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Rapid resurrection of chlorolichens in humid air: specific thallus mass drives rehydration and reactivation kinetics

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ABSTRACT

Identifying lichen traits that influence hydration and photosynthetic reactivation kinetics in humid air provides insight into niche preferences. Water vapor uptake and concurring reactivation of PSII efficiency (F_v/F_m) were monitored at high temporal resolution by means of programmed balance measurements and chlorophyll fluorescence imaging during 18 h trial periods in high relative humidity (RH). Desiccated, thin and/or highly branched forest epiphytes began to reactivate PSII in thallus apices and margins within two minutes of exposure to high RH. Specific thallus mass (STM) was a strong predictor of water vapor uptake rates across species and specimens. The forest epiphytes displaying the lowest STM reached the highest levels of saturation and showed the most rapid PSII reactivation. Thicker species from sun-exposed habitats required up to 11 times longer in high RH to reach peak PSII reactivation, particularly lichens collected from open, exposed rocks. There was a clear trade-off between water storage capacity and rapid saturation from water vapor/PSII reactivation. Thin chlorolichen growth forms are thus well-adapted to exploit humid air, while thick ones likely rely on liquid water.

1. Introduction

As poikilohydric organisms, lichens show a high selectivity for certain hydration sources and can influence ecosystem functioning by their substantial harvest of non-rainfall water (Stanton et al., 2014a; Stanton et al., 2014b; Pypker et al., 2017; Wang et al., 2017). Functional hydration traits in lichens exhibit notable variations across macro- (Ellis and Coppins, 2006) and microclimatic gradients (Merinero et al., 2014), and lichens' dependency on certain climates widely vary among species (Ellis, 2016). A diversity in morphological, anatomical and physiological lichen traits, in part reflects adaptations to exploit niche-specific water sources such as rain (Larson, 1981), dew (Kappen et al., 1980; Lakatos et al., 2012) and humid air (Lange and Tenhunen, 1982; Lange et al., 1986). Growth form influences lichen water economy and kinetics, although the basis for such links is not well understood. Traits with documented effects include branch density in hair lichens (Esseen et al., 2015), specialized morphological structures such as rhizinomorphs in Umbilicariaceae (Valladares et al., 1998), thallus size (Merinero et al., 2014) and even color, causing albedo during periods of solar radiation exposure (Sancho et al., 1994; Palmqvist, 2000). Also, anatomical traits, like the presence of a hypothallus in *Degelia plumbea* (Gauslaa and Solhaug, 1998) and the thickness of the photobiont and cortex layers (Gauslaa and Coxson, 2011), markedly influence water storage in lichens.

Research has demonstrated that dry mass per thallus surface area (STM) is a strong predictor of the ecologically relevant water-holding capacity (WHC), the amount of water per thallus area that drives the duration of photosynthetically active periods (as reviewed by Gauslaa, 2014). These functional traits also govern water kinetics in lichens, physical processes that appear uninfluenced by physiology (Blum, 1973; Lange et al., 1986; Jonsson Čabrajić et al., 2008), wherein the thallus water content, under natural field conditions, continuously fluctuates to equilibrium with the ever-changing water potential of the ambient air (Palmer and Friedmann, 1990; Gauslaa et al., 2012). The thallus mass-to-area ratio, for instance, has been shown to significantly affect both desiccation and water uptake rates in lichens (Larson and Kershaw, 1976; Larson, 1979, 1981), although this has so far only been studied in a few rock- and soil-dwelling lichens and at a low temporal resolution. Finely dissected, epiphytic growth forms, like hair lichens with a low mass-to-area ratio, are generally assumed to be quicker in achieving saturation from water vapor. Whereas chlorolichens can reactivate photosynthesis in humid air alone, cyanolichens need liquid water (Lange et al., 1986). Nevertheless, hydration preferences also vary considerably across green algal lichen species, and the functional hydration traits that drive chlorolichen performance require further study.

Chlorophyll fluorescence, a non-invasive method to assess maximal PSII efficiency (F_v/F_m), provides valuable insight into the performance

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of photosynthetic tissues (Maxwell and Johnson, 2000). In various photosynthetic organisms, chlorophyll fluorescence has been useful in analyzing responses in PSII performance to abiotic stressors like excessive temperature (Larcher et al., 1997) and high-light intensity (Gauslaa and Solhaug, 1996; Gauslaa and Solhaug, 2000), as well as to substrate acidity (Gauslaa et al., 1996) and water availability, both in the lab (Csintalan et al., 1999) and in situ (Schlensog and Schroeter, 2001). Chlorophyll fluorescence imaging has broadened the applications of traditional fiber optic measurements by allowing spatio-temporal visualization of photosynthetic activity. The method can be used to efficiently investigate, for example, how pathogens affect plant tissues (Berger et al., 2007) and explore the effects of drought and high temperature on crops (Brestic and Zivcak, 2013). In lichens, fluorescence imaging allows analyses of photosynthetic variability across entire thalli during changing environmental conditions (Barták et al., 2004, 2005, 2006), which is useful in linking PSII performance to thallus morphology and anatomy, as well as to lichen hydration status. The ability to visualize reactivation in the lichen thallus is particularly informative, since, unlike most vascular plants, the entire lichen body takes up water (Green et al., 2008). Such tools allow for an evaluation of how morphological, anatomical and physiological traits alter water economy and rapid photosynthetic responses to specific hydration sources, elucidating functional aspects of lichen growth forms.

Understanding functional lichen biology at the trait level is essential in improving our appreciation of the larger-scale forces that drive lichen performance and distribution. As primary contributors to ecosystem functions (Ellis, 2012; Asplund and Wardle, 2017), players in food webs (Pettersson et al., 1995; Kinley et al., 2006), interceptors of water and nutrients (Van Stan and Pypker, 2015) and facilitators of their hosts' water use (Stanton et al., 2014b), canopy chlorolichens are important. Identification of traits regulating their activity is needed. In this study, we simultaneously and continuously monitored water uptake and photosynthetic reactivation in a range of lichen growth forms. To avoid confounding effects of substrata, we selected upright or pendent fruticose and foliose lichens with freely exposed lower sides, spatially well-separated from the boundary layer of their substrate (tree bark or rock). We hypothesized that: (1) thin and highly dissected growth forms can more rapidly absorb and utilize water vapor for photochemistry, suggesting high performance capability even where humid air is often the main hydration source. We expected that a high temporal resolution of measurements will detect the minimum time needed for reactivation. (2) STM determines rates of vapor uptake and photosynthetic reactivation across lichen species. A documentation of close ties between STM and hydration rates in humid air across growth forms, species and/or specimens would improve our understanding of lichen life forms and their niches. Because intraspecific variation was expected, we intended to provide hydration estimates not of individual species per se, but of the traits that govern their basic ecophysiological responses. Additionally, by comparing kinetics using the hydration parameters water content per dry mass, water content per thallus area, and relative water content (see below), we aimed to analyze and discuss functional implications of these traits.

2. Materials and methods

2.1. Lichen material

Lichen thalli representing seven common species and genera were collected on 3 December 2016 from three sites in Ski and Ås, Akershus, SE Norway, from optimal habitats for each species. Four species were collected from lower twigs and trunks of *Picea abies* in mixed, old spruce and pine forest (site 1: 59°43′41″N 10°58′54″E; 150–170 m asl), including: *Platismatia glauca* (L.) W.L.Culb. & C.F.Culb.; *Bryoria capillaris* (Ach.) Brodo & D. Hawksw.; *Alectoria sarmentosa* (Ach.) Ach.; and *Usnea dasopoga* (Ach.) Nyl. Three species were collected from open roadside habitat in agricultural settings, including *Ramalina fraxinea* (L.) Ach. on *Acer platanoides* and *Crataegus* sp. (site 2: 59°43′00″N 10°54′57″E; 160 m asl), and *Dermatocarpon miniatum* (L.) W. Mann and *Lasallia pustulata* (L.) Mérat on vertical, SW-facing open rocks (site 3: 59°38′24″N 10°50′42″E; 85 m asl). None of the studied species were present in more than one visited habitat. Five, apparently healthy, intact thalli with a study-wide thallus area mean of 17.3 ± 1.1 cm^2 (thallus size did not significantly differ between the species-wise pools of collected thalli) were randomly selected from each species, left to air dry at room temperature in the lab and then stored at −18 °C until the measurements were taken (within three months). Freezing lichens prior to physiological tests has no noticeable effects on chlorophyll fluorescence measurements (Honegger, 2003).

2.2. Experimental design

The experiment was conducted in a dark, temperature-controlled room at 16 °C. To account for any possible bias due to external conditions or lichen viability during the measurement period, each of the seven species were tested in a repeated sequence rather than consecutively testing all samples from each species. Specimens were placed in a desiccator for 48 h prior to measurements. Dark-adapted specimens were quickly removed from the desiccator and individually hung by thin copper wire from a Sartorius CP Gemplus Series balance (Bradford, MA, USA), elevated on a platform above a transparent, 25 × 22 × 8 cm glass chromatography tank lined with wet filter paper and with a 1 cm layer of water at the bottom (Fig. 1).

Relative humidity (RH) within the tank was measured using a HOBO Micro Station Data Logger with a 12-bit Temperature/Relative Humidity Smart Sensor and monitored with HOBOware Pro v2.2.1 (Onset, Bourne, MA, USA). RH was recorded at close to 100% within the tank. However, no mass gain from condensation occurred on aluminum foil in any period of complete diurnal cycles, documented by continuous weighing of the foil in the tank. Therefore, the tank's RH was slightly below 100%, and all uptake of water was from vapor only.

A 13 mm diameter hole in the tank lid allowed for the suspension wire to pass unimpeded into the tank. A custom script was loaded and run with ImagingWin v2.46i (Heinz Walz GmbH, Effeltrich, Germany), coupled to a red LED Imaging-PAM M-series chlorophyll fluorometer (Heinz Walz GmbH, Effeltrich, Germany) aimed through the tank wall

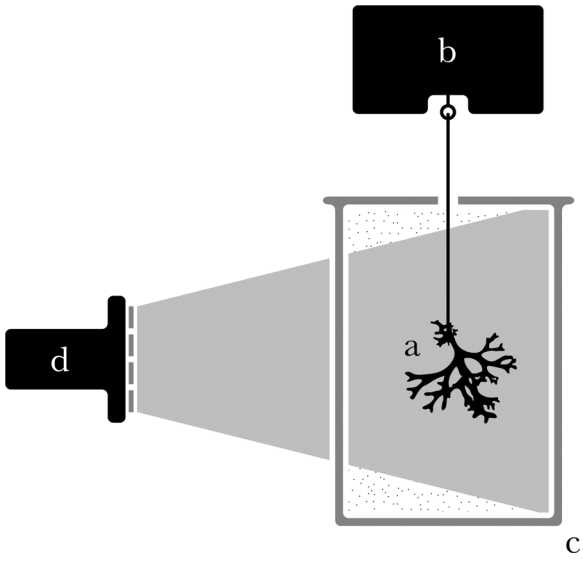

Fig. 1. Schematic of experimental setup. A (a) lichen thallus was hung from a (b) Sartorius CP Gemplus Series balance into a (c) high-humidity glass tank to measure mass gain from water vapor uptake. A (d) red-LED Imaging-PAM M-series chlorophyll fluorometer simultaneously recorded changes in maximal PSII efficiency (F_v/F_m). A ULM-500 quantum sensor was used to calibrate fluorometer measurements and to record irradiance of saturating pulses.