

## USE OF A NETWORKED DIGITAL CAMERA TO ESTIMATE NET CO<sub>2</sub> UPTAKE OF A DESICCATION-TOLERANT MOSS

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Simple visible-light digital cameras offer a potential for expanded forms of plant ecological research. The moss *Tortula princeps* undergoes changes in reflected visible light during cycles of drying and hydrating in the field, and the MossCam project has collected digital images of *T. princeps* at least daily since 2003. Laboratory studies can be used to calibrate these images to indicate field physiological conditions. Drying the moss 6 d in the laboratory resulted in a decrease of net CO<sub>2</sub> uptake to near 0; recovery after rewetting occurred within 10 min. The difference in reflectance between hydrated and dry *T. princeps* was maximal ca. 550 nm, and maximal net CO<sub>2</sub> uptake was linearly related to the green : red ratio of laboratory images when net CO<sub>2</sub> uptake was positive. Using the green : red ratio of field images and otherwise assuming ideal conditions, the total carbon gain for a 6-d period around a 1.3-mm rain event was ca. 208 mmol CO<sub>2</sub> m<sup>-2</sup>, equivalent to 69 d of respiration under dry conditions. Using a visible-light digital camera with micrometeorological data and laboratory-based gas exchange measurements, *T. princeps* can be used as a model species for simple field estimations of photosynthesis, carbon gain, and phenological events.

**Keywords:** red, green, and blue (RGB), charge-coupled device (CCD), webcam, visible light, *Tortula*.

### Introduction

The popularity of inexpensive visible-light digital cameras has increased dramatically in recent years, although the adoption of such cameras for plant ecological studies has been slow. One limitation to the use of consumer-grade digital cameras for remote sensing is the standard addition of integrated filters because of the infrared sensitivity of the charge-coupled device (CCD) sensors normally found in such cameras. Popular remote-sensing methods, such as the normalized differential vegetation index (NDVI) used in phenology and primary productivity studies, use wavelengths in the near infrared, and consumer-grade digital cameras cannot be used for such measurements. However, comparisons between standard remote-sensing methods and those using visible light that are captured by digital cameras indicate that such devices are useful for estimating the concentration of certain plant pigments and leaf nitrogen (Gamon and Surfus 1999; Wang et al. 2004). Indeed, plant canopy reflectance in visible wavelengths (400–700 nm) is predominantly influenced by chlorophyll and photosynthesis-related pigments (Vogelmann 1993; Blackmer et al. 1994; Gitelson et al. 2003).

Most of the plant studies that have involved the use of digital cameras have concentrated on agronomic applications.

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Adamsen et al. (1999) were some of the earliest users of digital cameras, and they examined senescence in a wheat canopy over 1-m<sup>2</sup> areas by using a ratio of green to red pixel values. Lukina et al. (1999) used digital images to estimate the vegetation coverage and biomass of wheat, and Purcel (2000) compared captured images with under-canopy measurements of light for estimating total ground cover of soybean. Using digital cameras for the measurement of yield and nutrient status of wheat (Hafsi et al. 2000; Jia et al. 2004a, 2004b) and corn (Daughtry et al. 2000; Graeff et al. 2001), for the measurement of the quality of turf grasses (Karcher and Richardson 2003), and for counting the number of flowers of cultivated *Lesquerella fendleri* (Adamsen et al. 2000) underscores the utility and potential applications for such devices.

Unfortunately, the number of plant ecological studies using consumer-grade digital cameras has been few. Hemispherical photography readily lends itself to the use of digital cameras (Leblanc et al. 2005), although the technique is primarily concerned with transmitted sun and sky light rather than reflected light from vegetation. Dymond and Trotter (1997) used digital color images of forests and pastures taken from aircraft to determine their reflectance properties, Richardson et al. (2001) examined the spectral reflectance of pine needles along an elevational gradient, and Hamerlynck et al. (2000) compared surface reflectance to the chlorophyll fluorescence characteristics of the desiccation-tolerant moss *Tortula ruralis*.

*Tortula* is a terrestrial moss genus that has been considered a model taxonomic group for work on the physiology of desiccation tolerance (Oliver et al. 2000). Desiccation-tolerant

bryophyte cells function for most of the time at full turgor when external water is present; during drying, external water is lost first and water stress is a relatively brief phase (minutes to hours) before full desiccation occurs and net CO<sub>2</sub> uptake ceases (Proctor 2000). The genus has been extensively studied because of its cellular protection, rapid shutdown, and resumption of gene expression during cycles of drying (Velten and Oliver 2001; Oliver et al. 2004). *Tortula* may also be an ideal candidate for ecological and phenological investigations using a digital camera because its natural cycles of drying and rehydration that result in changes in net photosynthesis and respiration are associated with changes in its reflectance of visible light.

The aims of this study were to determine (1) whether data from laboratory-collected color digital images of *Tortula princeps* could be correlated with simultaneous laboratory measurements of photosynthesis over the course of a drying cycle, and (2) whether this correlation could be applied to the images collected at the field site and then related to local micrometeorological conditions to estimate field photosynthetic rates. The ultimate goal of this study was to begin to develop methods for the application of visible-light digital cameras for plant ecological studies.

## Material and Methods

### Field Site and Species

The University of California James Reserve is located in the San Jacinto Mountains of southern California (33°48'30"N, 116°46'40"W) at 1658 m elevation in a mixed conifer and hardwood forest with a perennial mountain stream. The area receives ca. 485 mm precipitation annually. The reserve acts as a test bed for technology developed by the Center for Embedded Networked Sensing, a National Science Foundation-funded science and technology center located at the University of California, Los Angeles (<http://research.cens.ucla.edu>). The MossCam project, initiated in the spring of 2002, involves the University of California James San Jacinto Mountains Reserve and the Bryolab at the University of California, Berkeley. *Tortula princeps* (De Not.) is a desiccation-tolerant moss, globally widespread (Anderson et al. 1990), and is common in the area. Relatively low-resolution images (352 × 254 pixels until October 2004 and 704 × 480 pixels after that) of a stand of *T. princeps* located on the side of a large granite boulder are captured every 30 s by a networked color CCD-based video camera (the MossCam; model PC33C, Supercircuits, Liberty Hill, TX; fig. 1). Images are transferred by file transfer protocol to a video server (model 2400, Axis Communications, San Diego, CA) for transmitting digital video over the Internet and are archived in an open-source database (MySQL, Cupertino, CA) every 15 min. Additionally, data from an adjacent micrometeorological station located within 2 m of the MossCam that measures air temperature, relative humidity, wind speed, wind direction, leaf wetness, and precipitation are archived every 5 min, and all data are available via the James Reserve Web site, <http://www.jamesreserve.edu>. Images from the MossCam for the period of August 18–23, 2003, were chosen as a sample period for analysis because a 2.5-h rain event of 1.3 mm occurred at that site starting at ca. 1730 hours on August



**Fig. 1** MossCam project installation, showing the white environmental housing that covers the networked digital video camera that is pointed at a stand of *Tortula princeps* on a boulder at the James Reserve in the San Jacinto Mountains of southern California, United States. A, November 10, 2005, 1800 hours after ca. 20 d of no precipitation; B, the same day, 1900 hours after 0.5 mm recorded precipitation.

20 after 21 d of no rain. Images had been compressed to a 75% value compared to uncompressed images (100% value) using the Joint Photographic Experts Group (JPEG; ITU 1992) compression algorithm.

### Gas Exchange, Reflectance, and Image Analysis

Samples of *T. princeps* including the associated underlying soil were collected at the field site from boulders adjacent to the sample under observation by the MossCam. Samples used in gas exchange thus consisted of a moss-dominated community with *T. princeps* being the most conspicuous species in terms of exposed surface area. Samples were maintained in an environmental chamber (model E7H, Conviron, Pembina, ND) at a laboratory at the University of California, Los Angeles, at ca. 15°/10°C day/night temperatures with a photoperiod of 12 h and an instantaneous photosynthetic photon flux (PPF) of 300  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . *Tortula princeps* was defined as dry by air-drying in the environmental chamber for ca. 5 d; dry samples contained 5.9%  $\pm$  0.4% (mean  $\pm$  SE;  $n = 8$ )

water content (Proctor 2000) as measured with an analytical balance (PC440, Mettler-Toledo International, Columbus, OH) relative to samples placed in a drying oven at 60°C for 48 h; samples that had been in the drying oven were considered dead samples for reflectivity measurements. Samples were defined as hydrated when their water content was  $168.5\% \pm 13.3\%$  ( $n = 8$ ) of their dry weight, which was obtained by submerging samples in distilled water for 5 min to ensure complete hydration and then draining on paper towels for ca. 15 min to partially empty the external capillary spaces that reduce CO<sub>2</sub> diffusion (Dilks and Proctor 1979).

Instantaneous net CO<sub>2</sub> uptake was measured on samples ca. 50 mm in diameter (ca. 9 g hydrated weight) using an infrared gas analyzer (LI-6262, LI-COR, Lincoln, NE) in differential mode. Samples were placed in a transparent acrylic cuvette 150 mm long  $\times$  75 mm deep  $\times$  75 mm wide, and air flowing into the cuvette contained ca.  $370 \mu\text{mol CO}_2 \text{ mol}^{-1}$  air. The airflow rate was measured using a mass flowmeter (LFC-3, Technology Incorporated, Dayton, OH) and was adjusted so that the maximal decrease in mole fraction of CO<sub>2</sub> in the cuvette did not exceed  $25 \mu\text{mol mol}^{-1}$  air. To establish a 0 baseline for the gas analyzer, incoming air was passed simultaneously through both the reference and the sample sides of the gas analyzer every 10 min. Data from the gas analyzer and the mass flowmeter were collected every 10 min by a datalogger (23X, Campbell Scientific, Logan, UT) and were then periodically downloaded to a personal computer. Gas exchange data are expressed on a projected mat surface area basis.

The spectral distribution of reflected light from *T. princeps* was measured using a spectroradiometer (LI-1800, LI-COR) with an integrating sphere. Seven samples of *T. princeps* were maintained on moist paper towels in the environmental growth chamber for at least 1 wk before measurements. A central section of each sample was placed against the 130-mm<sup>2</sup> sample port of the integrating sphere equipped with an incandescent light source, and the sample's reflected light was scanned from 400 to 700 nm in 1-nm increments. The samples were then allowed to dry and the process was repeated. Two measurements per sample were taken, and the resulting data were averaged. Reflectance data were divided by that of a barium sulfate standard and averaged over 5-nm increments.

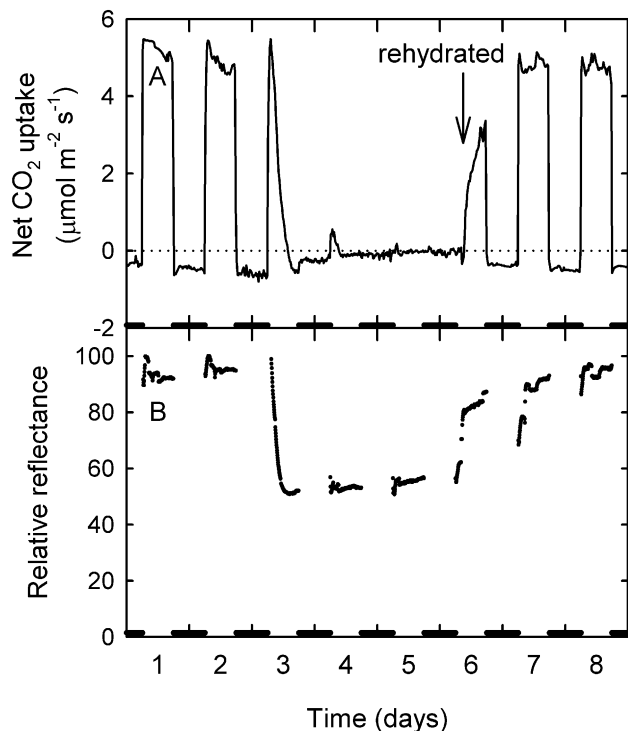
Color images of *T. princeps* during gas exchange measurements were automatically captured in the laboratory at a resolution of 640  $\times$  480 pixels every 5 min using a small color CCD camera (Quickcam, Logitech, Fremont, CA) designed to be attached to the serial port of a personal computer. Images from both the laboratory and the MossCam were carefully cropped to remove areas of the image that did not contain moss (the same 61.4% of the area that excluded rock and leaf litter was used in all field images for analysis; the usable area for images captured in the laboratory was ca. 36% but varied slightly because samples were not uniform in size) and were separated into their constituent red, green, and blue (RGB) channels using R, an open-source data analysis software package (<http://www.r-project.org>). Images from the MossCam were excluded from analysis when the occurrence of direct, diffuse, or reflected/scattered sunlight saturated the camera response and color information was lost.

The ratio of the averaged green channel pixel values to that of the red channel in cropped images produced the largest signal when comparing visually selected images of dry and hydrated moss captured from the MossCam during 2003, and thus this ratio was used with images collected in the laboratory to correlate with measurements of photosynthesis. Because of slight natural variations in the green : red ratio of samples of *T. princeps* measured in the laboratory, the green : red ratio values of hydrated samples and of dry samples were calibrated to those of hydrated and dry *T. princeps* images captured by the MossCam. The averages of 39 visually selected images of hydrated *T. princeps* (immediately following a precipitation event) and 206 images of dry *T. princeps* (following at least 7 d of drought) captured by the MossCam from May 2003 through May 2004 were used for calibration. Additionally, the regular patterns of changes in the green : red ratio of images associated with time of day due to changes in the spectrum of solar irradiance were corrected for by determining a green : red ratio offset every 15 min relative to noon for 10 d before the August 20, 2003, rain event when *T. princeps* was dry and assumed not to be changing in color.

## Results

Hydrated samples of *Tortula princeps* were allowed to dry over 6 d and then were rehydrated. Net CO<sub>2</sub> uptake decreased rapidly over a period of less than a day, and the rate of respiration measured during the following dark period was also less than when fully hydrated (fig. 2A). The maximum instantaneous net CO<sub>2</sub> uptake averaged  $5.3 \pm 0.7 \mu\text{mol m}^{-2} \text{ s}^{-1}$  (mean  $\pm$  SE;  $n = 6$ ), which occurred at a PPF of ca.  $500 \mu\text{mol m}^{-2} \text{ s}^{-1}$  at 15°C (data not shown). After the moss reached a relative water content of  $132\% \pm 13\%$  ( $n = 7$ ) of the dry weight, photosynthesis had essentially ceased. After rehydrating, *T. princeps* regained positive net CO<sub>2</sub> uptake within ca. 10 min (fig. 2A), and photosynthesis was 60% of maximal 8 h after rehydrating. The percent relative reflectance of the moss, as measured by the averaged pixel values of images captured during gas exchange relative to the initial, well-hydrated condition, decreased rapidly with drought, reaching a minimum of 50.8% when net CO<sub>2</sub> uptake became negative on the third day (fig. 2B). Total reflectance increased to 87.3% of its predrought level on the first day after rehydrating, in a similarly rapid manner as the resumption of net CO<sub>2</sub> uptake. The dry mass per projected area of isolated moss thalli (not including soil) was  $229.4 \pm 21.2 \text{ g m}^{-2}$  ( $n = 8$ ). The dry mass of isolated moss thalli averaged  $16.7\% \pm 3.7\%$  of the total mass of samples.

The spectral distribution of light reflected from hydrated *T. princeps* from 400 to 700 nm was maximal ca. 550 nm (fig. 3A;  $n = 7$ ). The relative intensity of light reflected from dry *T. princeps* was the lowest near 400 nm and the highest near 650 nm. Both hydrated and dry *T. princeps* had high reflectance above 675 nm (fig. 3A). The difference in relative reflectance between hydrated and dry live *T. princeps* was maximal ca. 550 nm, indicating that hydrated live *T. princeps* reflected more light in the green region of the spectrum, and minimal ca. 675 nm, indicating dry live *T. princeps* reflected more light in the red region (fig. 3B). Dead *T. princeps* also



**Fig. 2** Example time course for (A) instantaneous net CO<sub>2</sub> uptake over 8 d of a sample of *Tortula princeps* during drying and then rehydrating, indicated by arrow, and (B) relative reflectivity of the sample as measured by averaged pixel intensity of images captured every 5 min by a digital video camera.

increased in reflectivity when hydrated compared to dry; the maximal difference was ca. 630 nm, corresponding to a yellow region of the visible spectrum.

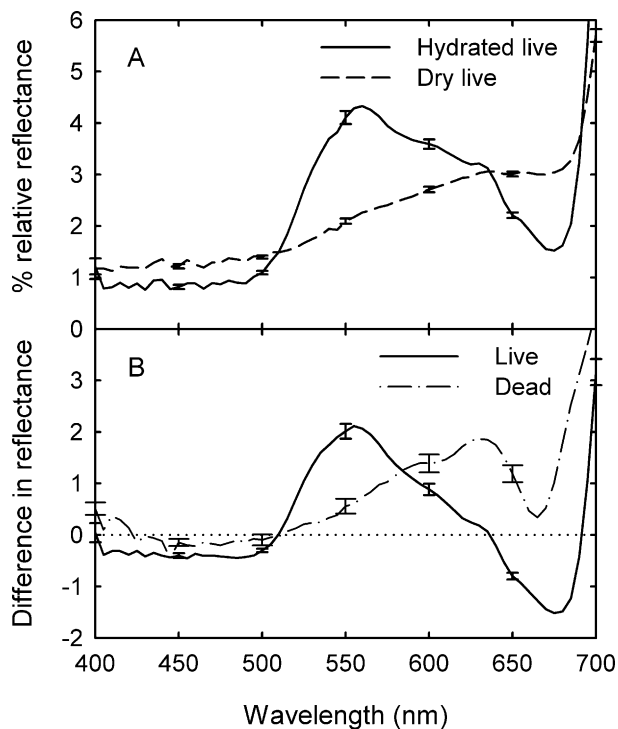
The frequencies of the pixel values in each of the RGB channels differed in visually selected images of hydrated ( $n = 37$ ) and dry ( $n = 206$ ) *T. princeps* captured by the MossCam in the field for the dates May 1, 2003, through May 1, 2004 (fig. 4) and were used for calibration of the laboratory-based measurements. The sum of the pixel values were higher for the hydrated *T. princeps* than for the dry, indicating a higher general reflectance of hydrated *T. princeps*, which corresponds to the measurements made in the laboratory with the smaller digital camera (fig. 2) and with the spectroradiometer (fig. 3). Pixel values at which the maximal frequency occurred for each of the red, green, and blue channels also differed between hydrated and dry *T. princeps* in the field (fig. 4). The average green : red pixel ratios of images of hydrated *T. princeps* was  $1.155 \pm 0.020$  and for dry was  $0.099 \pm 0.014$ .

The percentage of maximal net CO<sub>2</sub> uptake was linearly related to the green : red ratio of laboratory images captured simultaneously with gas exchange when net CO<sub>2</sub> uptake was positive (fig. 5). When net CO<sub>2</sub> uptake approached 0 and became negative during drying, the green : red ratio of images captured was a less accurate predictor of gas exchange. The most negative values of net CO<sub>2</sub> uptake ( $-28\%$  of maximal) occurred immediately after the transition from positive to negative net CO<sub>2</sub> uptake during drying; negative values near 0 occurred at the end of the drying experiment (fig. 5).

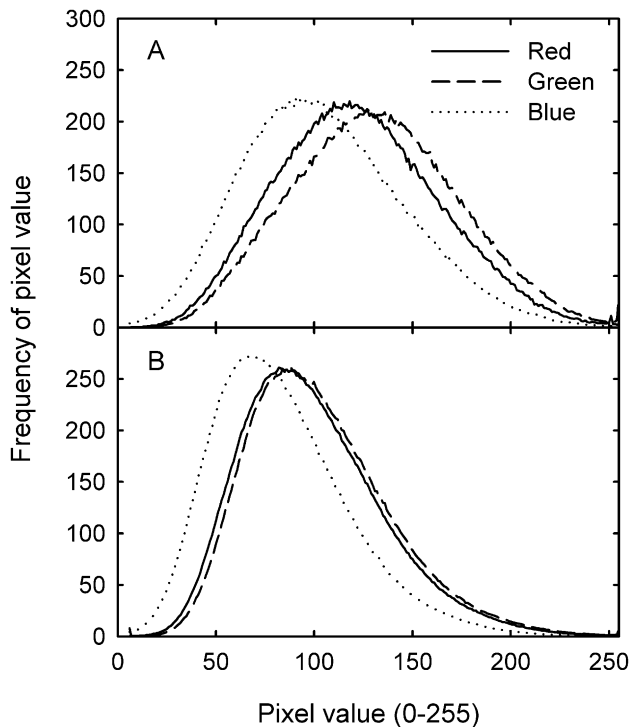
Using the green : red ratio as a predictor of net CO<sub>2</sub> uptake in the field and assuming saturating light conditions, no photoinhibition, and thallus temperature equal to air temperature, images from the MossCam collected before and after a 1.3-mm rain event on August 20, 2003, were analyzed. The predicted percentage of daytime maximal net CO<sub>2</sub> uptake averaged  $-1.4\% \pm 0.5\%$  before the rain event (fig. 6). Predicted percentage of maximal net CO<sub>2</sub> uptake was greatest at the same time as when the micrometeorological station indicated that precipitation had occurred, at 1754 hours. The predicted percentage of maximal net CO<sub>2</sub> uptake for the following day was low in the morning and evening hours and highest midday, and averaged  $66.1\% \pm 2.0\%$ . The second day after the rain event, predicted percentage of maximal net CO<sub>2</sub> uptake averaged  $20.9\% \pm 3.3\%$  and the third day averaged  $3.8\% \pm 0.5\%$  (fig. 6). Assuming a maximal photosynthetic rate in the field that is similar to that obtained in the laboratory under near ideal conditions (starting conditions in fig. 2), a nighttime carbon loss in the field when dry at  $0.012 \mu\text{mol m}^{-2} \text{s}^{-1}$  and when hydrated at  $0.45 \mu\text{mol m}^{-2} \text{s}^{-1}$ , then the total carbon gain for the period of August 18 to August 23, 2003, can be estimated at ca.  $230 \text{ mmol CO}_2 \text{ m}^{-2}$ .

## Discussion

Using micrometeorological data such as precipitation and air temperature in conjunction with laboratory-based gas exchange measurements, *Tortula princeps* can be used as a model species for simple field estimations of photosynthesis



**Fig. 3** Relative spectral reflectance of (A) hydrated and dry live *Tortula princeps* from 400 to 700 nm, and (B) the mathematical difference of hydrated/dry values for live and dead samples. Symbols are selected mean  $\pm$  SE;  $n = 7$ .



**Fig. 4** Frequency of pixel intensities of the red, green, and blue channels from visually selected images captured by the MossCam from May 1, 2003, to May 1, 2004, for (A) hydrated ( $n = 37$ ) and (B) dry ( $n = 206$ ) *Tortula princeps* used for calibration of the green : red ratio.

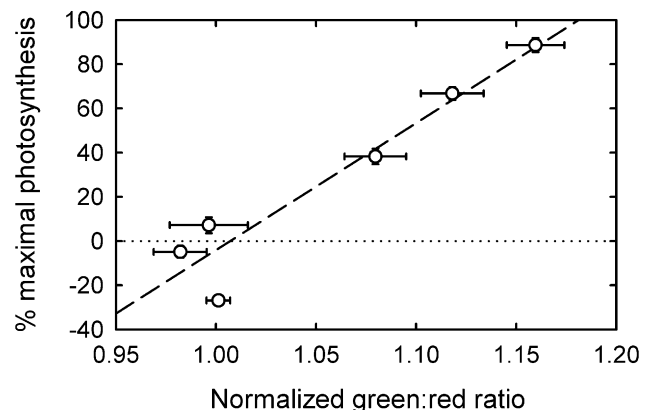
and phenological cycles employing a visible-wavelength digital color camera. Because the photosystems of *Tortula* survive essentially intact after drying (Proctor and Smirnov 2000), even though desiccation can be severe (*Tortula ruralis* can dry to ca. 5% dry weight; Proctor 2000), hydrating returns the photosystems to a functional state rapidly, often within 40 min or less (Proctor and Smirnov 2000). The changes in total reflectivity and color accompanying the desiccation and hydration of *T. princeps* and *T. ruralis* (Hamerlynck et al. 2000) can thus be used to quantify the potential capacity for photosynthesis and the timing of phenological events. To predict absolute photosynthesis values would entail the additional use of incident light, temperature, and possibly other measurements of the moss in the field. However, assuming saturating light levels with no photoinhibition and using air temperature and precipitation data for a week in August 2003, the amount of carbon gained by *T. princeps* calculated by its color change during a short rain event is equal to that consumed by respiration under similar dry conditions after ca. 69 d, indicating that even such short rain events may allow survival in areas where drought occurs regularly.

Gas exchange data in this study are expressed on a unit area basis of the moss mat in order to relate MossCam images to carbon uptake in a natural setting where the moss canopy and associated soil remained intact. For comparison to values in the literature, using a dry weight to area ratio of 229.4 results in a maximal instantaneous net CO<sub>2</sub> uptake

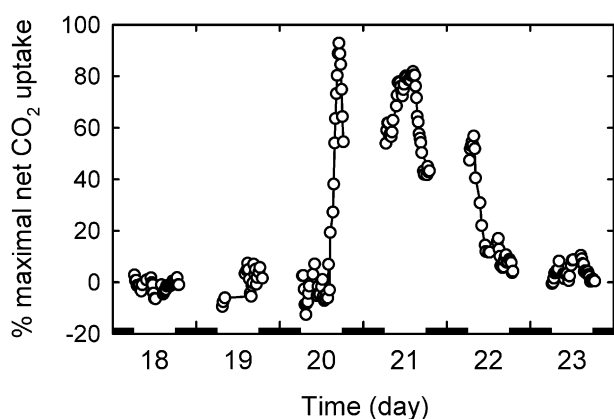
rate for *T. princeps* in the laboratory of  $24 \mu\text{mol kg}^{-1} \text{s}^{-1}$ , which is ca. 60% higher than values reported for *T. ruralis* when the thallus has been separated from the canopy and basal soil (Tuba et al. 1998a; Zotz and Rottenberger 2001). Photosynthesis tends to be maximized in plant canopies compared to individual leaves even though self-shading occurs (Friend 2001; Anten 2002), which may partially explain this difference. Additionally, the high respiration rates observed in this study compared to values in the literature are partly the result of the soil component being included in gas exchange measurements. For example, after removing the green portion of moss thalli from a sample, the respiration rate of the remaining sample, including brown basal portions of *T. princeps* and soil, was ca. 58% of that of the original sample.

Numerous studies have examined plant canopy and leaf spectral reflectance, although usually with hyperspectral techniques. Several remote sensing methods that do not rely on reflectivity in the infrared, however, may be adaptable to use with off-the-shelf digital cameras. The plant pigment ratio is composed of a 550-nm waveband and a 450-nm waveband close to carotenoid absorption and has a robust correlation to leaf chlorophyll and nitrogen concentration (PPR; Metternicht 2003; Wang et al. 2004). The modified chlorophyll absorption in reflectance index is the amount of the chlorophyll absorption at 670 nm relative to the reflectance at 550 and 700 nm (Daughtry et al. 2000) and relates primarily to chlorophyll concentrations. Unfortunately, such narrow bands of wavelengths may be difficult to reconstruct from the RGB color-encoded images that are produced by standard digital cameras.

Of more adaptability to visible light photography is the study by Gamon and Surfus (1999) that examined the RED : GREEN ratio, where RED referred to a broad band of wavelengths (600–699 nm) and GREEN to a broad green band (500–599 nm). They compared this reflectance index with extracted pigment levels and found species-specific relationships that were influenced by leaf structural properties, and they



**Fig. 5** Percentage of maximal net CO<sub>2</sub> uptake related to the green : red ratio calculated from images taken in the laboratory simultaneously with gas exchange. Percent net CO<sub>2</sub> uptake was divided into the six categories: 100% to 75% of maximal, 75% to 50%, 50% to 25%, 25% to 0%, 0% to -25%, and -25% to -50%. Color data were averaged within those categories. Data are mean  $\pm$  SE;  $n = 5$ .



**Fig. 6** Predicted net CO<sub>2</sub> uptake for *Tortula princeps* at the James Reserve before and after a rain event based on the green : red ratio of images captured between August 18 and August 23, 2003, by the MossCam.

concluded that there was a need for empirical calibration when using reflectance indices. Indeed, in the current study for *T. princeps*, chlorophyll and other pigment concentrations are assumed to remain unchanged over the short-term drying cycles imposed in the laboratory, as reported for *T. ruralis* (Tuba et al. 1998b), and so reflectance and color changes are instead necessarily coupled with the structural changes that occur during drying (Hamerlynck et al. 2000; Oliver et al. 2000).

The color index of green : red ratio (Adamsen et al. 1999) chosen for this study provided the best signal of color change in the field compared to several other methods investigated. Total reflectivity was not considered a good index because dead samples also increased in reflectivity upon hydration. The subtraction of images, shifts in peak frequencies of the RGB channels, as well as color conversions from RGB to hue, saturation, and luminance (HSL), hue, saturation, and brightness (HSB), and L\*a\*b color spaces (CIE 1976; Ohno 2000) all produced strong signals for comparison to gas exchange but had weaker final correlations. The HSB and L\*a\*b color spaces are nonlinear deformations of the RGB color space that produced noncontinuous data and so were particularly difficult to interpret, although they have been used successfully in other color comparison studies (Graeff et al. 2001; Karcher and Richardson 2003).

Positive net CO<sub>2</sub> uptake was linearly related to the green : red ratio for *T. princeps* in the laboratory. The color ratio was less good at differentiating low positive net CO<sub>2</sub> uptake rates from negative net CO<sub>2</sub> uptake rates, most likely because the samples of *T. princeps* did not dry uniformly. Higher-resolution images may increase this correlation during lower rates of photosynthesis, although the lossy compression algorithm used to reduce file size of field images before transmitting images over the Internet (JPEG; ITU 1992) may reduce the information that may be extracted from such images (Paola and Schowengerdt 1995).

Ambient light conditions affected the results from analyses of field images because of (1) the limited dynamic range of

the camera used and (2) the spectral changes in ambient light throughout the day. Field images of *T. princeps* that contained large areas of direct solar irradiance were discarded because pixel values in those areas were saturated, and thus color information was lost. Although methods exist for reconstructing saturated pixel values (Zhang and Brainard 2004), they were not attempted in this analysis. The green : red ratio of images also changed slightly and regularly with time of day when changes in the color of *T. princeps* were not expected (after 2 wk of drought); these were compensated for by a time-dependent normalization of pixel values to the average at noon. The natural spectra of forest light have recently received some attention (Chiao et al. 2000), and compensation for the effects of changes in the quality of light through the day on digital images (Marchant and Onyango 2002; Hernández-Andrés et al. 2004) holds promise for further work in the field. The use of standard artificial light sources while collecting images in the field has also been suggested in order to compensate for the variation in the quality and quantity of natural sunlight (Karcher and Richardson 2003), although care would have to be taken to avoid photomorphogenic and photoperiodic responses in natural settings.

Plant phenology measurements provide an important means of understanding the relationship between periodic phenomena, such as leaf flush or flowering, and microenvironmental and climatic conditions. Phenology relates strongly to primary productivity and the energy that enters into ecological food webs, and thus it is vital in understanding ecosystem function and the effects of climate and climate change (Badeck et al. 2004). The ability to remotely observe leaf flush, branch elongation, flowering, and fruit load while simultaneously collecting micrometeorological data may revolutionize the field of plant phenology. Even with the low-resolution camera on the MossCam, for example, reproductive structures (sporophytes) are visible, and their numbers could be automatically quantified, gaining insight to the annual reproductive success of this moss (Stark et al. 2000; Stark 2002). Ongoing studies at the James Reserve using mobile, robotic systems have indicated that other measurements on vascular plants, such as leaf number and total leaf area of *Rhododendron occidentale*, are tightly correlated with color information captured by visible-light digital cameras (data not shown).

A digital analysis of globally available ecological images is now possible with the large number of biologically related Internet-based cameras that are online. For instance, ca. 1,800 sites were encountered when “forest webcam” was searched for using a popular Internet search tool (Google, Mountain View, CA). More sophisticated image processing techniques, however, will be required when examining remote images where quality of image may not be controlled, such as polynomial modeling (Hong et al. 2001), principle components analysis (Tzeng and Berns 2005), and the spectral recovery from color data (Sharma and Wang 2002). For more controlled investigations, the use of calibrated color charts within images (e.g., Munsell Digital ColorChecker SG, GretagMacbeth, New Windsor, NY) or spectral calibration of the camera itself (Martínez-Verdú et al. 2002) have direct application to improving the quality of data collected from visible-light digital cameras used for ecological applications.



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### Literature Cited

- Adamsen FJ, TA Coffelt, JM Nelson, EM Barnes, RC Rice 2000 Method for using images from a color digital camera to estimate flower number. *Crop Sci* 40:704–709.
- Adamsen FJ, PJ Pinter, EM Barnes, RL LaMorte, GW Wall, SW Leavitt, BA Kimball 1999 Measuring wheat senescence with a digital camera. *Crop Sci* 39:719–724.
- Anderson LE, HA Crum, WR Buck 1990 List of the mosses of North America north of Mexico. *Bryologist* 93:448–499.
- Anten NPR 2002 Evolutionarily stable leaf area production in plant populations. *J Theor Biol* 217:15–32.
- Badeck F-W, A Bondeau, K Böttcher, D Doktor, W Lucht, J Schaber, S Sitch 2004 Responses of spring phenology to climate change. *New Phytol* 162:295–309.
- Blackmer T, JS Schepers, GE Varvel 1994 Light reflectance compared with other nitrogen stress measurements in corn leaves. *Agron J* 86: 934–938.
- Chiao C-C, D Osorio, M Vorobyev, TW Cronin 2000 Characterization of natural illuminants in forests and the use of digital video data to reconstruct illuminant spectra. *J Opt Soc Am* 17:1713–1721.
- CIE (Commission Internationale de l'Eclairage) 1986 Colorimetry. 2nd ed. CIE Publication 15.2. Central Bureau of the Commission Internationale de l'Eclairage, Vienna. 83 pp.
- Daughtry CST, CL Walthall, MS Kim, E Brown de Colstoun, JE McMurtry 2000 Estimating corn leaf chlorophyll concentration from leaf and canopy reflectance. *Remote Sens Environ* 74: 229–239.
- Dilks TJK, MCF Proctor 1979 Photosynthesis, respiration and water content in bryophytes. *New Phytol* 82:97–114.
- Dymond JR, CM Trotter 1997 Directional reflectance of vegetation measured by a calibrated digital camera. *Appl Opt* 36:4314–4319.
- Friend AD 2001 Modelling canopy CO<sub>2</sub> fluxes: are “big-leaf” simplifications justified? *Glob Ecol Biogeogr* 10:603–619.
- Gamon JA, JS Surfus 1999 Assessing leaf pigment content and activity with a reflectometer. *New Phytol* 143:105–117.
- Gitelson AA, Y Gritz, MN Merzlyak 2003 Relationships between leaf chlorophyll content and spectral reflectance and algorithms for non-destructive chlorophyll assessment in higher plant leaves. *J Plant Physiol* 160:271–282.
- Graeff S, D Steffens, S Schubert 2001 Use of reflectance measurements for the early detection of N, P, Mg, and Fe deficiencies in *Zea mays* L. *J Plant Nutr Soil Sci* 164:445–450.
- Hafsi M, W Mechmeche, L Bouamama, A Djekoune, M Zaharieva, P Monneveux 2000 Flag leaf senescence, as evaluated by numerical image analysis, and its relationship with yield under drought in durum wheat. *J Agron Crop Sci* 185:275–280.
- Hamerlynck EP, Z Tuba, Z Csintalan, Z Nagy, G Henegry, D Goodin 2000 Diurnal variation in photochemical dynamics and surface reflectance of the desiccation-tolerant moss, *Tortula ruralis*. *Plant Ecol* 151:55–63.
- Hernández-Andrés J, JL Nieves, EM Valero, J Romero 2004 Spectral-daylight recovery by use of only a few sensors. *J Opt Soc Am* 21:13–23.
- Hong GW, MR Luo, PA Rhodes 2001 A study of digital camera colorimetric characterization based on polynomial modeling. *Color Res Appl* 26:76–84.
- ITU (International Telecommunication Union) 1992 Information technology: digital compression and coding of continuous-tone still images: requirements and guidelines. Recommendation T.81. International Telecommunication Union, Genève. 186 pp.
- Jia L, A Buerkert, X Chen, V Roemheld, F Zhang 2004a Low-altitude aerial photography for optimum N fertilization of winter wheat on the North China Plain. *Field Crops Res* 89:389–395.
- Jia L, X Chen, F Zhang, A Buerkert, V Roemheld 2004b Use of digital camera to assess nitrogen status of winter wheat in the northern China plain. *J Plant Nutr* 27:441–450.
- Karcher DE, MD Richardson 2003 Quantifying turfgrass color using digital image analysis. *Crop Sci* 43:943–951.
- Leblanc SG, JM Chen, R Fernandes, DW Deering, A Conley 2005 Methodology comparison for canopy structure parameters extraction from digital hemispherical photography in boreal forests. *Agric For Meteorol* 129:187–207.
- Lukina E, M Stone, W Raun 1999 Estimating vegetation coverage in wheat using digital images. *J Plant Nutr* 22:341–350.
- Marchant JA, CM Onyango 2002 Spectral invariance under daylight illumination changes. *J Opt Soc Am* 19:840–848.
- Martínez-Verdú F, J Pujol, P Capilla 2002 Calculation of the color matching functions of digital cameras from their complete spectral sensitivities. *J Imag Sci Technol* 46:15–25.
- Metternicht G 2003 Vegetation indices derived from high-resolution airborne videography for precision crop management. *Int J Remote Sens* 24:2855–2877.
- Ohno Y 2000 Fundamentals for color measurements. Society for Imaging Science and Technology's NIP16: International Conference on Digital Printing Technologies, pp. 540–545. 6 pp.
- Oliver MJ, SE Dowd, J Zaragoza, SA Mauget, PR Payton 2004 The rehydration transcriptome of the desiccation-tolerant bryophyte *Tortula ruralis*: transcript classification and analysis. *BMC Genomics* 5, art. 89.
- Oliver MJ, J Velten, AJ Wood 2000 Bryophytes as experimental models for the study of environmental stress tolerance: *Tortula ruralis* and desiccation-tolerance in mosses. *Plant Ecol* 151:73–84.
- Paola JD, RA Schowengerdt 1995 The effect of lossy image compression on image classification. Pages 118–120 in IEEE Geoscience and Remote Sensing Society and International Union of Radio Science. 1995 International Geoscience and Remote Sensing Symposium: quantitative remote sensing for science and applications, Congress Center, Firenze, Italy, July 10–14. Vol 1. Institute of Electrical and Electronics Engineers, New York.
- Proctor MCF 2000 The bryophyte paradox: tolerance of desiccation, evasion of drought. *Plant Ecol* 151:41–49.
- Proctor MCF, N Smirnov 2000 Rapid recovery of photosystems on rewetting desiccation-tolerant mosses: chlorophyll fluorescence and inhibitor experiments. *J Exp Bot* 51:1695–1704.
- Purcell LC 2000 Soybean canopy coverage and light interception measurements using digital imagery. *Crop Sci* 40:834–837.
- Richardson AD, GP Berlyn, TG Gregoire 2001 Spectral reflectance of *Picea rubens* (Pinaceae) and *Abies balsamea* (Pinaceae) needles along an elevational gradient, Mt. Moosilauke, New Hampshire, USA. *Am J Bot* 88:667–676.
- Sharma G, S Wang 2002 Spectrum recovery from colorimetric data for color reproductions. Pages 8–14 in R Eschbach, GG Marcu, eds. Proceedings of SPIE. Vol 4663. Color imaging: device-independent color, color hardcopy, and applications VII. SPIE, Bellingham, WA.

- Stark LR 2002 Phenology and its repercussions on the reproductive ecology of mosses. *Bryologist* 105:204–218.
- Stark LR, BD Mishler, DN McLetchie 2000 The cost of realized sexual reproduction: assessing patterns of reproductive allocation and sporophyte abortion in a desert moss. *Am J Bot* 87:1599–1608.
- Tuba Z, Z Csintalan, K Szente, Z Nagy, J Grace 1998a Carbon gains by desiccation-tolerant plants at elevated CO<sub>2</sub>. *Funct Ecol* 12:39–44.
- Tuba Z, CF Protor, Z Csintalan 1998b Ecophysiological responses of homoiochlorophyllous and poikilochlorophyllous desiccation tolerant plants: a comparison and an ecological perspective. *Plant Growth Regul* 24:211–217.
- Tzeng D-Y, RS Berns 2005 A review of principal component analysis and its applications to color technology. *Color Res Appl* 30:84–98.
- Velten J, MJ Oliver 2001 Tr288, a rehydrin with a dehydrin twist. *Plant Mol Biol* 45:713–722.
- Vogelmann TC 1993 Plant tissue optics. *Annu Rev Plant Physiol Plant Mol Biol* 44:231–251.
- Wang ZJ, JH Wang, LY Liu, WJ Huang, CJ Zhao, CZ Wang 2004 Prediction of grain protein content in winter wheat (*Triticum aestivum* L.) using plant pigment ratio (PPR). *Field Crops Res* 90: 311–321.
- Zhang Z, DH Brainard 2004 Estimation of saturated pixel values in digital color imaging. *J Opt Soc Am* 21:2301–2310.
- Zotz G, S Rottenberger 2001 Seasonal changes in diel CO<sub>2</sub> exchange of three central European moss species: a one-year field study. *Plant Biol* 3:661–669.