Chapter 5

Best Practices for Measuring Photosynthesis at Multiple Scales

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Summary

Studies of bryophyte photosynthetic performance have generally adapted techniques developed for use in vascular plants and relied on underlying vascular plant functional models as guides. Within this context, bryophytes present intellectual and methodological challenges, but also opportunities relative to their vascular plant counterparts. For example, although the leaf is clearly a functional unit for vascular plants, the comparable bryophyte structure may or may not serve a similar purpose. Instead, shoot systems and their organization into canopies are often employed as the functional equivalent. Unfortunately, due to issues of scale and alternative functional demands on bryophyte shoots like external transport and nutrient

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uptake, neither the methodologies nor the underlying models that lead to an integrated understanding of photosynthesis in vascular plants apply well to bryophytes. This chapter will consider the appropriate functional units for studies of bryophyte photosynthesis and relate it to the growth form and life form literature. Methods to characterize photosynthetic "leaf" area, water content, and canopy structure will be evaluated relative to their use in characterizing rates of photosynthesis. In addition, various methods are used to study photosynthetic function and these will be considered in light of their appropriate spatial and temporal domains.

I. Introduction

Technological advances have allowed organismal plant physiologists to shift focus from leaves to canopies to ecosystems, from the lab to the field to remote sensing from space and from seconds to seasons or longer and into deep time using fossils and environmental reconstruction. Different methods to evaluate photosynthetic function apply across such broad spatial and temporal scales (Fig. 5.1) and many of these have been recently developed, improved, and/or made more widely available.

Investigators studying bryophyte function have often adapted techniques developed for use in vascular plants. Within this context, bryophytes present challenges caused by their size and slow rate of photosynthetic tissues (Martin and Adamson 2001), by dramatic dependence of photosynthesis on plant water status, and by lack of accepted standard practices. However, bryophytes also present opportunities not only as contributors to carbon dynamics of widespread ecosystems, but also as subjects to study the integration of leaf, shoot and canopy processes. This chapter will review the organization of bryophyte photosynthetic systems as it relates to photosynthetic function and propose standards that can guide measurement and reporting of photosynthetic rates.

Abbreviations: LAI – Leaf area index; P_{max} – Maximum rate of net photosynthesis; ϕ_{PSII} – Quantum yield of photosystem II; SAI – Shoot area index; STAR – Shoot silhouette to needle leaf area ratio

II. The Photosynthetic Organ in Bryophytes

A. Life Forms and Photosynthesis

Growth, development and organization of bryophyte shoot systems is modular and hierarchical (Fig. 5.2). In leafy forms, which comprise the vast majority of bryophyte species (100 % of >10,000 mosses, 85 % of 6,000–8,000 liverworts, although 0 % of 300 hornworts; Buck and Goffinet 2000; Crandall-Stotler and Stotler 2000; Vanderpoorten and Goffinet 2009), normally unistratose leaves (i.e., phyllids) are arranged on branches and stems, which in turn, organize into shoots and shoot systems by characteristic cell division at apices and/or by growth from subapical buds. Although developed from variants of a common plan, the morphological patterns that result differ considerably and have important functional consequences (Gimingham and Birse 1957; Scholfield 1981; Hedderson and Longton 1996; Kürschner et al. 1999; Cornelissen et al. 2007; Rice et al. 2008; Waite and Sack 2010; Elumeeva et al. 2011). The bryophyte canopy, affected by the size, density and arrangement of leaves, branches, shoots and shoot systems, is generally accepted as the primary functional unit of bryophytes as it relates to carbon and water dynamics (During 1992; Proctor 1990, 2000; Bates 1998; Cornelissen et al. 2007; Zotz and Kahler 2007; Waite and Sack 2010). Although the bryophyte canopy has served as the primary focus of functional studies, there lack standard methodologies that allow for easy comparison among studies.

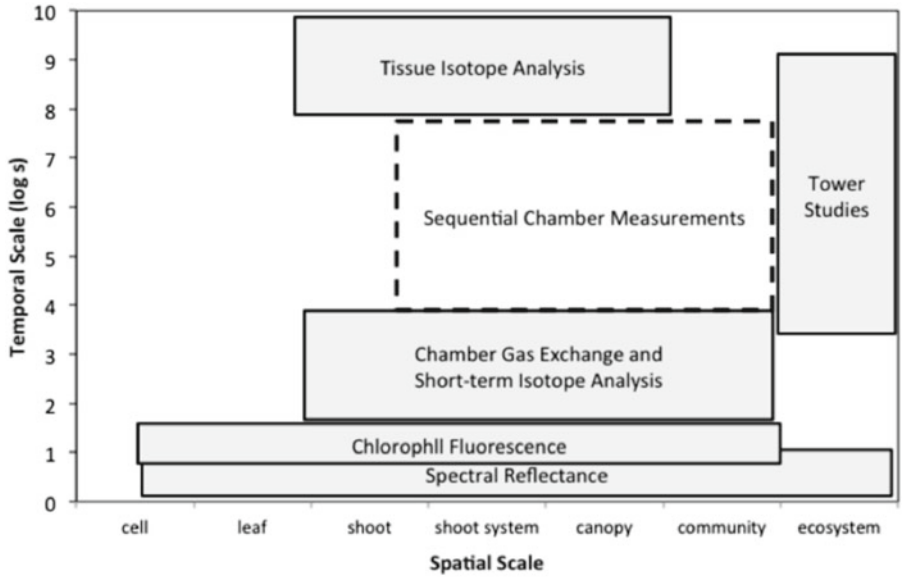

Fig. 5.1. Scale of photosynthesis measurements. Each set of techniques used to evaluate photosynthetic function occupies limited spatial and temporal domains. Most studies utilize chamber gas exchange in small diameter (<10 cm diameter) samples or chlorophyll fluorescence probes, which mainly evaluate <1 cm diameter regions. Used sequentially, or with larger chambers or imaging techniques, these may have extended application in space and time (dashed line), although see Bader et al. (2009) for limitations of temporal scaling in poikilohydric organisms. To evaluate photosynthesis at larger temporal and spatial scale, functional performance gets integrated over space and time, with a loss in resolution within those domains.

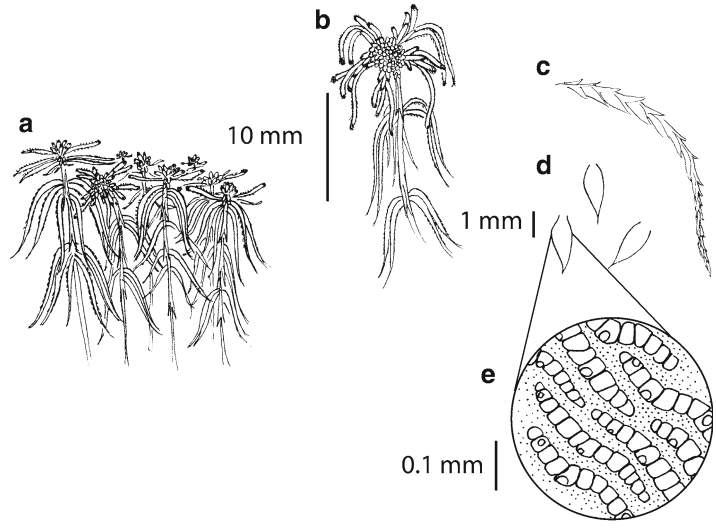

Fig. 5.2. Organization of Bryophyte photosynthetic systems. Variation in photosynthesis may be caused by differences in the structure and organization of units at many scales. The hierarchical arrangement of photosynthetic units shown for a *Sphagnum* species at decreasing scale from (**a**) canopy; (**b**) shoot; (**c**) branch; (**d**) leaf; and (**e**) cell (Drawing by S. Webb, adapted from Rice (2009)).

The desire to characterize shoot system organization has arisen from two arenas. One focus emerged from interest in identifying taxonomic characters and establishing homology for use in classification and systematics (Hedenäs 2002; Newton 2007). Although often considered in a functional context, it is clear that similar functional states can arise from different branching architectures and morphologies, although there is evident conservatism at the level of genera or family (Hedderson and Longton 1996; Hedenäs 2002). Alternatively, canopy structure has been considered in more functional terms in the discussion of life forms. This concept emerged from the notion of growth forms (Gimingham and Birse 1957; Gimingham and Smith 1971) that sought to characterize different canopy structures that related to their function, although with specific reference to the underlying patterns of growth and branching that create them. Life forms developed from this idea with a greater emphasis on function (Magdefrau 1982; During 1992; Bates 1998). In studies of polar (Gimingham and Smith 1971; Fowbert 1996), temperate (Gimingham and Birse 1957) and tropical (Kürschner et al. 1999) species, variation in growth or life form classifications associates significantly with environmental conditions, especially factors that affect water relations. Given the poikilohydric nature of bryophytes, plant water status, particularly the length of time plants remain hydrated, controls long-term carbon gain in many environments (Proctor 2000; Zotz et al. 2000; Rice and Schneider 2004; Mishler and Oliver 2009). Consequently, life forms may provide a suitable, general scheme for considering production. However, short-term dynamics affected by light interception and carbon exchange may not be adequately differentiated by the life form groupings, or if they are, there has been little research aimed at understanding these relationships (Bates 1998). If life forms are inadequate for quantifying canopy variation as it relates directly to photosynthetic processes, what alternatives are available? Recent research has explored the use of quantitative, continuous traits in the place of life form groupings to understand and predict canopy-level physiological function (Rice et al. 2008, 2011b; Cornelissen et al. 2007; Waite and Sack 2010; Elumeeva et al. 2011). In vascular plants, this approach has led to the development of broadly applicable models that link plant traits to photosynthetic function (Wright et al. 2004) and offers promise for the study of bryophytes.

Carbon and water dynamics of thalloid forms such as those in liverworts and hornworts have often been considered analogous to vascular plant leaf function. In complex, ventilated thalli like those in some genera in the Marchantiaceae, internal compartments increase the internal surface area relative to that of the leaf surface, thereby increasing maximal rates of photosynthesis (Proctor 1980; Green and Lange 1995; Meyer et al. 2008). With epicuticular waxes impeding water and CO_2 movement, pores on the thallus surface restrict, but do not exert short-term control over water and carbon diffusion. Species with simple, solid thalli, experience higher diffusion resistances and have lower rates of photosynthesis (Meyer et al. 2008), although carbon concentration mechanisms may overcome this limitation (Griffiths et al. 2004; see Chapter 6). When expressed on a chlorophyll basis, rates of net photosynthesis for complex thalli are comparable with vascular plant leaves, although they are much lower when expressed relative to dry mass (Green and Lange 1995). This difference is partly caused by the multiple functions of the thallus as it serves as the primary organ for water and nutrient uptake and storage, in addition to photosynthesis, a constraint shared with leafy bryophytes as well.

B. Functional Trait Relationships in Bryophytes

Although often considered analogous with vascular plant leaves as a photosynthetic unit with bryophyte leaves performing the role of mesophyll, recent studies have shown that many functional trait relationships observed in vascular plant leaves are not found in

bryophyte canopies. For example, the robust relationship observed in vascular plants between leaf maximum rates of photosynthesis and nitrogen (Hikosaka 2004; Wright et al. 2004) has not been observed in multi-species comparisons in either Hawaiian forest mosses (Waite and Sack 2010) or in a multi-species comparison of *Sphagnum* (Rice et al. 2008). However, bryophyte canopies show some similar trait relationships with both studies indicating strong negative relationships between canopy mass per area and maximum rates of photosynthesis expressed on a mass basis, a similar pattern found when comparing leaf mass per area and maximum assimilation on a mass basis for vascular plant leaves (Wright et al. 2004). In the forest mosses, low rates of maximum photosynthesis were associated with increased costa length and width, which correlate with increased structural support and plant height, characteristics that influence photosynthetic efficiency, the former by the allocation of non-photosynthetic tissues and the latter by decreasing the light efficiency of photosynthesis through self-shading (Waite and Sack 2010). In *Sphagnum*, allocation to non-photosynthetic hyaline cells, which contribute to enhanced water holding capacity, reduces photosynthetic efficiency on a mass basis. In *Sphagnum*, the distribution of mass within the canopy exerts primary influence on photosynthetic assimilation on a mass basis—species that concentrate mass in the upper-canopy achieve higher rates of maximal assimilation (Rice et al. 2008).

These traits that associate with biomass allocation patterns and affect support or water storage also have vascular plant leaf analogues. However, some shoot functions in bryophytes like nutrient uptake are more important than in vascular plants and these create alternative trait relationships. For example in *Sphagnum*, cell wall polyuronic acids, which are involved in ion exchange and sequestration, are responsible for up to 30 % of shoot dry weight (Clymo 1963; Popper and Fry 2003; Kremer et al. 2004) and shoot water storage is strongly and negatively correlated with maximum assimilation (Rice et al. 2008), relationships that will not affect the leaf economics spectrum of vascular plant leaves. Consequently, although they share some similarities, the bryophyte canopy represents a unique functional type.

C. Photosynthesis-Related Traits and the Carbon Balance of Bryophytes

While photosynthesis is obviously the key pathway for carbon sequestration by bryophytes, it is only one of the processes that determines the overall carbon gain of individual living bryophytes. Their net carbon gain will also depend on the allocation of photosynthates to (1) compounds and tissues promoting further photoassimilation versus those (2) promoting longer tissue lifespan through protective chemistry, including anti-herbivore defense (Coley 1988; Glime 2006; Cornelissen et al. 2007); or those (3) supporting organs for vegetative or generative reproduction (During 1979). Actual losses of tissues to physical damage, pathogens or herbivore attack will have direct negative effects on net carbon gain of individual bryophytes. At the ecosystem scale, the carbon balance of the bryophyte compartment depends on the balance between net carbon gains of living tissues and carbon losses from dead bryophyte tissues (Clymo and Hayward 1982; Gorham 1991; Clark et al. 1998; Cornelissen et al. 2007; Limpens et al. 2008). Microbial decomposition and fire (Kuhry 1994) are the predominant pathways for such losses. As for fire, a preliminary screening in a fire laboratory (methods in van Altena et al. 2012) indicated that some moss species were more flammable than others in the Dutch flora (NA Soudzilovskaia and JHC Cornelissen, in preparation); *Pleurozium schreberi* was more flammable in terms of rate of fire spread and fire temperatures and also continued to ignite at higher moisture content than *Hypnum jutlandicum* and *Polytrichum commune*, respectively. However, investigations on the differential effects of bryophyte species on fire regimes are still in their very infancy.

We know a bit more about bryophyte species and decomposition. It is now well established that bryophyte litter generally decomposes slowly compared to that of vascular plants, even in given environmental regimes (Hobbie 1996; Lang et al. 2009). But also within bryophytes as a group great variation in litter decomposition rate has been reported among higher clades and species (Lang et al. 2009). *In situ* decomposition rates of bryophyte litter of different species are strongly driven by both environmental (biotic and abiotic) conditions of their actual habitats and species traits, and their interactions (Clymo and Hayward 1982; Limpens and Berendse 2003; Turetsky et al. 2008; Lang et al. 2009). However, different bryophyte species also show consistent and large variation in litter decomposability at given environmental regime (Lang et al. 2009). For instance, *Sphagnum* species are generally among the most recalcitrant bryophytes around worldwide (Clymo and Hayward 1982; Scheffer et al. 2001; Dorrepaal et al. 2005; Lang et al. 2009) and this has been attributed to their anti-microbial phenolic chemistry (Verhoeven and Liefveld 1997) as well as to polysaccharide deposits in cell walls (Hajek et al. 2011). It is important to recognize that 'a *Sphagnum* is not a *Sphagnum*' as even within this genus ten-fold trait-driven variation in decomposition rates has been reported between different species, with hummock species tending to be more recalcitrant than hollow species (Johnson and Damman 1991; Rydin et al. 2006; Lang et al. 2009). Such differences have been attributed to chemical traits as well. Turetsky et al. (2008) pinpointed the ratio between structural and non-structural carbohydrates as a good predictor of interspecific variation in *Sphagnum* decomposition. Lang et al. (2009) also simultaneously compared multiple subarctic non-*Sphagnum* bryophyte species for litter decomposability in standard outdoor litter matrices. They found a comparable five-to six-fold range of litter mass loss rates both among moss species and among liverwort species. Such strong inherent variation in traits that drive litter decomposability has implications for the consequences of environmentally driven shifts in bryophyte species composition for ecosystem carbon budgets. However, the critical issue is ultimately how the concomitant shifts in carbon release play out relative to the species' productivity responses. In theory, if there were perfect one to one correspondence of productivity and decomposability across species, the net species effect on the carbon balance should be nil. This is still a virtually blank field of research as, to our knowledge, there are no multispecies studies that compare patterns of variation between photosynthetic rates, growth rates and decomposabilities. However, we do have a few preliminary pointers from combining different literatures based on high-latitude experiments with bryophytes. Skre and Oechel (1981) screened five boreal moss species for photosynthetic rates under a range of environmental conditions to derive P_{max}, under the assumption that at least one of the experimental environmental regimes would be close to the optimum for a given species. *Polytrichum commune* had the highest P_{max} (2.65 mg CO_2 g^{-1} h^{-1}) and this species was also the fastest decomposing moss species in the mentioned subarctic multispecies litter decomposability screening, where all species were exposed simultaneously to the same environment for microbial decomposition (Lang et al. 2009). *Hylocomium spendens* and *Pleurozium schreberi* had intermediate P_{max} (1.39 and 1.20 CO_2 g^{-1} h^{-1}) and these two species also had intermediate to high decomposability in the study by Lang et al. The two *Sphagnum* species tested by Skre and Oechel (1981), *S. nemoreum* and *S. subsecundum*, had particularly low P_{max} (0.25 and 0.57 CO_2 g^{-1} h^{-1}). While these species were not included in the decomposability screening, it is likely based on the *Sphagnum* evidence described above that these two species would have been very recalcitrant to decomposition compared to the other three species. This indirect comparison suggests a positive relationship between potential

photosynthetic rates and potential litter decomposition rates among bryophytes, which would match the evidence for photosynthesis-related traits and decomposability among vascular plants (e.g. Cornelissen and Thompson 1997; Cornwell et al. 2008). Also, Furness and Grime (1982) screened multiple bryophyte species in the NW European flora for relative growth rates (RGR) in biomass terms, in a standardized greenhouse setup. Four of their species were common with the litter decomposability screening of Lang et al. (2009) and broadly the RGR ranking corresponded with the litter decomposability ranking: *Racomitrium lanuginosum* < *Aulacomnium palustre* < *Hylocomium splendens* = *Polytrichum commune*. In contrast, within the genus *Sphagnum* four species (*S. balticum*, *S. fuscum*, *S. teres*, *S. riparium*) measured for productivity by Gunnarsson (2005) did not match in rank with decomposability measured by Lang et al. (2009). To sum up, it is obvious from these poorly matched combinations of studies that much work needs to be done before we can make any robust linkages between interspecific variation in photosynthesis and growth related traits of bryophytes on one side and their litter decomposabilities and flammabilities on the other; and on the interactions of these linkages with vascular plants and their litters. Progress in this field would greatly improve our predictive power of consequences of species shifts for the carbon balance of bryophyte-dominated ecosystems.

III. Standardizing Photosynthetic Measurements

The bryophyte canopy represents a unique functional type as it relates to carbon and water dynamics. In this section, we review tissue and canopy characteristics that may serve as appropriate standards to develop a better understanding of the mechanisms that cause variation in bryophyte photosynthesis.

A. Surface Roughness

Important differences in canopy structure that relate to boundary layer properties and, hence, water loss, have been summarized by measuring variation in shoot height in intact canopies. Hayward and Clymo (1983) calculated the variance in canopy height measurements obtained using a contact probe at 8 mm increments to parameterize an evaporation model for *Sphagnum* colonies. More recently, Rice et al. (2005) developed a non-contact laser scanning technique that provides fine-scale canopy height measurements that they use to calculate a surface roughness parameter based on semivariance analysis. This analysis provides a measure of the variance of canopy height measurements at the scale of canopy exchange elements (leaf, shoot or shoot system, depending on the species) and is less likely to be influenced by the spacing of canopy sampling. Krumnikl et al. (2010) demonstrate that even greater resolution can be obtained using stereoscopic imaging. Surface roughness obtained using scanning methods indirectly relates to the thickness of external boundary layers, but directly to conductance of water from the bryophyte surface (Rice et al. 2000). However, surface roughness has been shown to be unrelated to differences in canopy light dynamics as summarized by light extinction coefficients or to variation in canopy photosynthetic characteristics in a multiple species comparison of *Sphagnum* (Rice et al. 2008) or in intraspecific studies of gas exchange in *Pleurozium schreberi* (Rice et al. 2010). Although it is likely that surface roughness affects light capture, particularly at low angles of directional light, thereby influencing daily production, it presently remains of limited use in studies of photosynthesis.

B. Area- and Mass-Based Measurements

Depending on the purpose of study, rates of photosynthesis in bryophytes have been expressed relative to leaf area, shoot area, projected canopy area, canopy dry mass and/or chlorophyll concentrations. The distribution

within the canopy of leaf area, shoot area, or dry mass also can be used to characterize canopy structure in a way that relates meaningfully to function.

Although leaf area based rates of photosynthesis allow for functional comparisons with vascular plant leaves (Nobel 1977) or among different bryophyte leaf types (Krupa 1984), they have only been performed on species with large, non-overlapping leaves (e.g., *Mnium* spp., *Polytrichum* spp.). Given that leaves are not independent functional units for most species and that leaf area is sufficiently difficult to obtain, leaf area has not been a common metric to standardize or compare rates of photosynthesis in bryophytes. Most investigators, instead, employ either projected canopy area (i.e., ground area) if they are interested in ecological questions that have a spatial component (e.g., community interactions or ecosystem fluxes) or they utilize mass based measurements as this standardizes values relative to plant carbon. Recognizing that bryophyte shoots (i.e., stems and leaves) can serve as appropriate functional units, recently researchers have used shoot area as a standard as well. Below, we discuss these various measures as summaries of canopy traits and comment on their utility in studies of bryophyte photosynthesis.

In broad-leaved, vascular plant canopies, carbon exchange is often expressed relative to or compared with total canopy leaf area. Canopy leaf area is often summarized using the leaf area index (LAI, m^2/m^2), the total single-sided leaf area relative to the ground area. When expressed in this manner, the canopy photosynthetic rate is a function not only of leaf-level photosynthetic response, but of canopy properties that affect light availability (e.g., self-shading, leaf angle) and the distribution of physiological characteristics of leaves throughout the canopy, properties that may vary due to differences in leaf age, to acclimation to light levels within the canopy, and/or to allocation of resources like N differentially within the canopy (Chap. 9).

Given their dense, often overlapping needle-like leaves, conifers, perhaps, present a more appropriate model for understanding how to estimate leaf area for photosynthetic studies of bryophytes. In conifers, clustering of leaves with non-uniform orientation causes self-shading, but also allows deeper light penetration (Thérézein et al. 2007). Due to the interaction of light and leaves within conifer shoots, projected area of shoots insufficiently characterizes light dynamics. Instead, the shoot silhouette area to total needle area ratio (STAR) has been developed to better characterize shoot—light dynamics (Stenberg et al. 2001; Thérézein et al. 2007; Smith and Hughes 2009). In conifers, shoots that have a higher density of leaves as those grown in open conditions, have low values of STAR, whereas flattened or low density needles on branches lead to higher values. In addition, variation in STAR associates strongly ($r = 0.99$) with light interception efficiency in samples of Scots pine, *Pinus sylvestris*, grown in different light environments (Stenberg et al. 2001). Presently, there are no studies of bryophytes where STAR has been calculated, although it may be very useful to standardize across species or studies. Measurements require projected silhouette areas, which can be obtained on excised bryophyte canopy samples, together with whole canopy leaf area, which is possible, although difficult to measure as described above.

In bryophytes, leaf area is difficult to obtain although modern photographic and scanning methods have made it easier (see Bond-Lamberty and Gower 2007 for method). In general, LAI measurements range from 6 to over 140 (Simon 1987; Vitt 1990; Proctor 2000), with generally lower values associate with acrocarpous species with low leaf densities. Except for the low reported values, these are much greater than the leaf area of vascular plant canopies (range 1 to over 20, Barnes et al. 1998). Indeed, bryophyte LAI values correspond more closely with the mesophyll area in vascular plants, where ratio of mesophyll area to leaf area is normally between 10 and 40 (Nobel and Walker 1985). These considerations have led to the suggestion that bryophyte

canopies and vascular leaf mesophyll are functionally analogous. Unfortunately, differences in the scale of the exchange surface and stomatal control in vascular plant mesophyll limits the usefulness of this analogy for development of unified models of function. To our knowledge, there are few studies of photosynthesis that have reported leaf area or report results on a leaf area basis (for exceptions, see Nobel 1977; Krupa 1984). Given that bryophyte leaves often significantly overlap and do not function independently from adjacent leaves, leaf area is not normally a useful way to characterize bryophyte canopies.

Instead, area based measurements normally focus on ground area. Given that community (e.g. species colonization or replacement) and ecosystem (e.g., fluxes of H_2O or CO_2) processes have important spatial components where ground based measures relate to biological function, these are often the most ecologically relevant. However, these measurements do not allow for the development of an understanding about how the organization of the primary functional unit, the canopy, affects physiological function. In other words, this focus does not provide adequate information about mechanisms that link organismal form or within-canopy physiological variation to whole-organism function that would further our understanding of bryophyte photosynthesis.

Occupying the scale between leaf area and ground area is the area of the shoot system. In many species with small, overlapping leaves including most pleurocarps and many acrocarps, shoots represent a relevant unit for exchange of water and energy. Consequently, the shoot area index (SAI; shoot area per ground area) has been used to summarize light dynamics in studies of light attenuation within bryophyte canopies (van der Hoeven et al. 1993; Williams and Flanagan 1998; Rice et al. 2011a, b) as well as serving as a way to standardize rates of photosynthesis (Williams and Flanagan 1998; Rice et al. 2011a, b). The distribution of shoot area can also be measured vertically within the canopy and help lead to a mechanistic understanding of light and photosynthetic function within canopies. However, rapid light attenuation and senescence instead of acclimation of shoots to low light within the canopy limits the contribution of the canopy interior to whole-plant photosynthesis, which has been shown in *Tortula* (=*Syntrichia*) *ruralis* (Zotz and Kahler 2007), in *Pleurozium schreberi* (Tobias and Niinemets 2010) and in *Sphagnum balticum* and *S. fuscum* (Johansson and Linder 1980). However, with greater recognition of photoinhibitory processes that are localized in the upper canopy (Chap. 7), studies relating the vertical stratification of shoot area will be a valuable component of understanding canopy carbon dynamics. In addition, in many ectohydric species, SAI also varies directly with water holding capacity as shoots serve an important water storage function. This leads to their use as an important parameter in bryophyte production models that seek to integrate bryophyte carbon and water dynamics (Rice et al. 2010).

Shoot area can be obtained with similar techniques as leaf area using fine resolution scanners or imaging microscopy. Shoot area measurements are used to calculate a shoot area to dry weight ratio and SAI is estimated using canopy dry weight. The measure is normally the projected shoot area, not the sum of leaf area of a shoot, although conversions to reflect the surface area of non-flat shoots have been employed (Bond-Lamberty and Gower 2007). Expressed for green tissue relative to the ground area, SAI provides an indicator that is easily comparable among species, which can be expressed in an index relative to ground area and that can be measured at different depths within the canopy. Although this measure has been used to model light dynamics and as a unit measure for photosynthesis, comparative data are few. Van der Hoeven et al. (1993) found SAI values that ranged from 4 to 7 for *Calliergonella cuspidata*, *Rhytidiadelphus squarrosus* and *Ctenidium molluscum* (estimated for green tissue from data presented), although total canopy SAI including brown

tissue could be greater than 20. For *Pleurozium schreberi*, Williams and Flanagan (1998) and Rice et al. (2011a, b) obtained SAI values in the range of 1.6–4.8. In the latter study using 25 field collected samples, SAI was the strongest predictor of light saturated rates of photosynthesis, which were expressed on a ground area basis ($R^2 = 0.41$).

In many bryophytes including cushion forming and some acrocarpous mosses, SAI may be difficult to obtain due to the high density of shoots. Instead of converting to shoot area, canopy dry weight expressed alone or per unit ground area is a reasonable unit for species comparisons (Alpert and Oechel 1987; Zotz and Rottenberger 2001; Rice et al. 2008; Waite and Sack 2010) as well as for understanding physiological dynamics within canopies (Zotz and Kahler 2007). Mass based measures correlate well with SAI (Rice et al. 2011a, b).

C. Chlorophyll

In addition to characterizing variation in the amount or distribution of photosynthetic tissues, the photosynthetic efficiency of these tissues also affects whole plant carbon dynamics. Expressing photosynthetic rates relative to concentrations of light harvesting pigments provides an indication of the efficiency of light capture. Although vascular plants have approximately two to tenfold higher rates of photosynthesis expressed on a weight basis compared with bryophytes, the rates are much more similar when standardized using chlorophyll (Green and Lange 1995). In comparative studies, differences in total chlorophyll (a + b) concentrations show high positive correlations with mass or area based measures of photosynthesis (Rice et al. 2008; Waite and Sack 2010; Rice et al. 2011b). Chlorophyll also varies vertically within canopies and Tobias and Niinmets (2010) suggest patterns of decreasing chlorophyll concentrations they observed within *Pleurozium schreberi* canopies reflect senescence of photosynthetic tissues in older shoots within the canopy interior. Similar patterns were found in *Tortula* (=*Syntrichia*) *ruralis* (Zotz and Kahler 2007).

D. Effects of Water

At saturating light, photosynthesis in bryophytes shows strong dependence on water content. The response is typically unimodal, with decreased rates of photosynthesis at higher water contents due to additional external water films that increase diffusion resistance and at low water contents due to biochemical changes that accompany tissue desiccation (Dilks and Proctor 1979; Proctor 1980). Although the response curve is typically asymmetrical about the maximum, the details about the curve vary from one species to the next. Indeed, in *Sphagnum* optimal water contents varied from 12 to 26 g H_2O/g dry weight among ten species (Rice, unpublished data, 2008). Given that maximal rates of photosynthesis are often two to over three times higher than those at full water content, measurements of photosynthesis can be quite sensitive to plant water status.

Although some studies that focus on the physiological effects of water content report full or partial response curves, many studies typically report that they remove excess water from the plant surface using a drip-dry or blotting technique. In our experience, these treatments can satisfactorily place plants near a water content optimal for photosynthesis. However, given the sensitivity described above doing this alone is insufficient. Instead, full or partial water content curves should be performed to establish the water content where maximal photosynthesis occurs and this water content should be replicated in the pre-treatments. If situations where non-optimal water contents are preferred (e.g., ecological conditions where high or low water contents are found), the photosynthetic—water content relationships will establish a context for the particular measurements. This will aid in better comparisons among studies and species. (note: see Chap. 13 for recommendations to deal with rapid drying in photosynthetic chambers).

As a measure of plant water status, plant water content does not allow for useful comparisons among species. Indeed, plant water contents vary considerably among bryophytes and this variation can be caused by differences in cell wall thickness, specialized water-holding cells, organs like paraphyllia, leaf size, shape or arrangement, or by other aspects of shoot organization. These differ in regards to their effect on water in the apoplast, in the symplast or held externally. Consequently, water content as a measure of plant water status is not adequately comparative across species. Instead, techniques have been developed to quantify plant water potential and determine the water content where physiologically important states like cell turgor loss point are achieved (Proctor et al. 1998; Proctor 1999). For example, Hájek and Beckett (2008) performed photosynthetic drying curves on five *Sphagnum* species and evaluated photosynthetic activity using the chlorophyll fluorescence parameter ϕ_{PSII}. The water content where cell turgor was lost, represented the point where ϕ_{PSII} began to decline and there was a strong quantitative relationship between these parameters. The relative water content (relative to the water content when external water has fully evaporated) at turgor loss varied by almost a factor of two (0.36–0.62) among the species. Consequently, relative water content is a coarse measure of plant water status, at least in how it relates to physiological state.

E. Sampling

In terms of their morphology and physiology, bryophytes display high levels of phenotypic plasticity that can alter photosynthetic dynamics (Tobias and Niinemets 2010; Rice et al. 2011a, b). In addition, their response to desiccation or other physiological stress may lead to prolonged recovery that needs to be considered when evaluating photosynthesis, especially when most studies of photosynthesis in bryophytes use field collected material for evaluation. This approach combines environmental and genetic variation and provides insight into the behavior of plants acclimated to the conditions where they grow, conditions that are ecologically relevant. However, investigators need to be careful to ensure that samples are fully recovered from transient stress, unless of course, it is the recovery that is of particular interest. Species express different recovery times in relation to full or partial desiccation stress and the recovery may be affected by the duration and intensity of the stress (Proctor 2000; Proctor et al. 2007). Since the recovery times vary, it is prudent to perform preliminary trials with the study species to determine an appropriate pre-treatment.

Alternatively, there have been recent studies that utilize common garden conditions for physiological studies (Rice et al. 2008). Following adequate periods to allow for plant growth responses, these studies allow investigators to discriminate genetic differences in physiological performance. It is important that these studies focus on new tissue that developed following transplant or initiation of the environmental treatment. This might mean 4–8 weeks for some species like *Sphagnum* grown in optimum conditions to one or more growing seasons in bryophytes with slow growth rates.

IV. Best Practices for Studies of Photosynthesis

1. Employ the canopy as primary unit of study. If technical restrictions prevent this (e.g., using a chlorophyll fluorescence probe on a leaf or shoot), provide information on variation of the measure and its distribution within the canopy that would allow for scaling to canopy-level (see Chap. 9).
2. Provide sufficient information that would allow the conversion of measurements to be expressed on ground-area, dry weight of green tissue and chlorophyll bases. Also, when appropriate, report shoot area as this represents a useful comparative exchange unit for bryophytes.

3. Characterize plant water status and photosynthetic responses adequately. Studies should complete photosynthetic drying curves and report how water content during measurements relates to optimal water content. It is also worthwhile to perform more detailed analyses on the physiological water status by measuring plant water potentials and relating relative water contents to state transitions like the turgor loss point. Finally, it is useful to report on the recovery phase of photosynthesis from desiccation as this has important effects on plant carbon gain.
4. When scaling from short-term field measurements to seasonal or annual measurements, perform adequate sampling within days as well as over many days during the year. This will help overcome problems caused by variation in plant water content or other environmental factors such as light availability and temperature. Although this has not been explored quantitatively for bryophytes, suggestions made by Bader et al. (2009) for lichens should be considered.
5. If using field-collected samples in the lab, allow for sufficient acclimation and recovery from short-term physiological stress in the field. We have found some species require 4–6 day to achieve maximum rates of photosynthesis in mesic forest species. When genetic and not environmental variation is the study focus, employ a common garden approach.
6. Identify specimens to species, when possible. There remain too many studies that use ecological or generic groupings.

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