Tradeoffs between variability and local adaptation influence the ecophysiology of the moss, *Sphagnum magellanicum*

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Abstract

Bryophytes are a diverse plant group and are functionally different from vascular plants. Yet, their peculiarities are rarely considered in the theoretical frameworks for plants. Currently, we lack information about the magnitude and the importance of intraspecific variability to the ecophysiology of bryophytes and how these might translate to local adaptation—a prerequisite for adaptive evolution. Capitalizing on two ecologically distinct (hummock and hollow) phenotypes of *Sphagnum magellanicum*, we explored the magnitude and pattern intraspecific variability in this species and asked whether the environmental-mediated changes in shoot and physiological traits are due to phenotypic plasticity or local adaptation. Size, pigmentation, and habitat type that distinguished the species in the field did not influence the trait responses under a transplant and factorial experiment. Also, the magnitude and pattern of trait variability changed with the treatments, which suggests that trait responses were due largely to phenotypic plasticity. The trait responses also suggest that the ecophysiological needs for mosses to grow in clumps where they maintain a uniform growth may have an overriding effect over the potential for a fixed adaptive response to environmental heterogeneity, which would constrain local adaptation. We conclude that extending the trait-based framework to mosses or making comparisons between mosses and vascular plants under any theoretical framework would only be meaningful to the extent that growth form and dispersal strategies are considered.

Keywords: bryophytes, plant growth form, local adaptation, phenotypic plasticity, intraspecific trait, morphological integration, environmental heterogeneity

Introduction

Bryophytes are a diverse plant group—comprising of 15000–20000 species (Shaw et al. 2011) and are functionally different from vascular plants. Yet, plant ecological theories and hypotheses are often presented as universally applicable frameworks. The trait-based ecology is no exception; emphasizing the importance of intraspecific trait variability to plant performance and fitness (Bolnick *et al.* 2011; Violle *et al.* 2012; Siefert *et al.* 2015; Wright *et al.* 2016) but primarily from vascular plants perspective. Bryophytes lack complex morphological and physiological structures (e.g. roots and stomata) through which vascular plants actively interact with their environments for resource acquisition, conservation, and response to environmental heterogeneity (e.g. Hepworth *et al.* 2016). Even traits that seem comparable between bryophytes and vascular plants (e.g., leaf mass per area) are often difficult to quantify. However, bryophytes are capable of using facilitative interactions such as lateral movement (externally) of water across space (Rice 2012) or vertical movement of water through their litter matrices, as a means of responding to change in their moisture environment. This cooperation for resource acquisition and retention means that individuals are buffered from the direct effect of the environment (e.g. Elumeeva *et al.*, 2011). This highly integrated ecophysiological mechanism presents a rather unique inter- and intraspecific interactions where competition for resources such as moisture is weak (e.g., Hayward and Clymo, 1983; Rydin, 1993). Thus, intraspecific variability in this plant group may not necessarily have the same ecological meaning as it is understood for vascular plants.

Although there has been considerable research evaluating *Sphagnum* traits in the context of ecosystem function—particularly in linking *Sphagnum* species traits to aspects of water (Titus *et al.*, 1983; Schipperges and Rydin, 1998; Hájek and Beckett, 2008) and carbon cycling (Turetsky et al. 2008; Laing *et al.* 2014; Bengtsson *et al.* 2016), only a few studies (Sastad and

Flatberg 1993; Sastad *et al.* 1999) have quantified intraspecific variability in traits. This is due to the difficulty in quantifying traits and perhaps also due to determining what constitutes an individual in clonal bryophytes like *Sphagnum,* because functional traits may only be measured at the level of an individual (Violle et al. 2007). However, viewing an individual as a structurally unattached, morphologically complete tissue—comprising of the capitulum, branch, and stem—the notion of individual is not complicated. That is, unattached individuals are physiologically independent and therefore, interact independently with their environments.

Variability often exists within a population because of sexual reproduction without apparent or immediate ecological benefits or consequences. Thus, intraspecific trait variability at the population level may reflect both the intrinsic genetic variability and phenotypic plasticity. One approach to evaluating the mechanistic importance of intraspecific variability is to explore trait variability in the context of local adaptation (Kawecki and Ebert 2004). Plastic (non-genetic) responses to environmental heterogeneity could cause phenotypic differentiation within and among populations and this phenotypic differentiation may become genetically fixed by mutation and natural selection. Such differentiation on phenotypic responses to environmental heterogeneity is often the basis for local adaptation (Kawecki and Ebert 2004). Additionally, locally adapted individuals would continue to exhibit adaptive responses that make them successful in their home environment even when they are subjected to a new environment where such response is no longer advantageous (Price *et al.* 2003; Kawecki and Ebert 2004). Investigating moss traits within the general framework of local adaptation can be informative in estimating the pattern, magnitude, and importance of intraspecific trait variability in this plant group.

Here, we explore the source, magnitude, and importance of intraspecific trait variability in *Sphagnum* moss. We ask whether there are differences in intraspecific trait variability and trait values between conspecifics from contrasting environments and whether the differences are due to adaptation to the conditions in their respective origin (hummock or hollow). That is, whether these differences are due to adaptive differentiation (local adaptation) or phenotypic plasticity. We focus on *S. magellanicum*, which is an ecologically dominant and widely distributed *Sphagnum* species. *S. magellanicum* is typically found in hollows and on low hummocks where moisture availability is high. However, it is also found within the carpets of *S. fuscum* on high hummocks—away from the water table, where a combination of high irradiation and moisture deficit often impacts photosynthesis and growth (Harley *et al.* 1989; Murray *et al.* 1993; McNeil and Waddington 2003). The individuals of *S. magellanicum* found on hummocks often exhibit a reddish-brown pigmentation (as opposed to green), are less physically robust (e.g., slender stem and smaller capitulum) and relatively lower tissue water content compared with individuals found in hollows. This variation in phenotype is good for exploring intraspecific variability in the context of phenotypic plasticity versus local adaptation. Here, we capitalize on the pattern observed in the field to ask how intraspecific trait variability influences the breadth of environments where *S. magellanicum* is found. We test the following hypotheses.

- Since strong morphological integration (clump growth) of individuals is necessary for survival on hummocks, which also promotes fast height growth, the hummock-originated individuals would consistently invest in height growth at the expense of biomass when grown in a common garden.
- Because green leaves tend to be more efficient for light capturing than red (anthocyanin-rich) leaves under low light (Burger and Edwards 1996), we predict that hummock-

originated plants would have lower F_v/F_m under the shade treatment than hollow-originated plants. However, we expect the opposite when the plants are grown under full light on hummock because of the lack of protective pigmentation in the hollow-originated individuals.

- We hypothesize that hummock-originated individuals are locally adapted to low moisture availability and high irradiance that are prevalent in hummocks. Therefore we predict that morphological and physiological responses of hummock-originated plants would be less sensitive to light and drought treatments compared with hollow-originated plants.

Materials and Methods

In June 2016, we visited Wylde Lake bog in southern Ontario (43.91775, -80.40489) and collected individuals of *Sphagnum magellanicum* Brid. found on high hummocks, which are typically dominated by *S. fuscum* and thus represent an atypical environment for *S. magellanicum*. The sampling included plants from several hummocks with more than 100m between some of the hummocks because *S. magellanicum* is not typically found on hummocks and to collect enough samples for the experiments. Similarly, we collected individuals from hollow environments in which *S. magellanicum* was dominant. There was at least a 10m distance between sample collection points. Also, the bog sites within the conservation area have contrasting hydrology, with some closer to the marsh and thus have a high water table. We collected samples across the wet and dry sites, which are about 500m apart. The average vertical distance between the hummocks and the hollows was 40.4cm. The *S. magellanicum* from hummocks were smaller and reddish-brown in colour whereas those from hollows were more physically robust and completely green. Hollow samples were kept separately from those collected from hummocks. All samples were immediately transferred to the University of Guelph phytotron where *S. magellanicum* samples from each environment were cut by knife into top 5 cm segments to exclude deeper, non-living component of the tissues and to create a standard length for all the plants.

We employed two experimental approaches. In the context of local adaptation, each experiment contains aspects of a “home” versus an “away” treatment (Kawecki and Ebert 2004; Blanquart et al. 2013). In the first transplant experiment, hummock individuals transplanted onto the hummock mesocosms represent a “home” treatment while hollow individuals represent an “away” treatment. However, this transplant experiment is an incomplete design but it was not possible for us to maintain hollow mesocosms due to the extremely unconsolidated (low bulk density) nature of hollow surface soils and species homogeneity. The combination of the experiments nonetheless represents a range of environments that the species is typically exposed to and allows us to at least reduce the potential for superficial conclusions (Kawecki and Ebert 2004).

Hummock transplant experiment

We extracted four hummock monoliths, which comprised a continuous carpet of *S. fuscum* into surface peat to a depth of about 20 cm. The monoliths allowed us to incorporate the ecophysiological peculiarities (e.g. neighbourhood effect and vertical movement of moisture through litter matrices) of our study system into the experiment. Each monolith was gently placed in an 8.83-litre cylindrical pot. Each monolith was partitioned into equal halves with a stick, which was inserted horizontally into the surface of the moss carpet in each pot. Individuals of *S. magellanicum* from the two home environments (hummock versus hollow) were randomly assigned to a monolith and were inserted into the carpet of *S. fuscum*. Specifically, we inserted fifteen *S. magellanicum* hummock-originated individuals into one half of each monolith and fifteen hollow-originated individuals into the other half. Thus, across the four replicate monoliths, we transplanted 60 plants from each plant origin. The hummock transplant experiment represents the breadth of "home" environment for individuals that were collected on hummocks in terms of substrate conditions, while hollow-originated plants in this case, were transplanted onto an "away" substrate. Two monoliths were assigned to a shade treatment and two were assigned to full light treatment. The shade treatment involved two shade boxes of 3.25 m × 1.47 m × 0.63 m in dimension, built from PVC pipes. The shade boxes were covered with breathable 50% neutral density shade cloth. We used breathable shade cloth to avoid heat build-up under the shade boxes and in the pots, which would have required frequent watering, which would compromise our drought treatment. The 50% shade approximates the proportion of the photosynthetic photon flux density (PPFD) admitted into the *Sphagnum* carpet by the dominant vascular plant species (*Myrica gale*) at our site. This was obtained by measuring PPFD below and above the canopy using the point sensor of a LI-250 light meter (LI-COR, Lincoln, Nebraska). These measurements were used to compute percentage of light admitted into the moss surface. The above canopy PPFD ranged from 1206 – 2035 µmol m^{-2} s^{-1} whereas below canopy values ranged from 224 -1714 µmol m^{-2} s^{-1}. We quantified water content in the hummocks at 1, 6, and 12 cm depths from the top and also at the foot of the hummocks using a Hydrosense soil moisture meter (Campbell Scientific, Inc., USA). However, we did not find a difference in the moisture profiles of hummocks sampled along moisture gradient in our site, therefore, we did not vary moisture for this experiment.

Factorial light × moisture experiment

Our second experiment involved a 3 × 2 factorial pot experiment with two plant origins (hummock versus hollow), two light treatments (full light; 50% light) and two water treatments (saturated; low water). This experiment represents the breadth of "home" environment for hollow-originated individuals in terms of substrate conditions, while hummock-originated plants in this case were transplanted onto "away" substrates. The shade treatment was imposed as described above. The drought treatment was created by maintaining treatment pots at an average volumetric water content of about 12%, which is the mean summer volumetric water content at the top 1 cm of moss in the field site. This is close to the condition under which drying of *Sphagnum* tissues can be observed in the field. The saturated water treatment was maintained by monitoring and topping up the experimental pots with water, and volumetric water content consistently exceeded 21%. The water contents across all experimental pots were monitored with a portable Hydrosense soil moisture meter (Campbell Scientific, Inc., USA).

The experimental pots were filled with 3 cm of deep peat moss underneath a 1 cm layer of surface peat. The deep peat was from a commercial source while the surface peat was extracted from the field in an area near the *Sphagnum* collections in hollow. The pots were 227.4 cm^3 in size, with holes at the base through which water was fed into the pots. The pots were placed in trays, which were covered with transparent lids. There were 9 plants (one plant per pot) for each of the four treatment combinations (9 plants × 2 origin × 2 light × 2 moisture treatments), which we replicated twice. Thus, a total of 144 plants were used in the experiment. Because bogs are nutrient-poor and typically fed by rainwater, the plants were not fertilized and were watered exclusively with rainwater that was harvested in Guelph. The experiments were conducted at the University of Guelph greenhouse, which was kept at average 20°C night/26°C day temperatures and 16-h photoperiod. Daylight was supplemented with artificial light on cloudy days and in the evenings.

Quantification of traits

The two experiments ran fully from July 2016 to January 2017. At the end of the experiments, we measured a suite of traits on individuals from each treatment. We focused on morphological traits and shoot traits in particular because *Sphagnum* is poikilohydric and holds a large proportion of its moisture externally. We quantified two traits related to growth, including height and biomass. We also measured allocation of biomass into capitulum, branch, and stem. The capitulum is taken as the top 1 cm of the plant (Clymo 1970). Branch mass was determined by removing the tissues that line the stem (fascicles). Branch mass comprised of tissues (fascicles) that line the stem. The exposed stem was taken as the stem mass.

We also quantified the dark respiration as a measure of metabolic activity. Respiration rates were measured on six individuals per treatment, which were selected at the end of the experiment. For these individuals, we placed the entire plant in a dark glass jar. The jars were sealed with stopcocks and placed under their respective treatment environment. The CO_2 in the jar headspace was drawn three times at 3 hr intervals with gas-tight syringes. The CO_2 concentration was analyzed with an EGM-4 infrared gas analyzer (PP Systems, Hitchin, Hertfordshire, UK). We performed linear regressions of CO_2 concentration against time, using the slopes of these relationships as our measurement of respiration rate. We then used the dry mass of the samples to convert the slopes into μmol of CO_2 $g^{-1}minute^{-1}$.

Finally, we measured the dark-adapted fluorescence (F_v/F_m) as a measure of maximal photosynthetic efficiency. The dark-adapted F_v/F_m measurements were taken at the end of the experiment. Individuals from each treatment were placed in the dark for at least 6 hours to ensure that Q_A electron acceptors are fully oxidized and that reaction centers are in the 'open' state. We then quantified dark-adapted F_v/F_m on each plant using a pulse-modulated fluorometer (OS1p, Opti-Sciences, Hudson, NH).

Statistical analyses

Because the plants in the hummock transplant experiment were grown in only four pots, we tested for differences in trait values using mixed effect models, where we analyzed pot ID as a random effect to account for lack of independence. Multiple mean comparisons were obtained for models with interaction effects using "lsmeans" package in R. We tested for mean trait values in the factorial experiment using 3-way ANOVA and obtained multiple mean comparisons for interaction effects using Tukey HSD. In both analyses, we explored the models' residuals for normality and where there was a departure from normality (e.g., height and branch mass), the data were transformed using a logarithm transformation. We explored patterns of trait variability across experimental treatments by partitioning the variance in the data using the varpart function in R package "Vegan". We used this approach combined with redundancy analysis to examine how the experimental treatments influenced within-trait variability and total trait variability. All analyses were performed in R 3.2 (R core Development Team 2015) and all statistical tests were conducted at $\alpha = 0.05$.

Results

Hummock-transplant experiment

Hummock-originated plants had lower F_v/F_m than hollow plants (Fig. 1a) with no other significant main effects or interactions (Table 1). Height, capitulum mass, and respiration were consistently higher under the shade than the high light treatment (Fig. 1b & c). Total biomass and stem biomass was influenced by a plant origin × light interaction (Fig 1d). Hummock plants tended to have lower total and stem biomass than hollow plants but only in the shade treatment.

We found strong positive correlations between some of the traits. There were correlations for example between height and respiration rate and between respiration rate and biomass for both hummock and hollow plants ($r^2 = 0.24$, $p < 0.05$ and $r^2 = 0.56$, $p < 0.001$) and hollow plants ($r^2 = 0.30$, $p < 0.05$ and $r^2 = 0.73$, $p < 0.001$) (Fig. 2a & b).

For most traits, plant origin did not explain a significant amount of variation in individual traits (0–10%), while light explained between 0 and 46% (Table 2). Origin (hummock vs. hollow) explained significant variation for F_v/F_m and stem mass, while light explained significant variation in height, capitulum mass, and total biomass (Table 2). When analyzed for total variability across all traits (respiration, F_v/F_m, capitulum, branch, stem, and total biomass), plant origin only accounted for 2% of the variability ($p > 0.05$) whereas light accounted for 16% ($p < 0.001$).

Light × moisture factorial experiment

In the factorial experiment, traits were more generally influenced by the main effects of origin and moisture than their interaction effects or the main effect of light (Table 3). The post-hoc tests showed that capitulum mass was greater in hummock plants than in hollow plants under the high moisture treatment ($p < 0.05$) but did not significantly differ between the plant origins under the low moisture treatment. The opposite trend was true for branch mass as hollow plants had a greater branch mass than hummock plants under the high moisture treatment ($p < 0.05$) but there was no difference in branch mass between the origins under the low moisture treatment. The stem mass of hollow plants subjected to low moisture was greater than stem mass of hummock plants subjected to high moisture ($p < 0.001$). Height was fastest under the high moisture treatments regardless of light (Fig. 3a) compared with the low moisture treatment but lower in the light x low moisture treatment Biomass was greatest at the high light and high moisture treatment and tended to be lowest under the low moisture treatments across both light treatments (Fig. 3b). F_v/F_m was higher in hollow individuals than in hummock individuals.(Fig. 3c). Respiration was higher under high moisture than the low moisture treatment and did not vary with light (Fig. 3d).

Consistent with the hummock transplant experiment, we found strong positive correlations between respiration and biomass and between respiration and height for both hummock ($r^2 = 0.25$, $p < 0.001$ and $r^2 = 0.57$, $p < 0.001$) and hollow ($r^2= 0.53$, $p < 0.001$ and $r^2 = 0.65$, $p < 0.001$) plants (Fig. 4a & b).

Plant origin explained the most variation in stem mass (44%) relative to moisture and light. Except for height, the influence of the light treatments explained little or no variation among traits in this experiment. Moisture explained a significant amount of variation in all traits except for capitulum mass and was particularly important for respiration and branch mass variation. Plant origin and moisture explained similar levels of total variation across traits (Table 4). The data were also split into two independent datasets based on plant origin and were accordingly explored for variability due to light and moisture effects. Light explained 1% of total variability in hollow plant traits and 4% in hummock plant traits whereas moisture explained 22% of variability in hollow plant traits and 13% in hummock plant traits. However, the effect of light on variability of hollow plant traits was not statistically significant.

Discussion

We capitalized on two ecologically distinct (hummock and hollow) phenotypes of *S. magellanicum* to evaluate the magnitude and the importance of intraspecific variability in this species and asked whether the environmental-mediated changes in shoot and physiological traits are due to phenotypic plasticity or local adaptation. This is not an attempt to characterize *Sphagnum* physiology but rather to explore the importance of trait variability in controlling responses to environmental heterogeneity.

Effect of plant origin (hummock versus hollow) on Sphagnum traits

Although clonality is ubiquitous in bryophytes, their populations can be spatially and genetically diverse as the population of any non-clonal plants (e.g., Stenoien and Sastad 1999; Gunnarsson *et al.* 2007). Our hummock–hollow sampling design assumed that the two phenotypes of *S. magellanicum* used in our experiments are genetically disparate groups that have been shaped by adaptive differentiation (local adaptation). At the same time, a unique characteristic of *Sphagnum* is that it acquires and conserves moisture through stem and canopy integration (clump growth form), especially on hummocks. That is, *S. magellanicum* growing on hummocks may not grow considerably faster or taller than the typical height of *S. fuscum*-derived carpet (Hayward and Clymo 1983). Pure stands of *S. magellanicum* typically grow faster than those of *S. fuscum* (Breeuwer et al. 2008), which implies that *S. magellanicum* plants (regardless of their origin) growing on *S. fuscum*-dominated hummocks may not express their maximum growth rates. Thus, the generally weak effect of plant origin on the traits in the hummock transplant experiment relative to that in the factorial experiment suggests that the trait responses were due largely to phenotypic plasticity as opposed to local adaptation.

Indeed, environmental heterogeneity may cause phenotypic changes that are not genetically determined adaptive responses (Kawecki and Ebert 2004). That is, while the appearance of our hummock-dwelling phenotype is undoubtedly shaped by the hummock conditions, its distinguishing characters from the hollow-dwelling phenotype may not have genetic bases. Further, if the ecophysiological needs for morphological integration have an overriding effect over the potential for a fixed adaptive response to environmental heterogeneity, then the clump growth form of *Sphagnum* would likely constrain local adaptation.

Light controls on Sphagnum trait variation

High irradiation is a common source of stress influencing bryophytes performances (Post *et al.* 1990; Marschall and Proctor, 2004). Sphagnum species especially the hummock dwelling species are susceptible to photoinhibition due to the prevalence of low moisture and high irradiation (Murray et al. 1993; Hájek 2014; Bragazza 2008). Thus, because hummock species are rarely completely green except under shade, we considered the pigmentation in the hummock plants a photoprotection feature (Bonnett *et al.* 2010). Contrary to our predictions, the hummock plants had a relatively lower F_v/F_m across all experimental treatments compared with hollow the plants. Also, under the shade treatments, some of the hummock plants changed from reddish to light pink colour and some with a tint of green, which is consistent with the findings that pigmentation of *S. magellanicum* is plastic (Yousefi et al. 2017). However, Fv/Fm gives an insight into stress (e.g., moisture stress) tolerance of species and an indication of stress-induced damage to the photosynthetic apparatus (Maxwell and Johnson 2000; Manninen et al. 2011). Since *S. magellanicum* is known to occupy a wide range of environments (Breeuwer et al. 2008; Kyrkjeeide et al. 2016; also see Oke and Turetsky 2020) and was able to survive on high hummocks, we cannot attribute the generally low F_v/F_m to low-stress tolerance, which thus implies that damage to its photosynthetic apparatus is likely a cost to occupying the high hummocks; hence the rarity of the species in that habitat. However, we did not find any relationship between F_v/F_m and total biomass, which is often used as a proxy for fitness in plants (Younginger et al. 2017).

Shade tends to reduce transpiration (Muthuchelian *et al.*1989; Pons *et al.* 2001; Gent, 2007), which would diminish the need for morphological integration. Under the shade treatment of the hummock transplant experiment, the plants were more robust (e.g., bigger capitulum) and the moss canopy was generally rough and loose compared with light treatment, which was relatively smooth and compacted. This disparity in growth response due to the difference in light level likely contributed to the strong effect of light on trait variability in the hummock transplant experiment. Surprisingly, light was less important to trait variation in the factorial experiment. This could be because we only manipulated moisture in the factorial experiment, which is well established as having an important role in *Sphagnum* growth and distribution (McNeil and Waddington 2003; Oke and Hager 2017) and also was the dominant source of trait variation in the factorial experiment.

Implications of trait variability and local adaptation in Sphagnum

Trait variability is considered one of the mechanisms by which plant populations cope with environmental heterogeneity (Jung et al. 2014) and it is deemed the raw material for natural selection (Bolnick et al. 2011). For instance, high trait variability could aid the persistence of a population by allowing optimal response to selective pressures (e.g., Bürger 1999). In this study, most of the variability remained unexplained by our treatments. However, it is important to note that most traits measured in this study exhibited low levels of variation. It is also important to note that clonality is common in *Sphagnum,* especially at fine scales, which may lead to low phenotypic variation. Low phenotypic variation may be advantageous for morphological integration. Although our sampling design was intended to avoid repeatedly sampling clones, it is not uncommon for a *Sphagnum* population to be dominated by a single clone (Cronberg et al. 1997; Gunnarsson et al. 2007), which would then likely be overrepresented in our experiments.

Due to the generally low nutrient condition that limits spore germination in peatlands (Sundberg and Rydin 2002), *Sphagnum* populations are maintained largely by clonal growth (Cronberg *et al.* 1997; Gunnarsson *et al.* 2007). That is, dispersal by spore in *Sphagnum* is long-distant and random (Whitaker and Edwards 2010). This is true for many moss species (Miles and Longton 1992), which means that there is a low accruable benefit in passing down the local selective advantage through spores. While the short-distance dispersal through clonal growth is less random, it likely results in low phenotypic variability. The low phenotypic variability may have an ecophysiological value in stem and canopy integration for moisture retention and survival. However, as observed in the field and as demonstrated in the current study, morphological integration is quite common in *Sphagnum* even among species with different growth rates (Clymo and Hayward 1982; Hayward and Clymo 1983; Ingerpuu and Vellak 2013). This means that stem and canopy integration is more likely a function of plasticity rather than low phenotypic variability per se. Thus, given their mode of dispersal and the clump growth form, locally adapted growth responses may not be beneficial to mosses. In any case, extending the trait-based framework to mosses or making comparisons between mosses and vascular plants under any theoretical framework would only be meaningful to the extent that growth form (including lack of roots) and dispersal strategies are considered.

Our findings that trait responses and variability depend on the prevailing environment highlights the limitation of investigating or drawing conclusions about local adaptation from responses to a single environment. Additionally, because phenotypic changes may not necessarily have a genetic basis, it is possible in a common garden experiment to confuse or conflate adaptive changes arising from phenotypic plasticity with that arising from local adaptation (Gienapp et al. 2008).

Finally, there is an on-going taxonomic revision to *S. magellanicum*. The species is considered a complex, comprising at least three species—*S. divinum* and *S. medium* in eastern North America, and *S. magellanicum* sensu stricto in South America (Hassel et al. 2018). These species have distinct morphological, molecular, and distributional characters. The preliminary study suggests that *S. medium* has an amphi-Atlantic distribution while *S. divinum* is circumpolar in its distribution. Since the pigmentation of "*S. magellanicum"* (as we currently know it) lacks genetic basis (Yousefi et al. 2017) and considering the pattern of distribution of these species relative to our field site in Southern Ontario, it is unlikely that we sampled across a mix of *S. medium* and *S. divinum* in a way that would bias our findings. Also, considering that origin had little effect on trait variability, a more likely scenario is that we sampled one species or the other. However, because further study is required on the distribution and identification of these subspecies (Hassel et al. 2018), we are unable to accordingly characterize our species and therefore maintain the name *S. magellanicum* for the purpose of this study.

Conclusion

In summary, we explored the magnitude and pattern of trait variability in *S. magellanicum* from contrasting habitats in the context of phenotypic plasticity and local adaptation. We found that the trait responses were due largely to phenotypical plasticity with little influence on whether plants originated from hummocks or hollows. We also found that trait variability depends on the prevailing light or moisture environment. However, most trait variation remained unexplained by our experimental treatments. Collectively, our results suggest that using traits to draw inferences about the ecology of *Sphagnum* would require an understanding of the mechanisms driving traits and the pattern of trait variability. Lastly, because morphological integration may have an overriding influence on growth traits, it is not clear under what conditions might local adaptation occur or benefit this plant group. We hope that future studies will further explore this area of inquiry in mosses, with consideration for their growth form and recruitment strategies.

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Table 1 Results of a mixed effect model for the hummock transplant experiment showing F and p-values specific to each trait. Bold texts are significant values ($p < 0.05$). O = Origin, L = Light and DF = treatment and sample degrees of freedom

Treatments	**DF**	**Respiration** (umol^{-1}g^{-1}min)	**F_v/F_m**	**Height (cm)**	**Total biomass (g)**	**Capitulum mass (g)**	**Branch mass (g)**	**Stem mass (g)**
O	1, 59	0.21, 0.651	**4.4, 0.040**	2.4, 0.128	2.6, 0.115	1.3, 0.267	0, 0.915	**6.8, 0.012**
L	1, 59	**9.2, 0.038**	1.3, 0.264	**50.2, <0.0001**	**12.4, 0.026**	**21.1, <0.0001**	0.77, 0.382	3.1, 0.146
O*L	1, 59	1.5, 0.222	0.1, 0.764	2.5, 0.122	**7.1, 0.010**	3.9, 0.053	1.9, 0.172	**4.1, 0.048**

Table 2 The effects of plant origin and light treatment on individual trait variability as well as total trait variability for hummock transplant experiment. Bold figures are statistically significant trait variability values ($p < 0.05$) under each parameter while the p-values are the overall p-values of the model.

Traits	Origin (%)	Light (%)	F-values	P-values
F_v/F_m	**6**	0	2.9	0.066
Respiration	0	**17**	2.9	0.067
Height	1	**46**	24.0	<0.001
Capitulum mass	0	**29**	10.3	0.002
Branch mass	0	0	0.5	0.651
Stem mass	**10**	0	4.7	<0.001
Total biomass	3	**19**	6.9	0.004
Total traits	2	**16**	6.5	<0.001

Table 3 Results of a 3-way ANOVA for the factorial experiment showing F and p-values for the traits. Bold texts are significant values ($p < 0.05$). O = Origin, L = Light, M = Moisture and DF = treatment and sample degrees of freedom

Treatments	**DF**	**Respiration (umol^{-1}g^{-1}min)**	**F_v/F_m**	**Height (cm)**	**Total biomass (g)**	**Capitulum mass (g)**	**Branch mass (g)**	**Stem mass (g)**
O	1, 143	0.0, 0.831	**14.5, 0.002**	**4.6, 0.032**	**8.1, 0.0051**	3.3, 0.070	**14.8, 0.0001**	**144.4, <0.0001**
L	1, 143	1.1, 0.31	3.5, 0.061	**20.5, <0.0001**	0.55, 0.459	2.1, 0.145	0.0, 0.930	1.3, 0.251
M	1, 143	**16.4, 0.0002**	**22.8, < 0.0001**	**48.6, <0.0001**	**32.1, <0.0001**	0.1, 0.791	**62.8, <0.0001**	**26.25, <0.0001**
O*L	1, 143	3.4, 0.071	2.3, 0.133	1.3, 0.218	0.3, 0.617	0.35, 0.553	0.3, 0.582	2.5, 0.113
O*M	1, 143	0.9, 0.336	2.2, 0.137	1.2, 0.267	0.0, 0.886	**15.6, 0.0001**	**6.7, 0.010**	**8.4, 0.0041**
L*M	1, 143	0.2, 0.625	2.7, 0.098	**7.9, 0.0056**	**4.4, 0.037**	2.9, 0.088	2.3, 0.128	**7.7, 0.006**
O*L*M	1, 143	0.13, 0.721	0.39, 0.529	0.75, 0.385	0.0, 0.869	3.0, 0.082	1.4, 0.241	1.9, 0.172

Table 4 Percentage trait variability due to plant origin as well as experimental light and moisture treatments in the factorial experiment. Bold figures are statistically significant trait variability values ($p < 0.05$) under each parameter while the p-values are the overall p-values of the model.

Traits	Origin (%)	Light (%)	Moisture (%)	F-values	P-values
F_v/F_m	**7**	1	**12**	12.1	<0.001
Respiration	0	0	**26**	5.7	0.005
Height	2	**8**	**21**	22.0	<0.0001
Capitulum mass	2	0	0	2.0	0.112
Branch mass	**6**	0	**26**	21.3	<0.001
Stem mass	**44**	0	**7**	46.6	<0.001
Total biomass	**3**	0	**15**	10.8	<0.001
Total traits	**11**	1	**14**	16.5	<0.001

Figure legends

Fig.1 Results of mixed effects models examining trait variation in *Sphagnum magellanicum* to treatments in hummock transplant experiments (a) F_v/F_m averaged by plant origin (b) respiration averaged by light treatment (c) height averaged by light treatment (d) biomass averaged by a light × plant origin treatment interaction. Same letter notation depicts no differences between means based on Tukey HSD post-hoc tests.

Fig. 2 Correlational relationships between respiration and total biomass (a) and the relationship between respiration and height (b) for *Sphagnum magellanicum* in the hummock transplant experiment.

Fig. 3 Effects of treatments in the factorial experiment on *Sphagnum magellanicum* traits (a) height averaged by a light × moisture treatment interaction, (b) biomass averaged by a light × moisture treatment interaction, (c) canopy F_v/F_m averaged by plant origin and moisture treatments, and (d) respiration averaged between the moisture treatments. Same letter notation depicts no differences between means based on post hoc tests. There was no origin × light interaction on F_v/F_m.

Fig. 4 Correlational relationships between respiration, biomass, and height for *Sphagnum magellanicum* in the factorial experiment for hummock and hollow originated plants.

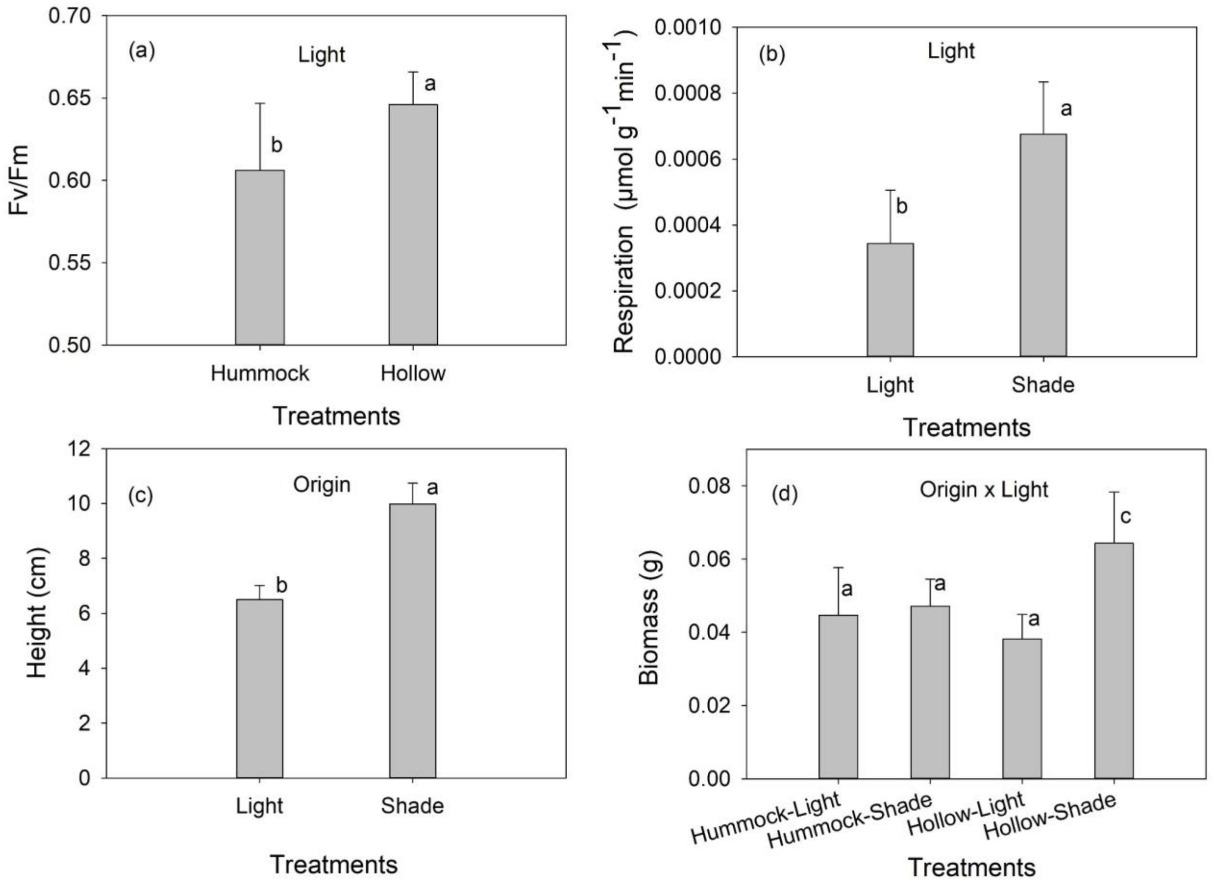

(a)
Light
Fv/Fm
0.70
0.65
0.60
0.55
0.50
a
b
Hummock
Hollow
Treatments
(b)
Light
Respiration (µmol g^{-1}min^{-1})
0.0010
0.0008
0.0006
0.0004
0.0002
0.0000
a
b
Light
Shade
Treatments
(c)
Origin
Height (cm)
12
10
8
6
4
2
0
a
b
Light
Shade
Treatments
(d)
Origin x Light
Biomass (g)
0.08
0.06
0.04
0.02
0.00
a
a
a
c
Hummock-Light
Hummock-Shade
Hollow-Light
Hollow-Shade
Treatments

Fig. 1

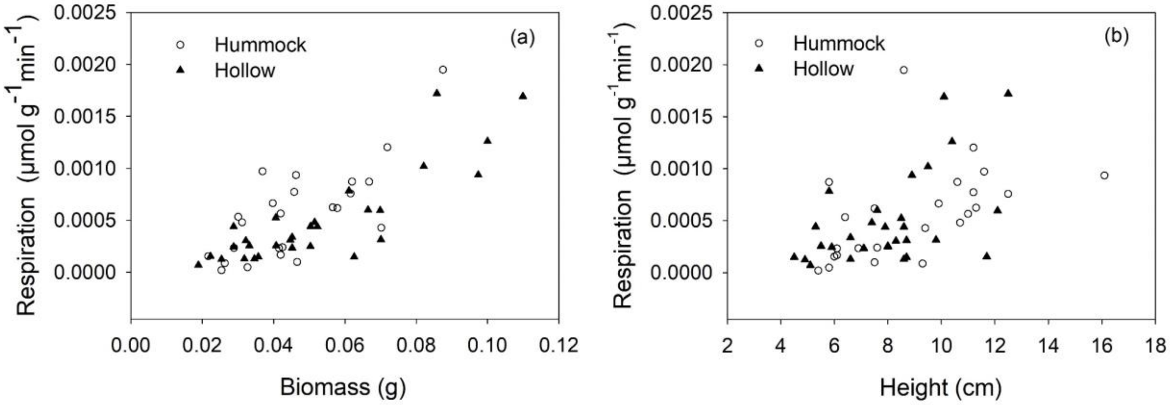

Respiration (µmol g-1 min-1)
0.0025
0.0020
0.0015
0.0010
0.0005
0.0000
Hummock
Hollow
(a)
0.00
0.02
0.04
0.06
0.08
0.10
0.12
Biomass (g)
(b)
2
4
6
8
10
12
14
16
18
Height (cm)

Fig. 2

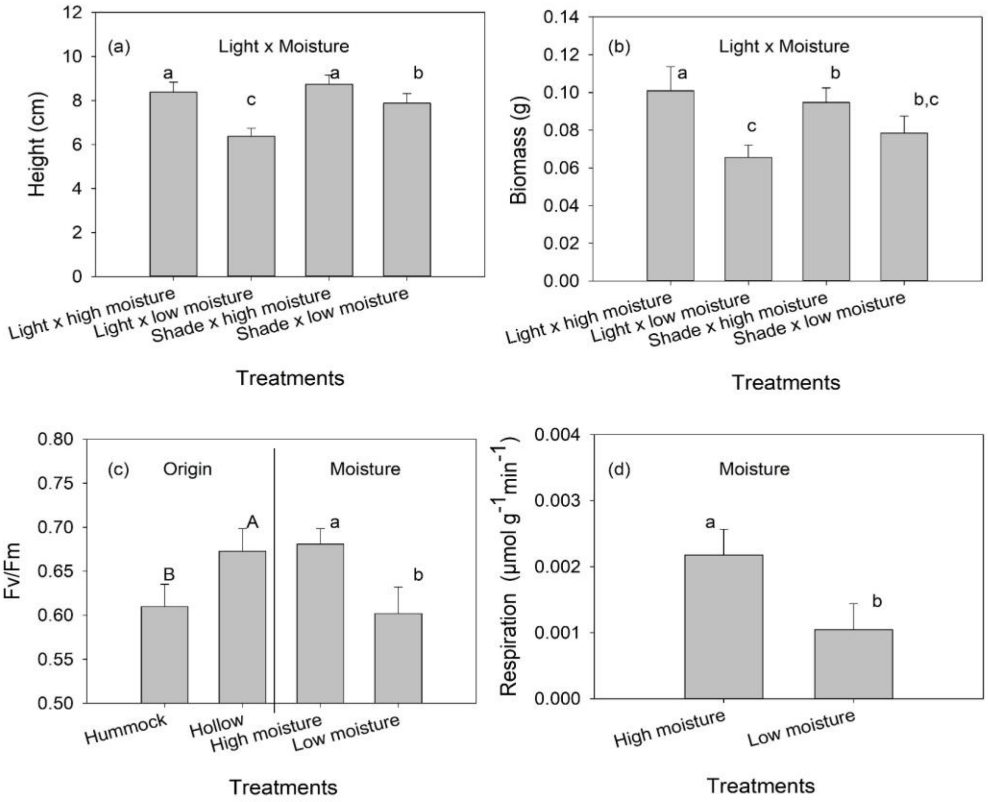
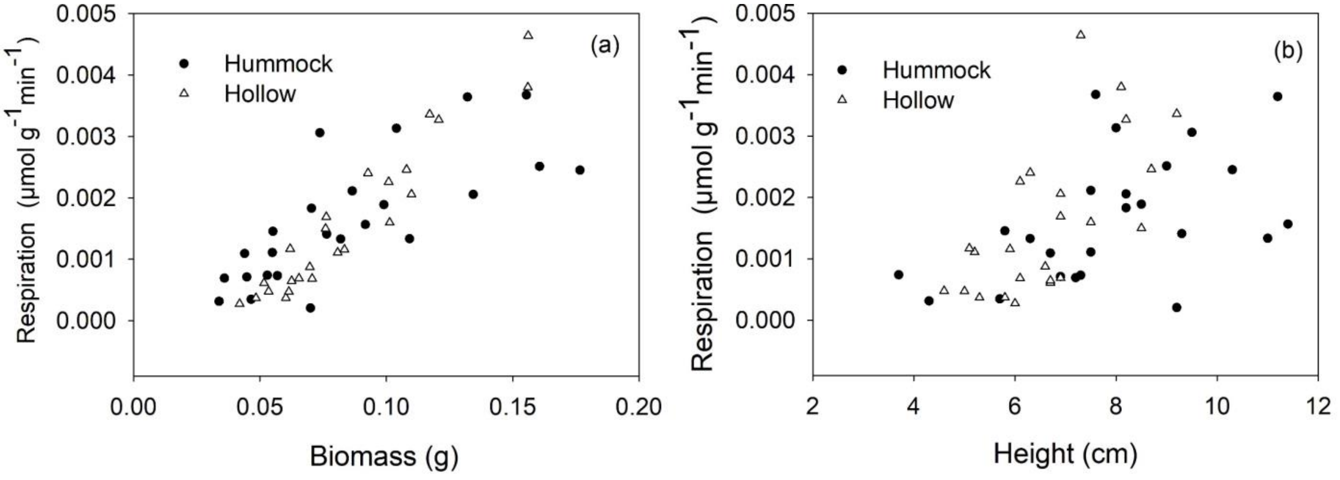

(a)
Light x Moisture
Height (cm)
0
2
4
6
8
10
12
a
c
a
b
Light x high moisture
Light x low moisture
Shade x high moisture
Shade x low moisture
Treatments
(b)
Light x Moisture
Biomass (g)
0.00
0.02
0.04
0.06
0.08
0.10
0.12
0.14
a
c
b
b,c
Light x high moisture
Light x low moisture
Shade x high moisture
Shade x low moisture
Treatments
(c)
Origin
Moisture
Fv/Fm
0.50
0.55
0.60
0.65
0.70
0.75
0.80
B
A
a
b
Hummock
Hollow
High moisture
Low moisture
Treatments
(d)
Moisture
Respiration (µmol g^{-1}min^{-1})
0.000
0.001
0.002
0.003
0.004
a
b
High moisture
Low moisture
Treatments

Fig. 3



Respiration (µmol g^{-1}min^{-1})
0.005
0.004
0.003
0.002
0.001
0.000
(a)
● Hummock
△ Hollow
0.00
0.05
0.10
0.15
0.20
Biomass (g)
(b)
2
4
6
8
10
12
Height (cm)

Fig. 4