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Does the removal of non-photosynthetic sections lead to a down-regulation of photosynthesis in mosses? A first experiment

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ABSTRACT
When measuring photosynthesis in mosses, the non-photosynthetic (brown) sections are usually removed and only the green sections are measured. However, how this pretreatment affects photosynthesis rates is unclear. Therefore, we studied the effect of removing the non-photosynthetic sections in three moss species with distinct morphological and stem-anatomical structures, comparing net CO_2 assimilation rates (AN) of detached green and brown sections to those of intact shoots. Right after separation, the summed AN of the separated sections was significantly lower than those of the intact shoots for *Pogonatum nudiusculum* Mitt. and *Pleuroziopsis ruthenica* (Weinm.) Kindb. ex E. Britton, while no significant difference was found for *Actinothuidium hookeri* (Mitt.) Broth. However, AN recovered within a day, and the progressive reduction of AN expected if carbon-sink removal was an important mechanism was not observed. Our study indicates that removal of non-photosynthetic sections results in an underestimation of the photosynthetic capacity of the

KEY WORDS
Actinothuidium hookeri, *Pleuroziopsis ruthenica*, *Pogonatum nudiusculum*, gas-exchange, hydraulic integrity, sample preparation, sink regulation.

green moss sections, but only for the species with relatively complex internal transport structures, and only immediately after the separation. The fast and transient response suggests a mechanism via an electrical signal induced by wounding or reduced hydraulic integrity, rather than through a reduced carbon-sink strength. More comprehensive investigations on signaling and other mechanisms regulating moss photosynthesis will contribute to more accurate measurement methods as well as a deeper understanding of moss ecophysiology and the contribution of mosses to carbon fluxes in terrestrial ecosystems.

MOTS CLÉS
Actinothuidium hookeri, *Pleuroziopsis ruthenica*, *Pogonatum nudiusculum*, échanges gazeux, intégrité hydrique, préparation des échantillons, régulation des puits.

RÉSUMÉ
L'élimination des sections non photosynthétiques entraîne-t-elle une régulation négative de la photosynthèse chez les mousses ? Une première expérience.
Lors de la mesure de la photosynthèse chez les mousses, les sections non photosynthétiques (brunes) sont généralement retirées et seules les sections vertes sont mesurées. Cependant, la façon dont ce prétraitement affecte les taux de photosynthèse n'est pas claire. Nous avons donc étudié l'effet de l'élimination des sections non photosynthétiques chez trois espèces de mousses présentant des structures morphologiques et anatomiques de tige distinctes, en comparant les taux d'assimilation nets de CO_2 (AN) des sections vertes et brunes détachées à ceux des pousses intactes. Juste après la séparation, la somme des taux d'assimilation de CO_2 des sections séparées était significativement inférieure à celle des pousses intactes pour *Pogonatum nudiusculum* Mitt. et *Pleuroziopsis ruthenica* (Weinm.) Kindb. ex E. Britton, alors qu'aucune différence significative n'a été trouvée pour *Actinothuidium hookeri* (Mitt.) Broth. Cependant, l'AN s'est rétabli en un jour, et la réduction progressive de l'AN attendue si l'élimination des puits de carbone était un mécanisme important n'a pas été observée. Notre étude indique que l'élimination des sections non photosynthétiques entraîne une sous-estimation de la capacité photosynthétique des sections de mousses vertes, mais seulement pour les espèces ayant des structures de transport interne relativement complexes, et seulement immédiatement après la séparation. La réponse rapide et transitoire suggère un mécanisme via un signal électrique induit par une blessure ou une intégrité hydraulique réduite, plutôt que par une force de puits de carbone réduite. Des études plus complètes sur la signalisation et d'autres mécanismes régulant la photosynthèse des mousses contribueront à l'élaboration de méthodes de mesure plus précises ainsi qu'à une meilleure compréhension de l'écophysiologie des mousses et de leur contribution aux flux de carbone dans les écosystèmes terrestres.

INTRODUCTION

Mosses have much lower mass-based photosynthetic rates than most vascular plant leaves (Waite & Sack 2010; Glime 2017; Wang *et al.* 2017). Several reasons have been proposed to explain this, including CO_2 diffusion limitation at the gas-exchange surface (Green & Lange 1995; Carriquí *et al.* 2019), low nutrient contents (Wang et al. 2017), but also the small carbohydrate sink activity and pool size (Wang *et al.* 2015). A more methodological reason for low observed photosynthetic rates in mosses may be that gas-exchange studies in these small plants generally have to be conducted at the individual or population scale, rather than the leaf scale. Thus, the measured photosynthetic rates of mosses include the respiration of branches and stems (though many mosses have green stems and branches that can carry out photosynthesis). They could even include respiration of the non-photosynthetic sections (brown stem sections and rhizoids), but these sections are often removed and only the green section is measured. This practice improves the accuracy and comparability of photosynthesis measurements (for comparisons among moss species or between mosses and other plants), since the ratios of photosynthetic to non-photosynthetic sections of moss shoots vary a lot among species and habitats. However, potential side-effects of this pretreatment are unknown.

For measuring photosynthesis in vascular plants, cutting the photosynthetic section (normally the leaves) from the rest of the plant is usually avoided, because the reduced hydraulic connection would result in stomatal limitation (Gauthier & Jacobs 2018; Meng *et al.* 2019). Moreover, the removal of active sinks and the consequent accumulation of carbohydrates in leaves can suppress leaf photosynthesis (Herold 1980; Jang & Sheen 1994; Paul & Foyer 2001; Ainsworth & Bush 2011; Salmon *et al.* 2020). Indeed, the activity of carbohydrate sinks, like growing tissue and respiration, has been proposed to control photosynthesis rather than the other way around, though this is an ongoing debate (Fatichi *et al.* 2014; Körner 2015; Rodrigues *et al.* 2019; Avila *et al.* 2020). Although Marschall (2010) has reported also for mosses that photosynthesis can be controlled by carbohydrate pools (exogenous sugars addition down-regulates the photosynthetic activities), it is still unclear how removing non-photosynthetic moss tissue, a potential carbon sink, affects photosynthetic rates.

In contrast to vascular plants, mosses can take up water and CO_2 through their entire surface and their photosynthesis does not depend on hydraulic connections (though they can

help maintain activity in dry air in some structurally complex species, such as Polytrichaceae, Brodribb *et al.* (2020)) or stomatal opening (Green & Lange 1995; Proctor 2001). Also, most major active carbon sinks of mosses, such as the growing apices, leaves, and sporophytes, are all located on the photosynthetic sections of the moss shoots. The sink activity of the non-photosynthetic sections of moss shoots is supposed to be weak: they do not have tuberous organs to store large amounts of nutrients and assimilates, and leaves on the older moss sections generally turn to brown and black and decay gradually, while recycling their nutrients to the young growing sections (Wells & Brown 1996; Lang *et al.* 2014). Therefore, theoretically, the removal of non-photosynthetic section is unlikely to down-regulate the photosynthesis of mosses. However, in many species the older non-photosynthetic moss shoot sections play important roles in supporting the photosynthetic sections mechanically, in allowing upward water and nutrient transportation (Ayres *et al.* 2006), and sometimes by sprouting new shoots (Maslova *et al.* 2015). It is thus not surprising that experiments using ^{14}C labeling have shown the translocation of carbohydrates from photosynthetic to brown moss sections for *Hylocomium splendens* (Hedw.) Schimp., *Pleurozium schreberi* (Brid.) Mitt., and species of *Polytrichum* Hedw. and *Sphagnum* L. (Collins & Oechel 1974; Reinhart & Thomas 1981; Skre *et al.* 1983).

Although mosses are simply structured compared to vascular plants, high morphological and anatomical variation exists among species (Mägdefrau 1982). Moss species with generally simple stems are exohydric, i.e., they transport water and nutrients externally or through their cortex with less specialized parenchyma cells (Alpert 1989; Sokolowska *et al.* 2017). In contrast, some erect species, such as those from the Polytrichaceae family, have a central strand with specialized conducting cells for water and assimilates, the hydroids and leptoids (Goffinet *et al.* 2008). Undoubtedly, the internal transportation capacities of species with such complex structures are much higher than those with simple stem anatomies (Jiang *et al.* 2012; Brodribb et al. 2020). Such species are therefore either endohydric (with mostly internal water conductance) or mixohydric (using a combination of internal and external conductance). Independent of the mode of water conductance, mosses can take up water directly through most of their surface. Therefore, despite the importance of endohydric transport in some species for maintaining photosynthesis in dry air (Stanton *et al.* 2014; Brodribb *et al.* 2020), for gas-exchange measurements in mosses cutting off the connection to the soil has been assumed uncritical if samples are maintained at the same water contents. Still, the importance of hydraulic integrity for gas exchange measurements in anatomically complex species merits stronger attention. Currently, the importance of stem anatomy for the responses to the removal of non-photosynthetic sections, as hydraulic support and as carbohydrate sink, is unknown.

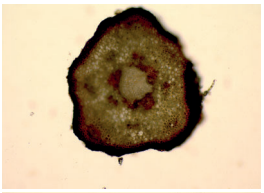
To test the effects of removing the non-photosynthetic section on the photosynthesis of mosses, we determined and compared the net CO_2 assimilation rates (AN) of the intact shoots, detached green photosynthetic and brown non-photosynthetic sections of three moss species with distinct morphological and stem anatomical structures. We aimed to investigate: 1) whether the sum of AN of green and brown sections is different from that of intact shoots; 2) how the AN of green sections and the sum of the separated sections change from directly after separation to eight days later; and 3) whether species with different morphological and stem anatomical structures have different responses to the separation of green and brown shoot sections. We hypothesized that 1) photosynthesis is down-regulated after removing the carbon sink represented by the brown-section, resulting in a higher AN in intact shoots than in the separated brown and green shoot sections combined; 2) carbohydrates accumulate in the green sections with time due to sink removal, resulting in a progressive decrease in AN in separated shoots, (or alternatively: if caused by a wound-induced electrical signal, AN would be reduced immediately and transiently); and 3) species with more internal transport capacity should show stronger differences between intact and separated shoots. If our hypotheses are supported, this has important implications for our understanding of the controls on photosynthesis in mosses, as it would indicate that even in these poikilohydric organisms, photosynthesis depends on the integrity of the whole plant.

MATERIAL AND METHODS

Study site, focal species, and sampling

Three common mountain moss species (*Actinothuidium hookeri* (Mitt.) Broth., *Pleuroziopsis ruthenica* (Weinm.) Kindb. ex E. Britton, and *Pogonatum nudiusculum* Mitt.) were sampled from a broadleaf-coniferous forest (elevation 3014 m, MAT 4.9°C, MAP 1626 mm) on the eastern slope of Gongga Mountain (29°33'-29°35'N, 101°58'-102°03'E) in Sichuan province, China in August 2017 (Wang *et al.* 2019). *Actinothuidium hookeri* (Thuidiaceae) is endemic to eastern Asia, with shoots up to 15 cm long, yellowish-green above, light brown at the base, growing as wefts in large interwoven patches (Wu *et al.* 2002). New stems can emerge from the older shoot sections (Fig. 1A; Appendix 1) (Liu *et al.* 2015, 2020). *Pleuroziopsis ruthenica* (Pleuroziopsidaceae) is large, with creeping primary rhizome-like stems up to 8 cm giving rise to several erect secondary stems up to 7 cm long (Fig. 1B) (Maslova *et al.* 2015). The life form is dendroid, the colour is yellowish-green, brownish-green when old, slightly glossy (Wu *et al.* 2001). *Pogonatum nudiusculum* (Polytrichaceae) is medium-sized, somewhat rigid, gregarious. It grows as a turf with stems single, about 3 cm long (Wu *et al.* 2005) (Fig. 1C). New orthotropic shoots are formed from the previous-year shoots, which turn to a horizontal position with age. These three moss species have distinct stem anatomical structures (Table 1; Appendices 2, 3). The central strand of *A. hookeri* only accounts for a small fraction of the stem cross-section, while *Pleuroziopsis ruthenica* and *Pogonatum nudiusculum* have obvious central strands consisting of hydroids and leptoids.

TABLE 1. — Information about the three moss species collected on the eastern slope of Gongga Mountain and used to test the effect of removing brown moss sections on photosynthesis rates. Larger stem cross-sections with anatomical labels can be found in Appendix 2. The category of life forms followed Mägdefrau (1982).

Species	Family	Life form	Substrate	Photo	Stem cross-section
Actinothuidium hookeri (Mitt.) Broth.	Thuidiaceae	Weft	Soil		
Pleuroziopsis ruthenica (Weinm.) Kindb. ex E. Britton	Pleuroziopsidaceae	Dendroid	Soil		
Pogonatum nudiusculum Mitt.	Polytrichaceae	Turf	Soil		

Four samples (replicates, more than 20 shoots for each sample) of each species were obtained from separated patches (any two of the patches were more than 20 m apart). The mosses were collected with the underlying substrate, sealed in plastic bags, and brought to the laboratory. All of the samples were kept in a cool and shady environment (outside of the building, with *c.* 50% sky exposure but out of direct sunlight, *c.* 100 µmol photons m^{-2} s^{-1} photosynthetically active radiation (PAR) at noon, and a daily air temperature range of about 16-24°C) for 5 days before the gas-exchange measurements. The samples were sprayed with water twice a day. The specimens were deposited at the bryophyte herbarium of Shanghai Normal University (SHTU).

GAS-EXCHANGE MEASUREMENTS

Before the measurements, the samples were washed with distilled water to clean the dust and mud. Dead tissues (decayed, can be removed easily) were removed and only living tissues (including both green and brown sections) were collected. For each replicate of each species, several similar-sized shoots (eight for *A. hookeri* and *Pogonatum nudiusculum*, and six for *Pleuroziopsis ruthenica*) were selected as the final sample.

CO_2 gas-exchange was measured using a Li-Cor 6400-22 L with a Lighted Conifer Chamber (Li-Cor, Inc., Lincoln, NE, United States) at a temporary laboratory near the sampling site. We first measured *A. hookeri* and *Pogonatum nudiusculum* during 8 days and then repeated the experiment with *Pleuroziopsis ruthenica.* Following 30 min of light induction of moist samples under 150 µmol photons m^{-2} s^{-1} PAR, the intact shoots were rewetted in distilled water for 1 min, and the residual water on the tissue surface was carefully removed with a paper towel. The samples were weighed, arranged, and put in the chamber but avoiding overlap between shoots. The measurement chamber conditions were 25°C block temperature, 400 ppm CO_2 concentration, 400 µmol m^{-2} s^{-1} PAR light intensity (close to the mean saturating light intensity estimated from photosynthetic light-response curves for these three species (Wang *et al.* unpublished data)), 500 µmol s^{-1} flow rate and the relative humidity following ambient conditions (the initial relative humidity was around 50%). Each measurement lasted for about 2-3 min for AN to reach a steady state. After this initial measurement, the samples were reweighed to make sure that the water content of the sample was similar before and after the measurement. The sample was then cut into green and brown sections (Fig. 1). The separated samples were rewetted and before each measurement, the fresh weight was determined to ensure that the water content was the same as for the intact shoots. The AN of the two sections were measured (in less than five minutes after cutting) and then the samples were kept in a cool and shady environment again and sprayed with water as before. The measurements were repeated after 6 h, 1 d, 2 d, 5 d, and 8 d for *A. hookeri* and *Pogonatum nudiusculum*, and just after 6 h, 2 d, and 5 d for *Pleuroziopsis ruthenica* due to the unstable electric power. After the last measurement, the samples were oven-dried at 70°C for 48 h, and the dry weight of green and brown sections was determined.

DATA ANALYSIS

To compare AN before and after separation of the green and brown sections, we calculated the sum of AN, expressed as nmol CO_2 uptake per second per sample (not per mass or

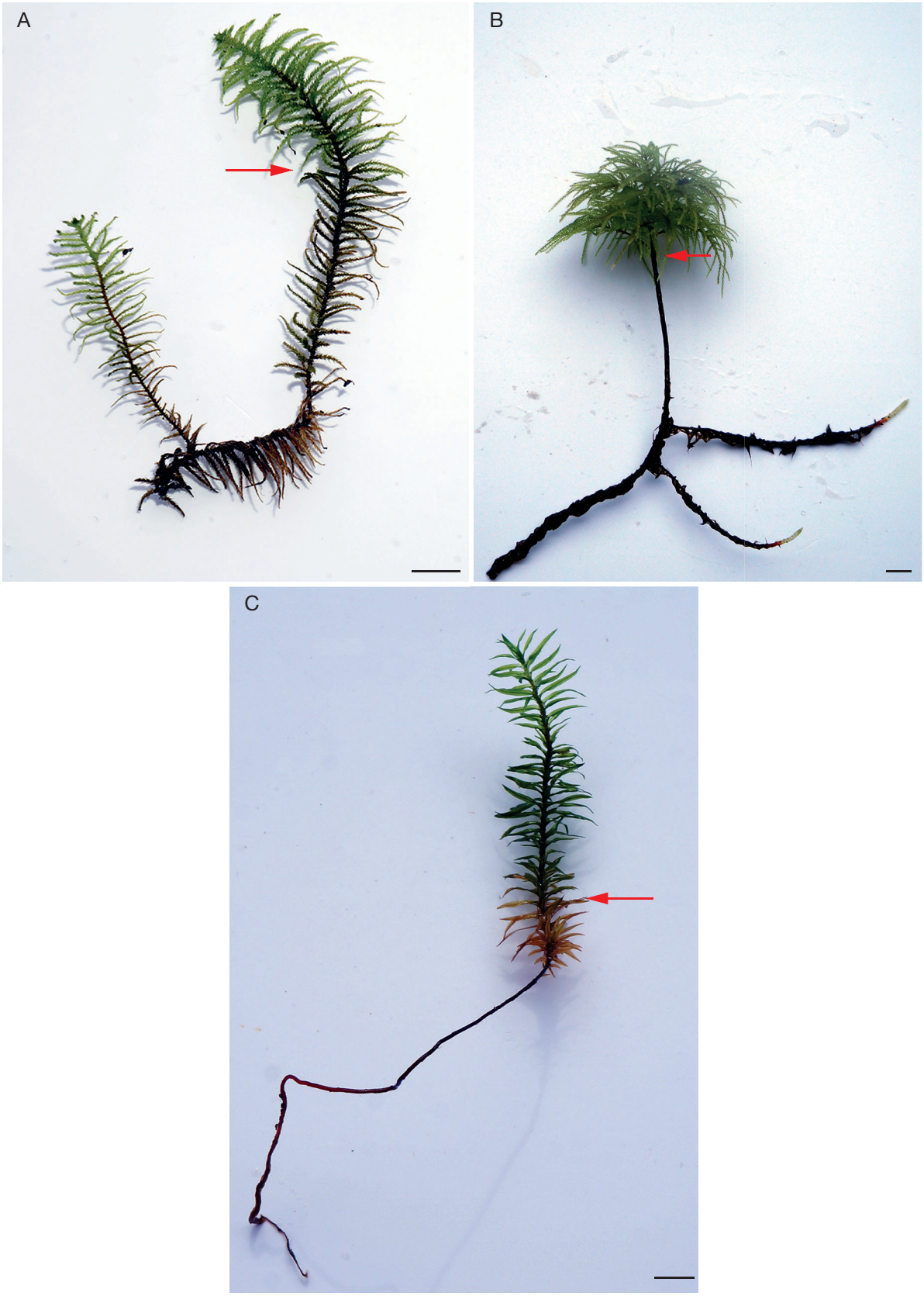


Fig. 1. — Shoots of the three moss species used for the "brown-section-removal" experiment, collected on the eastern slope of Gongga Mountain. **A**, *Actinothuidium hookeri* (Mitt.) Broth.; **B**, *Pleuroziopsis ruthenica* (Weinm.) Kindb. ex E. Britton; **C**, *Pogonatum nudiusculum* Mitt. The **red arrows** indicate the points where the brown and green sections were separated.

area), of the separated sections and tested the difference with AN of the intact shoots using a paired-samples t-test for each species. The sample-based AN gives a more reliable comparison than area- or mass-based AN, because the addition of sample-based AN of green and brown section gives the sample-based AN of the combined sample, whereas mass-based AN cannot be simply added for differently-sized samples. We additionally report the mass-based AN, however, for comparison with published photosynthetic rates. We determined the biomass only at the end of the experiment, after complete drying of the samples, and used this value to calculate the mass-based rates throughout the experiment. New growth was not observed in the samples, and a previous study reported that the *in situ* growth rate of *A. hookeri* was only 0.058 mm d^{-1} during the growing season (Wang *et al.* 2007), but a biomass increase during the experiment cannot be ruled out.

To detect changes in AN through time after separation, mixed-model with sample as the random factor was used to compare the differences in both sample- and mass-based AN of green and brown sections and the sum of these two sections (sample-based) for each species among different measurement time. Statistical analyses were performed using PASW Statistics 20.0 (IBM, Armonk, NY, United States), Microcal Origin 9.0 (Northampton, Massachusetts, United States). Differences were considered significant when $p<0.05$.

RESULTS

Right after the separation, the AN values of the sum of the separated sections were significantly lower than those of the intact shoots for *Pleuroziopsis ruthenica* and *Pogonatum nudiusculum* , while no significant difference was found for *A. hookeri* (Fig. 2A-C). The difference between the mean values of AN of the sum of the freshly separated sections and intact shoots was 4.1 and 1.4 nmol CO_2 s^{-1} per sample, corresponding to about 57 and 27% of AN in the intact shoots, for *Pleuroziopsis ruthenica* and *Pogonatum nudiusculum*, respectively. As compared to AN, the respiration of the brown sections was very low (0.85 and 0.35 nmol CO2 s^{-1}, respectively for these two species), due to their low biomass combined with low respiration rates (Fig. 2E, F). Therefore, it can be assumed that, to a great extent, the lower AN of the sum of the separated sections as compared to those of intact shoots was due to the decreased AN of the green sections, which may have been caused by reduced photosynthesis and/or increased respiration after cutting. The difference disappeared at 6 h for *Pleuroziopsis ruthenica* and *Pogonatum nudiusculum*, while for *A. hookeri* a difference was found after 6 h (27% lower AN in separated shoots than those of the intact shoots) (Fig. 2A), which was due to increased respiration in the brown sections rather than decreased photosynthesis in the green sections (Fig. 2D).

All three moss species showed increasing trends in sample-based AN for green sections and the sum of the separated sections and the mass-based AN of the green sections with time after excision, but there was much variation between samples and most points in time did not differ significantly (Fig. 2). The AN of the brown shoots varied little, with lower (more negative) sample- and mass- based values at 2 d for *Pogonatum nudiusculum* and *A. hookeri*.

DISCUSSION

Net photosynthesis was reduced immediately after separating green and brown moss shoots for two out of the three species, but this effect disappeared within the first six hours after the separation. Our experiment shows that the practice of removing brown moss sections before photosynthesis measurements can lead to an initial underestimation of carbon assimilation rates.

The fast and transient response of AN suggests that it was related to wound-induced electrical signal rather than sink removal. This response was similar to the response of sunflower leaves to the cutting of a primary leaf vein, which caused a decrease in AN (but no simultaneous decrease in stomatal conductance, suggesting that stomatal closure was not the cause) immediately after cutting, with a fast partial recovery in the next five minutes and a complete recovery after about 15 minutes (Hanson *et al.* 2013). This fast response was attributed to an electrical signal induced by the wound or the reduced hydraulic integrity, causing a reduction in mesophyll conductance. Similar electrical signals and cell-level changes in conductance to CO_2 may have caused the decrease in AN also in *Pleuroziopsis ruthenica* and *Pogonatum nudiusculum*, presenting a possible physiological regulation mechanism. Although it has been shown that wound-like cues can result in long-distance electrical signaling in the moss *Physcomitrella patens* (Koselski *et al.* 2020), the role of such signaling in regulating photosynthesis is unknown in bryophytes. In vascular plants, such electrical signals travel mostly via the phloem (Lüttge 2013), the functional equivalent of which in mosses would be the leptoids present in the more endohydric species. The resulting hypothesis that leptoids, or more generally the specialized conducting cells, including also the hydroids, perform a similar role in signal transduction is consistent with our observation that the response only occurred in the two species with complex stem anatomies. If this is a general pattern, this would indicate another example of analogous functions between vascular systems and moss conductive systems.

The fast response makes sink-regulation a less likely explanation for the observed decrease in AN. To our knowledge, the speed of sink-manipulation responses are usually measured at the scale of hours or days. However, it is not clear how much time is required for the photosynthetic tissue to build up a sufficient amount of carbohydrates to regulate photosynthesis. Previous research found that the reduction in net photosynthesis after leaf/branch detachment (under water) can be observed within three minutes for trees (Gauthier & Jacobs 2018), but this is most likely induced by electrical signaling (see above) or due to stomatal regulation rather than to sink

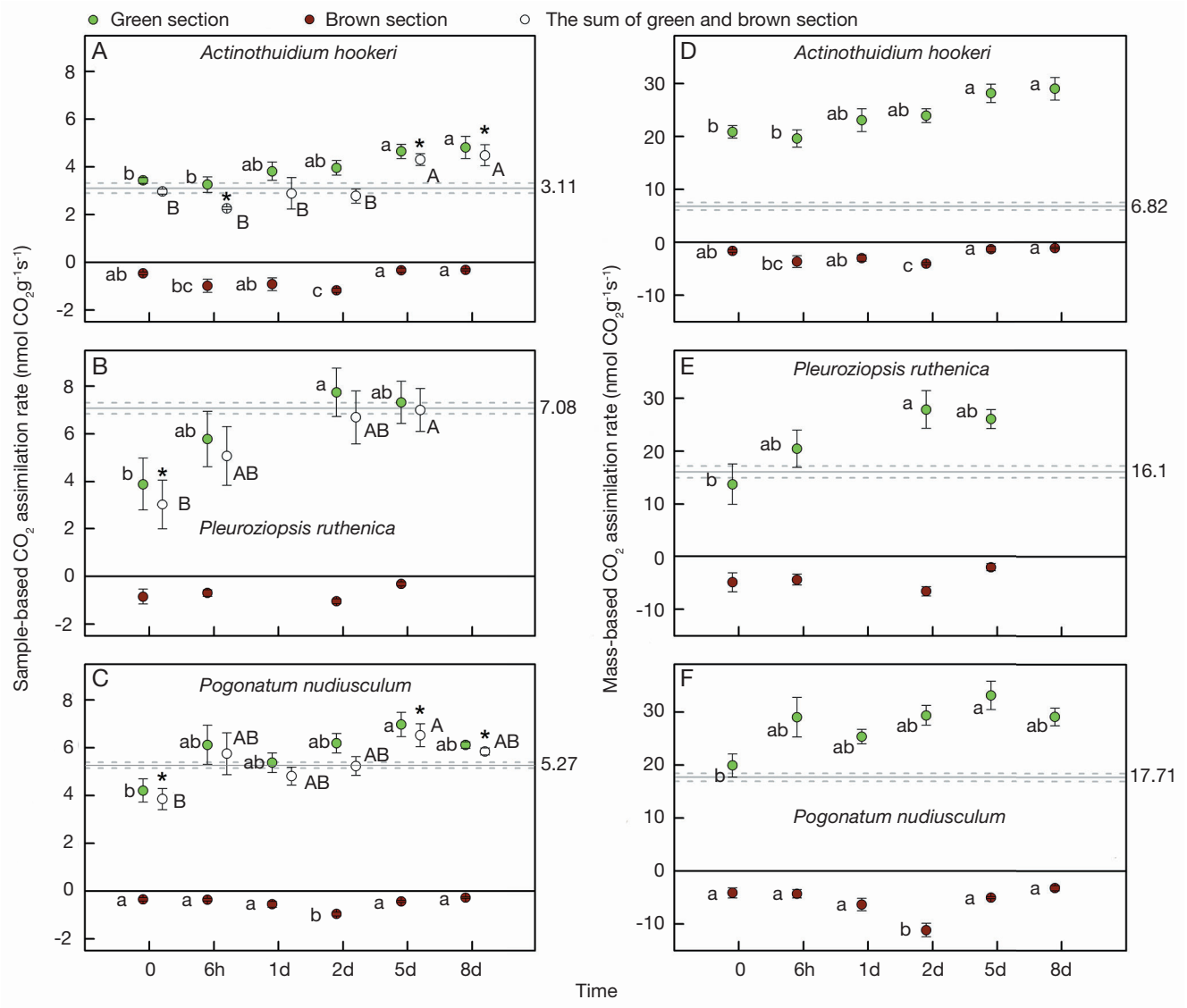


FIG. 2. — Comparisons of the sample- (**A**-**C**) and mass- (**D**-**F**) based CO_2 assimilation rates of green and brown moss sections, and the sum of these two sections (sample-based plots) and the changes through time for three moss species. Shown are the mean values ± standard errors (smaller than the symbol in some cases) of different sections determined at different time points, starting at a few minutes after separation. Sample-based plots are expressed as the CO_2 exchange per sample to allow a direct comparison of intact and separated sections. **Gray solid** and **dashed lines** show the mean values of intact shoots (values shown to the right), ± standard errors. Asterisks indicate significant differences in assimilation rates between intact shoots and the sum of the separated sections (paired t-test, $p<0.05$, $n = 4$). And the capital and lowercase letters indicate significant differences among time points ($p<0.05$).

removal. In mosses, stomatal regulation does not play a role, and in our experiment both the intact and the cut shoots were actively wetted (by immersion in water, i.e., through the entire surface) to maintain a similar water content before and after cutting in our experiment. Therefore, although cutting might result in air bubbles in the hydraulic system (Venturas *et al.* 2015), we had not expected to observe a strong effect of water deficiency on photosynthesis during the measurement. However, cellular changes induced by an electrical signal probably do not depend on the moss water status (in contrast to stomatal responses, the electrical signal did not depend on air humidity in the mentioned experiment with sunflower leaves in Hanson *et al.* (2013)). Electrical signals due to wounding or hydrological responses at the cut are thus again the explanation most consistent with our observations.

The tendency of AN to increase in the days after cutting also contradicts the sink-removal hypothesis. Under this hypothesis we would have expected a build-up of carbohydrates with time, leading to a progressive reduction of photosynthesis. Apart from the initial recovery from the incision, the increasing trends in A for the green sections were probably due to new growth, even if in our samples growth was not discernable with the bare eye. New growth would both provide a new C-sink, potentially boosting photosynthesis, and it would simply increase the biomass, increasing the carbon gain per sample. Future studies should consider monitoring

(non-destructively of course, e.g. through repeated weighing under standardized humidity conditions) the growth of both detached and intact shoots.

In summary, the current study found that the removal of non-photosynthetic sections resulted in a fast lowering of the measured assimilation rate in two moss species with well-developed conductive tissue, but not in a more exohydric species. The fast response and absence of a progressive suppression of photosynthesis contradict the hypothesized role of sink regulation but rather point at a mechanism involving a fast electrical signal induced by the cutting, either as a wound response or due to changes in the water potential at the cut. Additional experiments are clearly needed to better understand these processes and their importance among the bryophytes (liverworts as well as mosses). In any case, the results suggest that photosynthetic rates of green moss sections may be underestimated, at least in some species and for some time after cutting, when separating them from the brown sections for gas-exchange measurements. This needs to be considered when drawing conclusions about moss photosynthetic capacities. The effect appears to be restricted to moss species with relatively complex internal transport structures, although a wider range of species needs to be tested before making this generalization.

Future work should investigate how to diminish or avoid the effects of removing the non-photosynthetic sections on photosynthesis measurements in mosses. If these effects are indeed restricted to endohydric species, they may be avoided by leaving the samples intact and connected to a water source and clipping them into a cuvette as one would with a vascular-plant leaf, though this is practicable only for large and upright mosses. Apart from a methodological solution, further manipulations of carbohydrate sinks and sources as well as hydraulic connectivity and potentially relevant environmental cues (e.g. wounding, light, changes in air or soil water potential) are clearly needed to elucidate whether and under what conditions photosynthesis in mosses is controlled by electrical signaling, carbohydrate feedbacks, and/or hydraulic constraints, and hence how similarly mosses can really function to vascular plants. A deeper understanding of photosynthesis and its measurement in mosses are important not only for our understanding of land-plant evolution and functioning but will also contribute to a better evaluation of their contribution to carbon fluxes in terrestrial ecosystems.

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Author's contributions
ZW and WB designed the work. ZW, CP and YH collected data, ZW performed the data analysis, ZW, MB and SG wrote the manuscript. The authors declare no conflict of interest.

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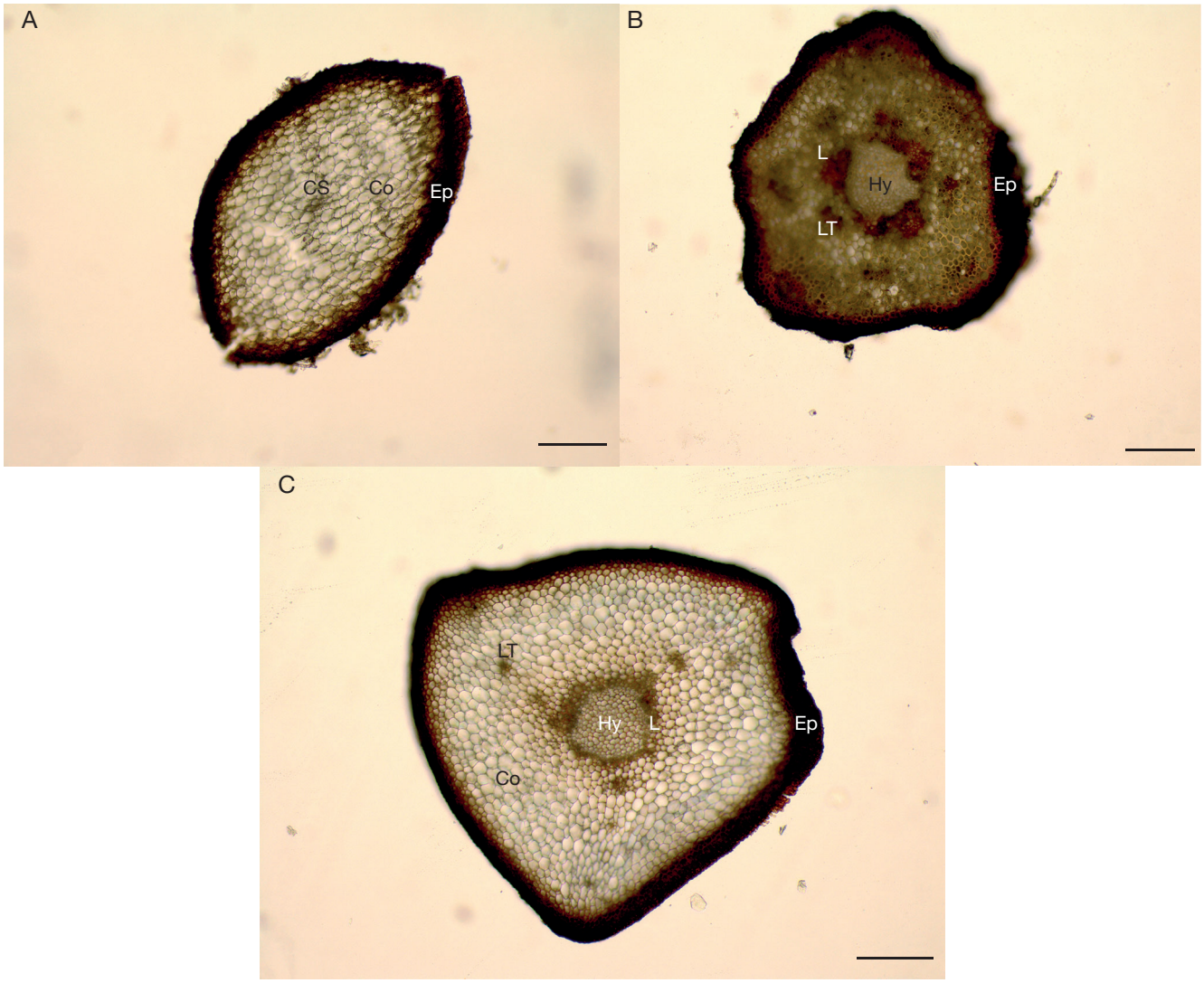
APPENDICES

Appendix 1. — Shoots of *Actinothuidium hookeri* (Mitt.) Broth. collected on the eastern slope of Gongga Mountain. The photos show that the shoots with similar age can emerge from different ends of the older section. The ages of the shoots are recognized according to the "bending of stem" (Liu *et al.* 2020).

Appendix 2. — **A**, *Actinothuidium hookeri* (Mitt.) Broth.; **B**, *Pleuroziopsis ruthenica* (Weinm.) Kindb. ex E. Britton; **C**, *Pogonatum nudiusculum* Mitt. The stem cross-sections of the three mosses collected on the eastern slope of Gongga Mountain, Sichuan province, China. One medium-sized individual from each replicate was selected to observe the stem cross-section. The samples were submerged in a petri dish for 30 minutes and the excess water was removed using a paper towel. The stem cross-section was made at the point where the brown and green sections were separated (Fig. 1) using a dissecting microscope and was photographed using an optical microscope. The cross-section anatomical structures following Maslova *et al*. (2015). Abbreviations: **Co**, cortex; **CS**, central strands; **Ep**, epidermis; **Hy**, hydroids; **L**, leptoids; **LT**, leaf trace.



Appendix 3. — The mean values (Mean ± SE) of anatomical characteristics measured from the stem cross-sections of the three mosses collected on the eastern slope of Gongga Mountain, Sichuan province, China (n = 4 for each species). The area of different parts was measured using Image J. The differences of each parameter among species were compared using one-way ANOVA. No significant differences were found among the species for each parameter ($p \leq 0.05$).

	Total area (μm²)	**Epidermis thickness (μm)**	**Epidermis area (μm²)**	**Epidermis percent (%)**
Actinothuidium hookeri (Mitt.) Broth.	1.61E + 5 ± 3.29E+4	21.04 ± 3.52	3.74E + 4 ± 9.02E + 3	23.14 ± 3.13
Pleuroziopsis ruthenica (Weinm.) Kindb. ex E. Britton	1.63E + 5 ± 8.38E + 3	20.13 ± 2.26	3.19E + 4 ± 4.72E + 3	19.78 ± 2.96
Pogonatum nudiusculum Mitt.	1.21E + 5 ± 2.48E + 4	18.19 ± 0.05	2.42E + 4 ± 3.87E + 3	20.54 ± 1.01
	Cortex area (μm²)	Cortex percent (%)		
Actinothuidium hookeri	1.23E + 5 ± 2.51E + 4	76.56 ± 3.13		
Pleuroziopsis ruthenica	1.15E + 5 ± 1.20E + 4	70.25 ± 4.55		
Pogonatum nudiusculum	8.35E + 4 ± 1.96E + 4	68.28 ± 1.78		