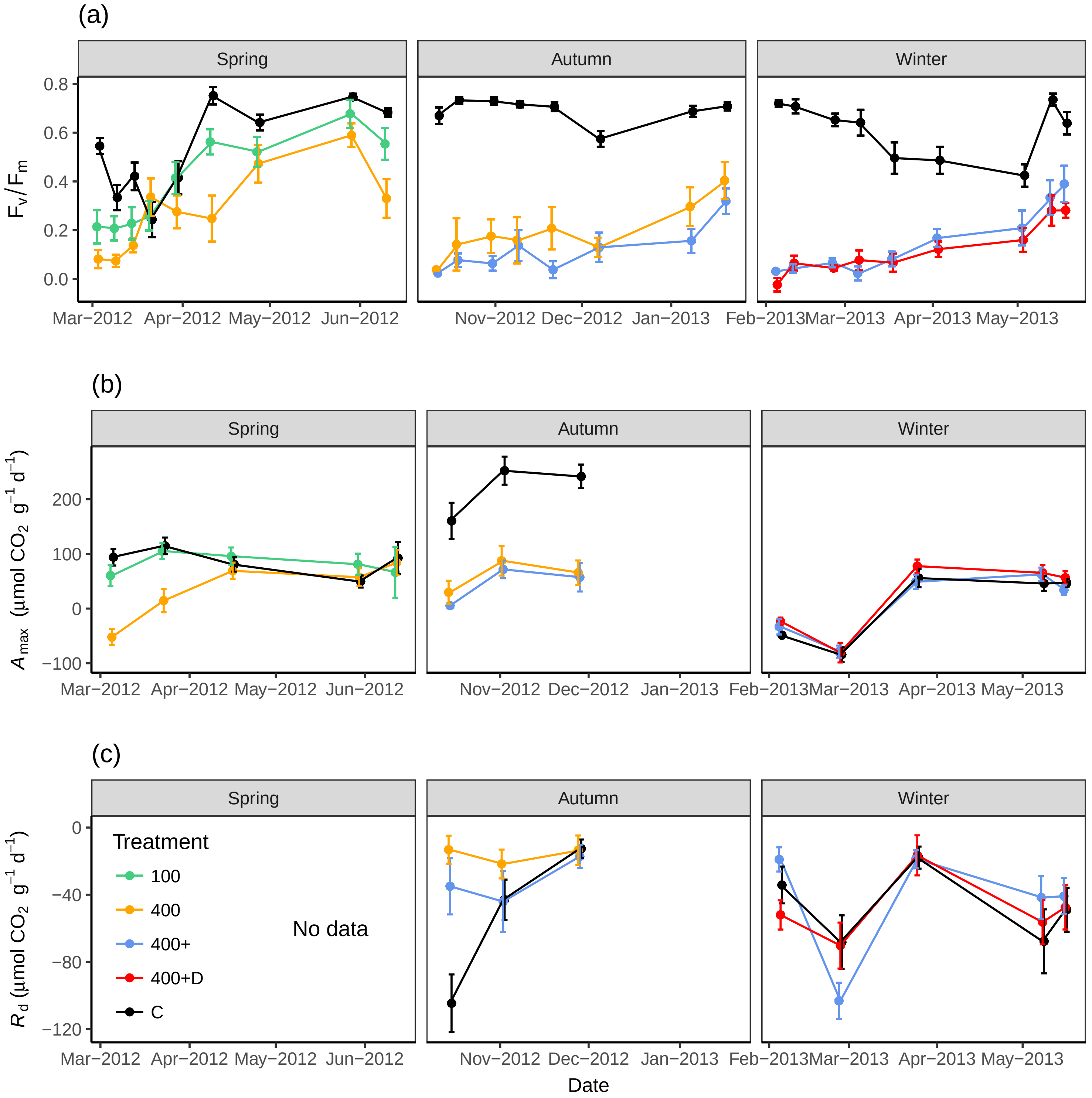
Figure 3: Examples of bleached *Sphagnum capillifolium* subjected to temperature treatments designed to simulate conditions recorded in *Calluna vulgaris* fires. (a) Pot showing the characteristic pale areas of bleaching of the capitula (red arrow). Bright green capitula are growth innovations from the stem below. (b) Several stems with individual branches bleached (red arrow). The lack of colouration in the lower stem is a normal response to low light levels. (c) *S. capillifolium* plant showing bleached capitulum (red arrow) and new growth innovation near the top of the plant (green side stem). (d) *S. capillifolium* plant showing bleached stem (red arrow) and new growth innovation near the bottom of the plant (green side stem) which were characteristically smaller and thinner than those arising further up the stem. When bleaching occurred, capitula became brittle to touch and easily broke away from the stem.

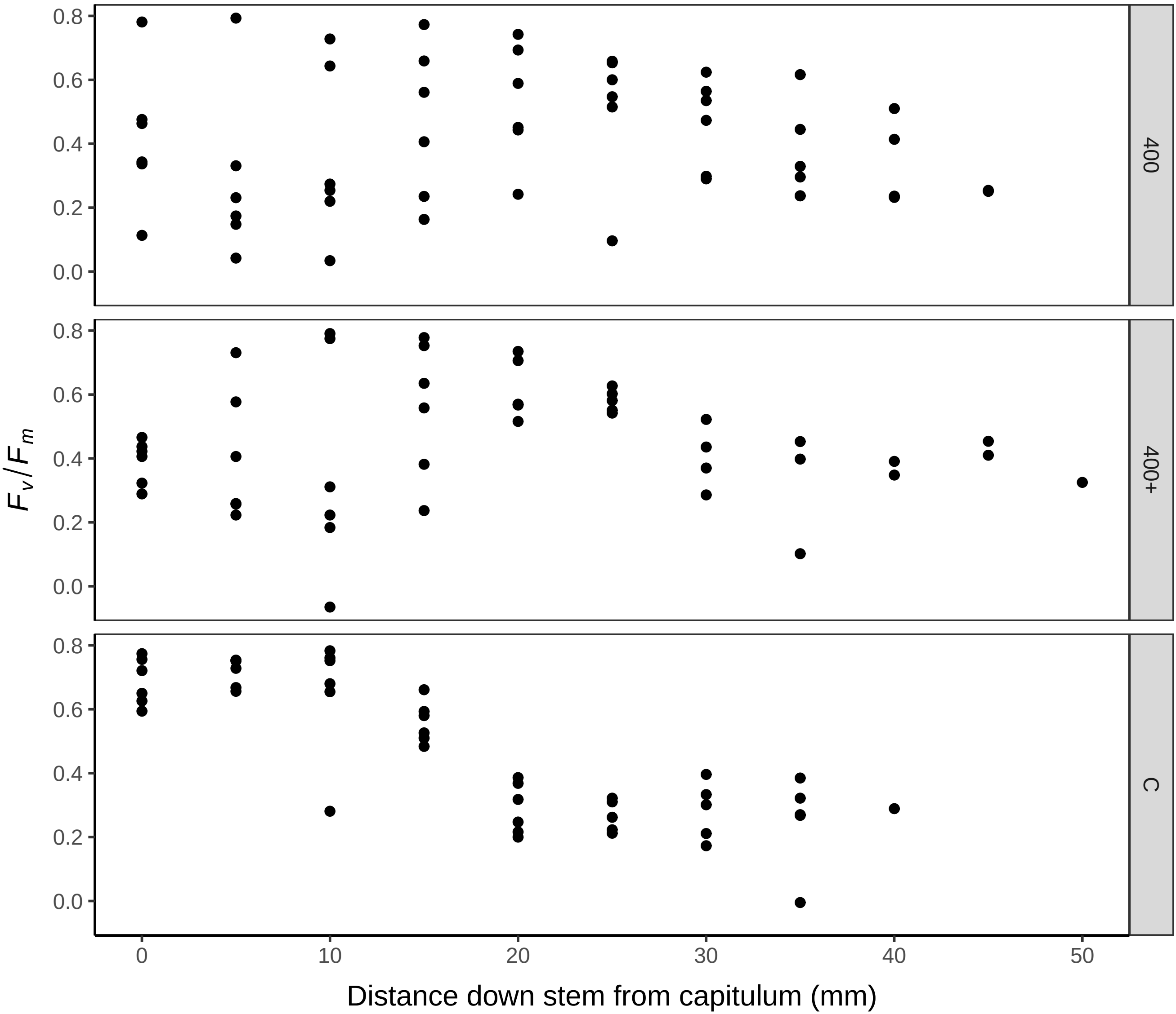
Figure 4 Depth of bleaching down stems of *S. capillifolium* (mean ± SEM) exposed to temperature treatments designed to simulate conditions recorded in *Calluna vulgaris* fires in a sub-sample of pots from experiments carried out in autumn and winter (n= 6 stems per pot, 16 pots per treatment per run of the experiment). Treatments were either a control without temperature treatment (C), exposed to 400 °C for 3 seconds (400), exposed to between 350 °C and 450 °C for 30 seconds (400+), or dried prior to exposure to between 350 °C and 450 °C for 30 seconds (400+D). No permanent bleaching was recorded in any control pots. All pots were harvested on day 100. Means with different letters are significantly different (Welch Two Sample t test: t=-4.6, df=39.6,*P*=<0.05 and t=-5.1, df=25.1, *P*=<0.05 respectively).

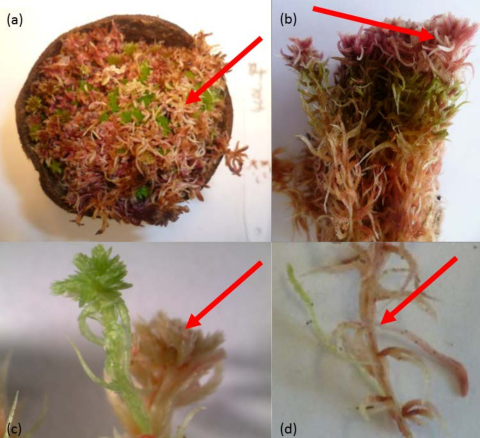
Figure 5: Capitulum decay in S. *capillifolium* plants subjected to temperature treatments designed to simulate conditions recorded in *Calluna vulgaris* fires. Bars show the number of stems showing capitulum decay (bleaching and/or reduced structural integrity) at each sampling time for each treatment during autumn and winter (n=8 stems per treatment per sampling time per run).). Treatments were either a control without temperature treatment (C), exposed to 400 °C for 3 seconds (400), exposed to between 350 °C and 450 °C for 30 seconds (400+), or dried prior to exposure to between 350 °C and 450 °C for 30 seconds (400+D). No capitulum decay occurred in control pots.

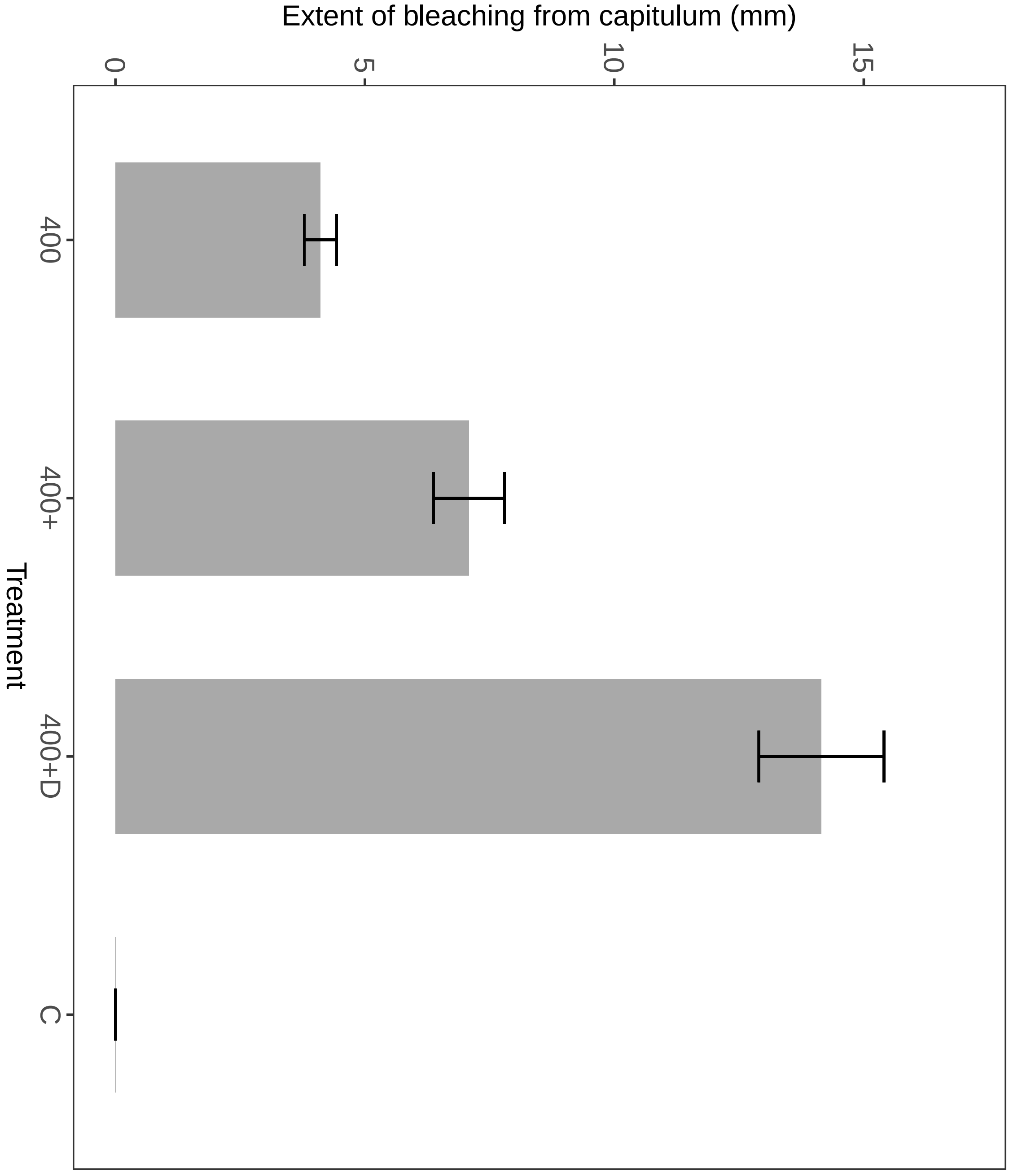
Figure 6: Bars show the number and location of regenerating stems of *S. capillifolium* following temperature treatments designed to simulate conditions recorded in *Calluna vulgaris* fires. Plots show a subsample of 16 pots per treatment per run showing the total number of new side and base innovations in spring and winter experiments. The total number of new innovations (base+side) was significantly higher in the 400 treatment compared to the 100 and control treatments in spring (Welch Two-sample t test: t=-3.3, df=38, *P*=<0.05 and t=-3.2, df=37, *P*=<0.05 respectively). There were significantly more new innovations in the 400+ and 400+D treatments compared to the control treatment in autumn (Welch Two Sample t test: t=2.6 df=39, p=<0.05 and t=3.4, df=36, *P*=<0.05 respectively). No significant difference was found between the 400+ and 400+D treatments.

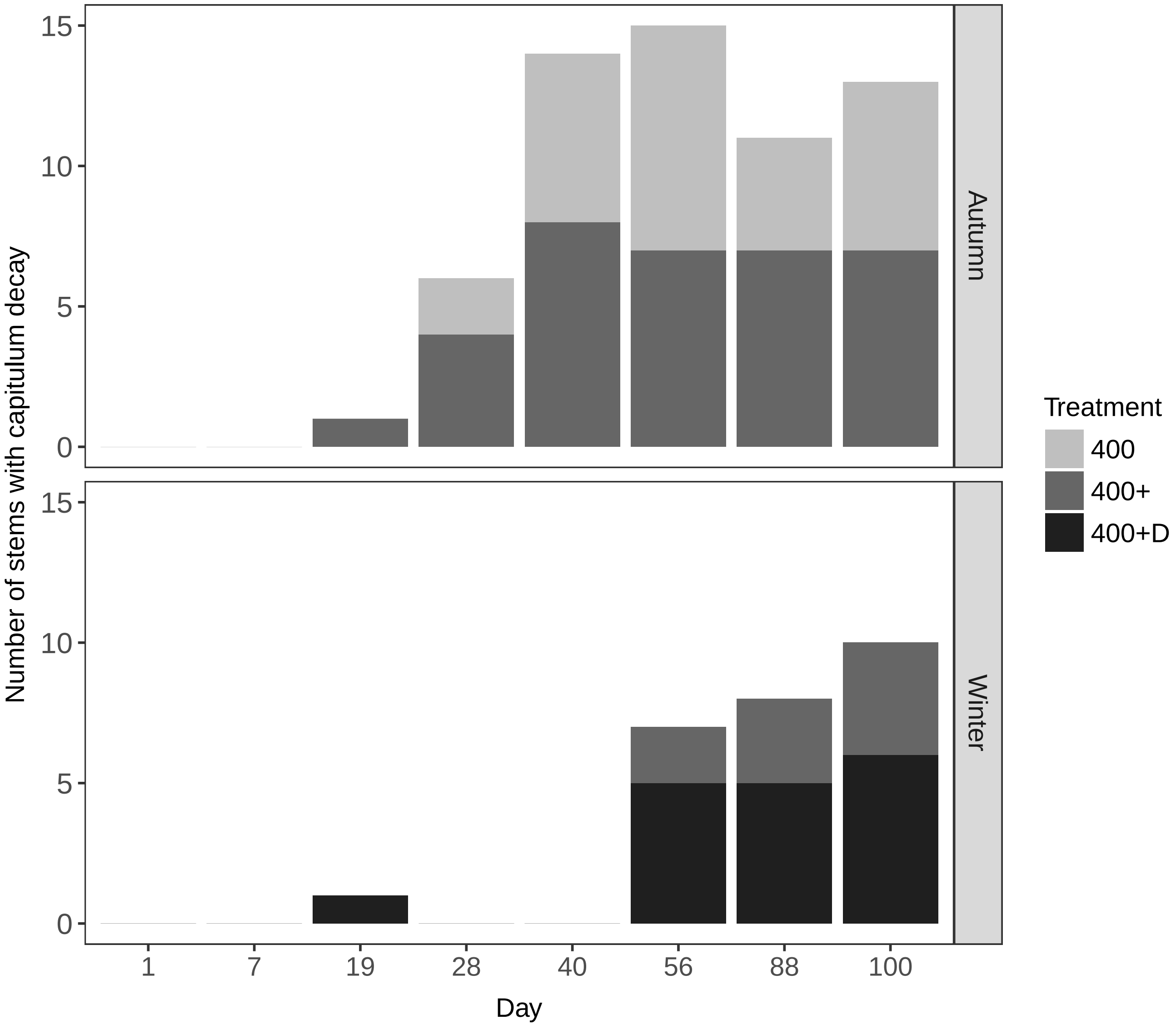
Figure 7: The location of regenerative growth in relation to the mean depth of bleaching in *S. capillifolium,* following temperature treatments designed to simulate conditions recorded in *Calluna vulgaris* fires. Points show the mean ± SEM from a subsample of 16 pots per treatment per run from the winter experiment. Treatments were either a control without temperature treatment (C), exposed to between 350 °C and 450 °C for 30 seconds (400+), or dried prior to exposure to between 350 °C and 450 °C for 30 seconds (400+D).

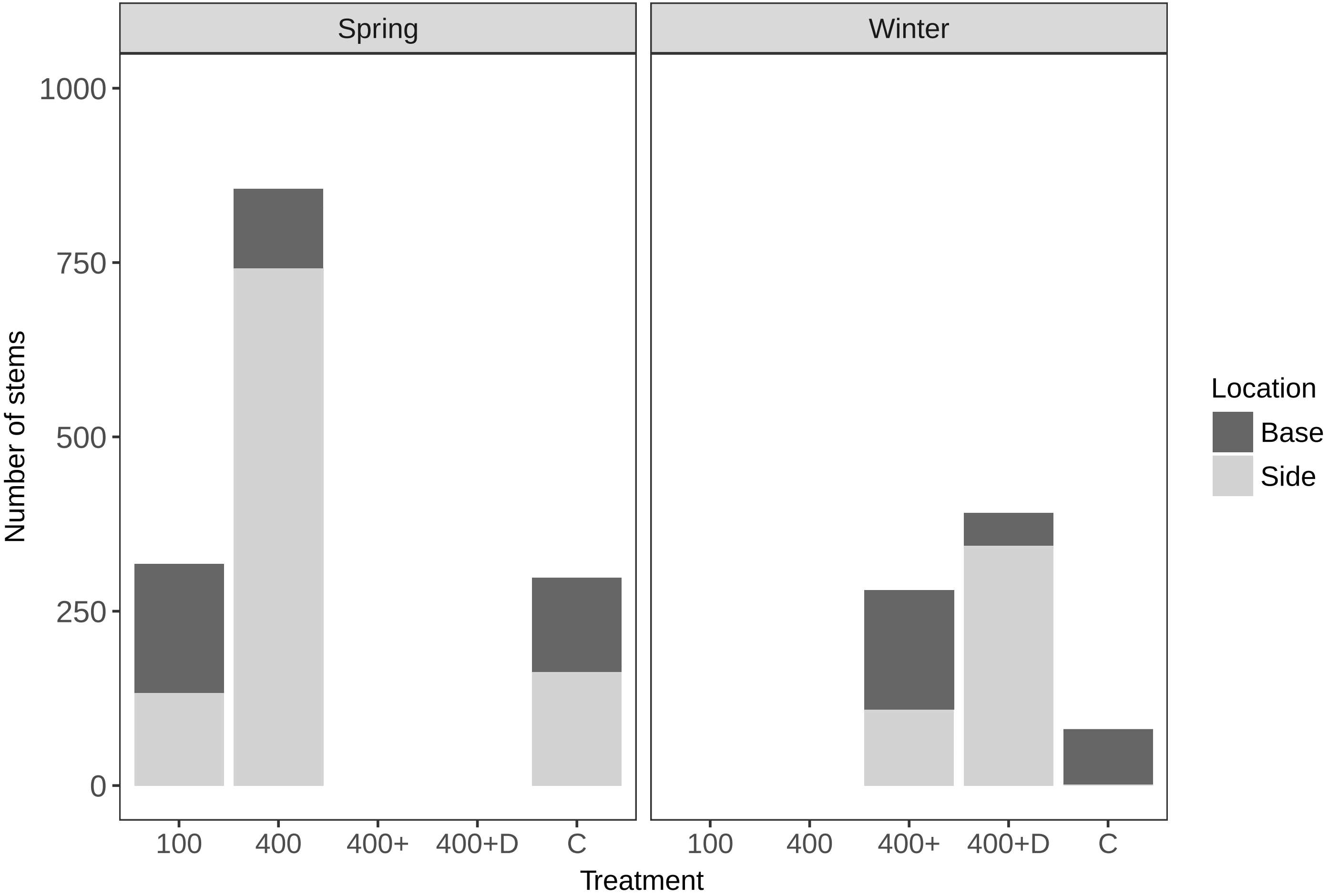

(a)
Spring
Autumn
Winter
F_v/F_m
0.8
0.6
0.4
0.2
0.0
Mar−2012
Apr−2012
May−2012
Jun−2012
Nov−2012
Dec−2012
Jan−2013
Feb−2013
Mar−2013
Apr−2013
May−2013
(b)
Spring
Autumn
Winter
A_{max} (µmol CO$_2$ g^{-1} d^{-1})
200
100
0
−100
Mar−2012
Apr−2012
May−2012
Jun−2012
Nov−2012
Dec−2012
Jan−2013
Feb−2013
Mar−2013
Apr−2013
May−2013
(c)
Spring
Autumn
Winter
R_d (µmol CO$_2$ g^{-1} d^{-1})
0
−40
−80
−120
Treatment
100
400
400+
400+D
C
No data
Mar−2012
Apr−2012
May−2012
Jun−2012
Nov−2012
Dec−2012
Jan−2013
Feb−2013
Mar−2013
Apr−2013
May−2013
Date

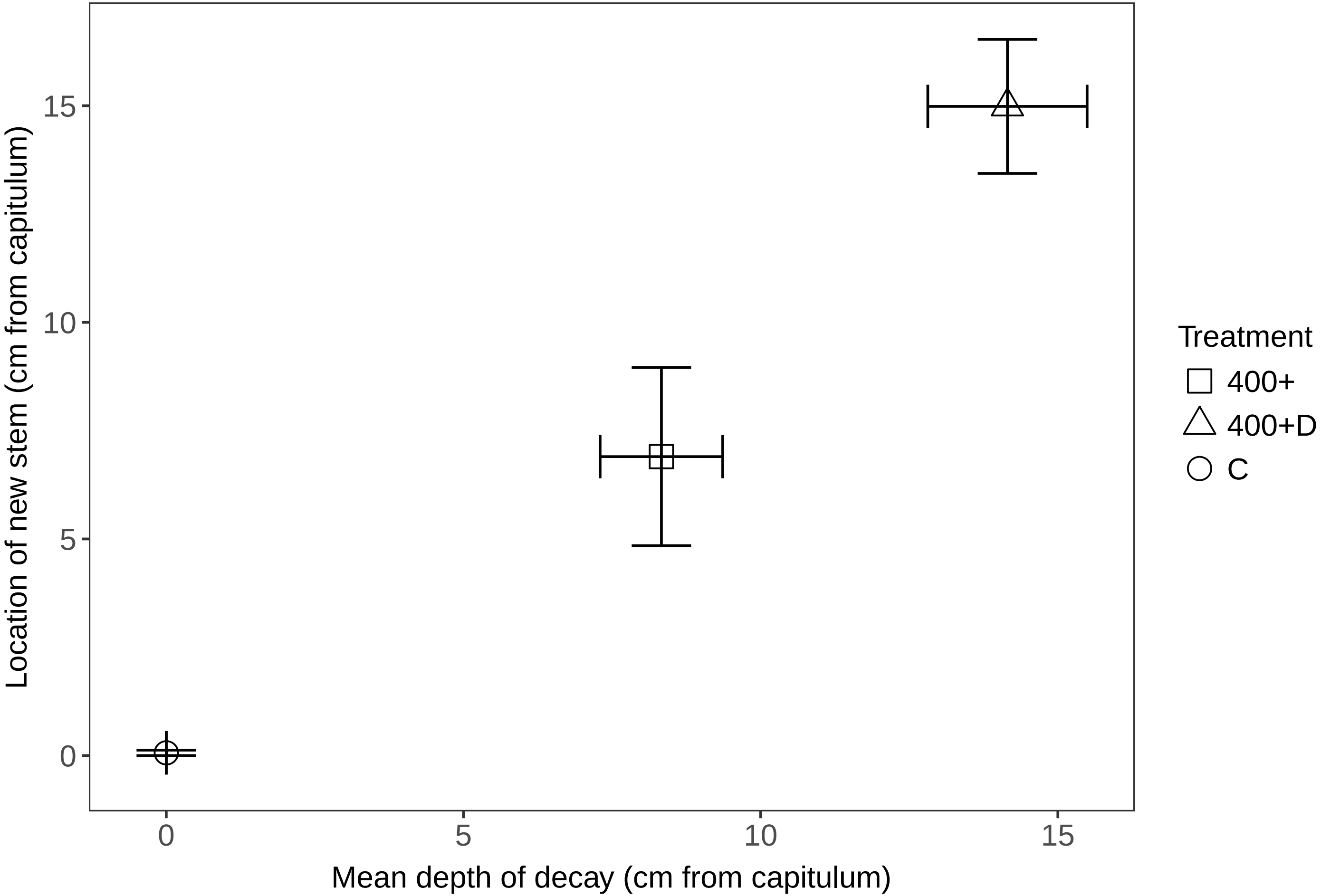

0.8
0.6
0.4
0.2
0.0
400
400+
C
F_v/F_m
0
10
20
30
40
50
Distance down stem from capitulum (mm)


(a)
(b)
(c)
(d)


Extent of bleaching from capitulum (mm)
0
5
10
15
400
400+
400+D
C
Treatment


Number of stems with capitulum decay
15
10
5
0
Autumn
Winter
1
7
19
28
40
56
88
100
Day
Treatment
400
400+
400+D


Spring
Winter
1000
750
500
250
0
Number of stems
100
400
400+
400+D
C
100
400
400+
400+D
C
Treatment
Location
Base
Side


Location of new stem (cm from capitulum)
0
5
10
15
Mean depth of decay (cm from capitulum)
0
5
10
15
Treatment
□ 400+
△ 400+D
○ C