Phytohormones are pivotal signaling compounds in higher plants, in which they exert their roles intracellularly, but are also released for cell-to-cell communication. In unicellular organisms, extracellularly released phytohormones can be involved in chemical crosstalk with other organisms. However, compared to higher plants, hardly any knowledge is available on the roles of phytohormones in green algae. Here, we studied phytohormone composition and extracellular release in aero-terrestrial Trebouxio phyceae. We investigated (a) which phytohormones are produced and if they are released extracellularly, and if extracellular phytohormone levels are (b) affected by environmental stimuli, and (c) differ between lichen-forming and non-lichen-forming species. Three free-living microalgae (Apatococcus lobatus, Chloroidium ellipsoideum, and Myrmecia bisecta) and three lichen-forming microalgae (Asterochloris glomerata, Trebouxia decolorans, and Trebouxia sp.) were studied. Algae were grown on solid media and the following cellular phytohormones were identified by ultra-high-performance liquid chromatography coupled with tandem mass spectrometry (UHPLC-MS/MS): indole-3-acetic acid (IAA), indole-3-butyric acid (IBA), abscisic acid (ABA), gibberellin A4 (GA4), and zeatin (ZT). Furthermore, IAA, IBA, ABA, jasmonic acid (JA), gibberellin A3 (GA3), and GA4 were found to be released extracellularly. IAA and ABA were released by all six species, and IAA was the most concentrated. Phytohormone release was affected by light and water availability, especially IAA in A. glomerata, Trebouxia sp., and C. ellipsoideum. No clear patterns were observed between lichen-forming and non-lichen-forming species. The results are envisaged to contribute valuable baseline information for further studies into the roles of phytohormones in microalgae.

Key index words: chlorophytes; culture; dehydration; extracellular; indole-3-butyric acid; light; phytohormones; stress response

Abbreviations: ABA, abscisic acid; AM, arbuscular mycorrhizae; BBM, Bold’s Basal Medium; BRs, brassinosteroids; CKs, cytokinins; DL, dim light (20 μmol photons · m⁻² · s⁻¹); DL + DH, dehydration under dim light; DM, dry mass; GA3, gibberellin A3; GA4, gibberellin A4; GAs, gibberellins; HL, high light (150 μmol photons · m⁻² · s⁻¹); HL + DH, dehydration under high light; IAA, indole-3-acetic acid; IBA, indole-3-butyric acid; JA, jasmonic acid; JAs, jasmonates; LOD, limit of detection; PTFE, polytetrafluoroethylene (Teflon); SA, salicylic acid; SLs, strigolactones; TM, Trebouxia medium; UHPLC-MS/MS, ultra-high-performance liquid chromatography coupled with tandem mass spectrometry.
liquid chromatography coupled with mass spectrometry; UPW, ultrapure water; ZT, zeatin

Phytohormones, including auxins, abscisic acid (ABA), salicylic acid (SA), jasmonates (JAs), gibberellins (GAs), cytokinins (CKs), ethylene, brassinosteroids (BRs), and strigolactones (SLs), are known to play key roles in development, growth, reproduction, and stress response of higher plants (Santner et al. 2009). The term “phytohormone” was defined by Went and Thimmann (1937) for higher plants, and significant efforts were made to understand their roles in model plants such as *Arabidopsis thaliana* and crops especially (Grappin et al. 2000, Reinhardt et al. 2000, Nagpal et al. 2005, Mirmansari and Smith 2014, Umehara et al. 2015, Verma et al. 2016). Phytohormones are also produced by bacteria, cyanobacteria, green algae, and fungi (Costacurta and Vanderleyden 1995, Sergeeva et al. 2002, Tudzynski and Sharon 2002, Grube et al. 2009). For example, ABA (Hirsch et al. 1989), auxins, GAs, BRs (Stöhr et al. 2013a), CKs (Stöhr et al. 2013b), jasmonic acid (JA; Fuji et al. 1997), and SA (Onofrejova et al. 2010) have been found in green algae. According to Bradley (1991) and Tarakhovskaya et al. (2007), the roles of phytohormones in algae might be similar to those in higher plants. Nevertheless, only few studies are available regarding the biological roles of phytohormones in green algae, and most of them report on responses of chlorophytes to exogenous treatment with phytohormones. For summary of the effects of exogenous treatment with IAA, ABA, CKs, and polyamines on a broad range of aquatic algae, see Bradley (1991). An involvement of ABA in response to oxidative (Hirsch et al. 1989), osmotic (Hinojosa-Vidal et al. 2018), and alkaline (Yoshida et al. 2003) stress factors was suggested in different chlorophytes.

Green microalgae grow in aquatic (Gray et al. 2007) or periodically wet environments (Candotto Carniel et al. 2015), and there are also aero-terrestrial members, mostly in the Trebouxiophyceae, that are even capable of surviving desiccation (Kraner et al. 2005, 2008, Holzinger and Karsten 2013, Candotto Carniel et al. 2016, Banchi et al. 2018). These organisms can release metabolites into the environment, and their secretome includes proteins, organic acids, lipids, polysaccharides, and various low-molecular-weight molecules (Liu et al. 2016). To the best of our knowledge, only Maršálek et al. (1992) and Mazur et al. (2001) reported on the occurrence of indole-3-acetic acid (IAA) and ABA in liquid growth media of four Trebouxiophyceae species, but the influence of different environmental conditions on the release of phytohormones by green algae was not tested. Indole-3-carbaldehyde, known as a precursor (Bandurski et al. 1995) and degradation product of IAA (Gazarian et al. 1998), was also determined in the exudates of four different isolated lichen “photobionts” (i.e., lichen-forming microalgae; but note that lichens can also have cyanobacterial photobionts), all within the genus of *Trebouxia* (Meeßen et al. 2013). In summary, our understanding of the metabolism and biological significance of phytohormones in chlorophytes is far from understood. However, phytohormones are key signaling molecules, and evidence is emerging that they could be important players in inter-kingdom signaling, as described in the pioneering papers of Hughes and Sperrando (2008) and Xu et al. (2015).

In this study, we investigated six terrestrial microalgae in the Trebouxiophyceae. These include the free-living algae *Apatococcus lobatus*, *Chloroidium ellipsoideum*, and *Myrmecia bisecta*. To obtain some first insights into the putative roles of phytohormones in inter-kingdom signaling, photobionts of the lichens *Cladonia graii*, *Xanthoria parietina*, and *Tephromela atra* were studied (i.e., *Asterochloris glomerata*, *Trebouxia decorolans*, and *Trebouxia sp.*, respectively). The lichen photobionts were chosen because they are symbionts of well-studied lichen models, and grow sufficiently fast for producing enough biomass for the intended experiments (note that lichen symbionts are notoriously slow-growing organisms when grown in axenic cultures). The free-living species were chosen because of their ecological preferences in conjunction with key physiological and reproductive traits (Nugari et al. 2009, Kulichova et al. 2014, Candotto Carniel et al. 2015, Bertuzzi et al. 2017): they typically occur in soil, bark, and rock micro-habitats, they tolerate dehydration (as do the lichen photobionts) and share a cell cycle that involves the formation of autosporangia followed by the release of autospores, and rare sexual reproduction, and they also grow sufficiently fast for producing the biomass required for the intended experiments. Eight representative phytohormones were analyzed, IAA, indole-butyric acid (IBA), ABA, SA, JA, gibberelin A3 (GA3), gibberelin A4 (GA4), and zeatin (ZT), focussing on cellular phytohormone composition and levels, and extracellular release under different environmental conditions with varying light intensities and water availability. The latter were assumed to mimic environmental conditions that have been proposed to play a role for the process of lichenization (Stocker-Wörgötter 2001), defined as the transition from a free-living to a symbiotic state. The following three hypotheses were tested: (a) phytohormones can be released extracellularly; (b) extracellular release can be influenced by environmental stimuli; and (c) lichen-forming and free-living algae secrete a different set of phytohormones.

**MATERIALS AND METHODS**

*Strain identity and culture conditions.* Axenic stock cultures of *Apatococcus lobatus* (SAG 2037; 18S accession number:
to the same conditions as described for DL and HL treatments, but with a dehydration step prior to rehydration on BBM agar. Cultures were dehydrated by transferring the PTFE membranes with the cultures into sterile 9 cm petri dishes (without lid) within humidity-proof, sealed boxes (Ensto, 300 × 300 × 132 mm) above 750 g of sterile silica gel (Rettberg, 3–5 mm diameter with a water capacity of 30%) together with a humidity sensor (EL8-USB-2-LCS T°C/RH sensor). Four petri dishes per species, each containing three 25 mm membranes with algal cultures, were placed into one box. The boxes were scored in a growth chamber (as above) at DL or HL for 22 h ± 30 min to dehydrate the algal cultures to a final water content of 0.1 g H₂O · g algal DM⁻¹. Thereafter, filter cultures were rehydrated by transferring them carefully onto solid BBM in sterile microboxes with air exchange filters (as above), and incubated either at DL or HL for 7 d and 20°C. All equipment was either autoclaved or surface-sterilized with 70% EtOH (VWR Chemicals, Vienna, Austria) for three times and dried under a sterile hood.

Blank treatments. To test if phytohormones in extracellular leachates were released by algae and were not artifacts, blank samples (i.e., filters without algae) were prepared with 50 µL of liquid TM, exactly as described for DL, HL, DL + DH, and HL + DH treatments, also including a pre-treatment of PTFE filters on TM for 6 and 18 d.

Sample harvesting. At the end of the treatments, all cultures were visually inspected for bacterial and fungal contamination with a stereomicroscope (Olympus SZ51, Vienna, Austria). Membranes with uncontaminated algal cultures were carefully separated from agar with a spatula. Samples were taken to assess cellular phytohormones (i.e., intracellular and cell wall-bound phytohormones) and phytohormones released from algal cells into the medium. Hereafter, “cellular” and “extracellular” phytohormones are indicated by the subscript letters “C” and “E,” respectively, after the abbreviations for the various treatments. For quantification of extracellularly released phytohormones, the agar containing the extracellular exudates was transferred into 2 mL Eppendorf tubes and immediately frozen in liquid nitrogen. For cellular phytohormone quantification, algal cultures were removed from the membranes using a spatula and placed in 2 mL Eppendorf tubes. To remove residual extracellular hormones from the outer surface of algal cells, cultures were washed with 1 mL of liquid BBM (pH 6.8). After gentle vortexing for 5 s and centrifugation at 500 g for 2 min at 20°C (Sigma®-18 KS, Sartorius AG, Göttingen, Germany), the supernatant was removed from algal biomass with a syringe. Both cellular and extracellular samples were immediately frozen in liquid nitrogen, lyophilized for approximately 90 h ± 30 min and stored at −80°C for further UHPLC-MS/MS measurements.

Chemicals. All chemicals used were of highest purity (HPLC or LC-MS-grade) and obtained from Sigma-Aldrich or VWR Chemicals, both Vienna, Austria, unless mentioned otherwise. All equipment used for culturing, harvesting, or ultra-high-performance liquid chromatography-mass spectrometry/mass spectrometry (UHPLC-MS/MS) was rinsed three times with LC-MS-grade acetonitrile or ultra-pure water (UPW), as appropriate, before use and then dried in a fume hood.

Sample preparation for UHPLC-MS/MS measurements. For UHPLC-MS/MS analysis, an adapted version of the method described in Buchner et al. (2017) was used. For cellular hormone measurements, 5 µg of lyophilized algal biomass was weighed with an analytical balance (XS 105; Mettler Toledo®). 20 µg of lyophilized agar was used to determine extracellular hormone levels. Algal biomass or agar was placed in 2 mL safe-lock Eppendorf tubes and extracted in 1.5 mL of ice-cold acetone/water/acetic acid (80:20:1, v/v/v)
after addition of 25 μL stable isotopically labeled internal standard solution (0.5 μM ABA-d6, 0.5 μM SA-d4) by shaking (TissueLyser II; Qiagen, Düsseldorf, Germany) at 30 Hz for 5 min using one 5 mm glass bead (pre-cleaned with methanol) for each Eppendorf tube, followed by centrifugation at 10,000g, 4 °C for 12 min. Supernatants were evaporated to dryness using a SpeedVac SPD111 vacuum concentrator (Thermo Fisher Scientific Inc., Waltham, MA, USA) for 1 h to remove the acetone, followed by freezing in liquid nitrogen and lyopholization for 14 h ± 5 min. The lyopholized pellet was resuspended in 75 μL of acetonitrile by vortexing for 10 s and 5 min ultra-sonication in an ice-cooled water bath. Then, 75 μL of UPW were added followed by vortexing for 10 s and 5 min ultra-sonication in an ice-cooled water bath. The extracts were filtered through 0.2 mm PTFE filters before injection into the UHPLC-MS/MS system.

**UHPLC-MS/MS analysis.** The phytohormones ABA, SA, JA, GA₃, GA₄, IAA, IBA, and ZT were identified and quantified by UHPLC-MS/MS, using an eXpert ultra LC1000 UHPLC system (Eksigent, Dublin, CA, USA) coupled to a QTRAP 4500 mass spectrometer (ABSCIEX, Framingham, MA, USA). For compound separation, a reversed-phase column (Luna Omega C18 100 A, 50 x 2.1 mm, 1.6 μm, Phenomenex, Torrance, CA, USA) with a SecurityGuard ULTRA Cartridge (UHPLC Fully Porous C18 with 2.1 mm inner diameter) connected ahead was used. The mobile phases contained 0.1% formic acid (v/v; solvent A) and acetonitrile containing 0.1% formic acid (solvent B). Samples were injected starting with 5% solvent B followed by a gradient to 70% solvent B (5 min), rinsing with 100% solvent B (5:01 to 6 min) and equilibration to 5% solvent B (6:50 to 8 min). The injection volume was set to 5 μL, the flow rate to 0.5 mL min⁻¹, and the column temperature to 30°C. Compounds were detected by the mass spectrometer operated in positive and negative ion mode using multiple reaction monitoring (MRM). Ion spray voltage was set to 5.5 kV (pos mode) or -4.5 kV (neg mode), gas 1 (nebulizer gas, N₂) to 40 psi and gas 2 (heater gas, N₂) to 50 psi at a temperature of 500°C. Both quadrupole mass analyzers were operated at unit resolution. Peaks were automatically detected based on retention time and MRM transition. Peak areas were normalized relative to the internal standards to account for variations during sample preparation and analysis. Concentrations were calculated according to the calibration curves created with authentic standards using the software Analyst 1.6.3 and MultiQuant 2.1.1 (AB SCIEX, Framingham, MA, USA).

Blank samples containing only the solid growth medium were also processed. ABA, JA, GA₃, GA₄, IAA, IBA, and ZT were below the limit of detection (LOD). Trace amounts of IAA slightly above the LOD were found in 6 of 32 blank samples. However, SA was detected in all blank samples (i.e., the solid growth media contained SA at concentrations above the LOD); therefore, quantification of this hormone was considered unreliable.

**Statistics.** For each species and treatment (DL, HL, DL + DH, and HL + DH), six biological replicates were measured. To test data of cellular vs. released hormone levels in DL, numerical analyses were conducted with R (version 3.5.1) and RStudio (version 1.1.383). QQ plots were used to test for normal distribution. For comparison of DLₐ (i.e., cellular phytohormone levels after treatment with DL as explained above) and DLₑ (i.e., extracellular phytohormone levels after treatment with DL) data, a non-parametric two-sided Mann–Whitney U Test with continuity correction was used at P < 0.01. Heat map visualization was performed with Morpheus software (https://software.broadinstitute.org/morpheus).

To test data for phytohormone release under different environmental stimuli (comparison of DLₑ, HLₑ, DL + DHₑ, and DH + HLₑ), two outliers were removed and the non-parametric Kruskal–Wallis test (P ≤ 0.05) followed by Dunn’s post-hoc Test (P ≤ 0.05) with Benjamini–Hochberg correction was conducted to verify significant differences.

**RESULTS**

Assessment of phytohormone composition under DL. We first assessed growth rates of all algal species cultured under DL (Fig. 1), which was the control condition suitable for all six species. *Chloroidium ellipsipolyedum* and *Myrmecia bisecta* grew considerably faster than the other four species (Fig. 1). For subsequent experiments, cultures in a comparable growth phase were used: *C. ellipsipolyedum* and *M. bisecta* were taken on day 6 and *Asterochloris glomerata*, *Trebouxia decolorans*, *Trebouxia sp.*, and *Apatococcus lobatus* on day 18, prior to transfer to microboxes and exposure to control conditions (DL) or treatments (HL, DL + DH, or HL + DH).

Next, cultures in the exponential growth phase (Fig. 1) were exposed to control conditions in DL for 7 days (Fig. 2 and Table 1), and phytohormones present in algal cells and their exudates identified. Cells of the lichen-forming alga *Trebouxia* sp. contained IAA, ABA and IBA. These three phytohormones were also released from cells, and in addition, JA and GA₃ were found in the extracellular exudates. In *Asterochloris glomerata*, IAA and ABA were found cellularly and these two phytohormones, together with JA, were detected in the extracellular exudates. In cells of *Trebouxia decolorans*, IAA, ABA, and ZT were present, but only IAA and ABA were found extracellularly. Cells of the free-living species *Myrmecia bisecta* contained IAA, ABA, and GA₄, and these phytohormones were also found in the extracellular exudates together with JA and GA₃. In *Chloroidium ellipsipolyedum* cells, IAA and ZT were found, and in extracellular exudates IAA, ABA, JA, and GA₄ were detected. In cells of *A. lobatus*, only ABA was detected, which was also found in extracellular exudates together with IAA and JA (Fig. 2).

Normalized to g algal DM, IAA levels were around 10 times higher in exudates compared to cellular levels for all species, those of ABA were between 5 and 100 times higher, and that of GA₄, only found in *Myrmecia bisecta* cells and their exudates, was around 50 times higher. Cellular levels of JA, GA₃, and GA₄ were below or at the LOD in all species, but at least one of these hormones was found in exudates, at levels varying from trace amounts to high abundance (Table 1).

In summary, both IAA and ABA were present in the cells of four species studied, with the exception of *Chloroidium ellipsipolyedum* and *Apatococcus lobatus*, in which ABA and IAA were not detectable, respectively. Trace amounts of ZT were found in cells of *Trebouxia decolorans* and *C. ellipsipolyedum*, and of GA₄ in *Myrmecia bisecta*. IAA and ABA were released by all species, and JA was released in detectable
amounts by all species except *T. decolorans*. GA$_3$ was detected in exudates of only two species, *Trebouxia* sp. and *M. bisecta*. GA$_4$ was detected extracellularly in *M. bisecta* and *C. ellipsoideum*, and IBA was only found at levels above the LOD in cells and exudates of *Trebouxia* sp. (Fig. 2).

Effects of high light and dehydration–rehydration treatments on extracellular phytohormone release. High light influenced phytohormone release only in two free-living microalgae: IAA release increased 4-fold in *Chloroidium ellipsoideum* (Fig. 3i; Kruskal–Wallis test: chi-squared = 15.01, df = 3, *P* < 0.05), whereas GA$_3$ release by *Myrmecia bisecta* decreased (Fig. 3d; Kruskal–Wallis test: chi-squared = 15.37, df = 3, *P* < 0.05). No significant differences (Kruskal–Wallis test: *P* > 0.05) under HL were observed for all other phytohormones and microalgae.

Compared to DL controls, dehydration treatments (DL + DH and/or HL + DH) had the highest impact on algal phytohormone release. Both dehydration treatments (DL + DH and HL + DL), but not HL, led to bleaching of *Myrmecia bisecta* (observed visually). No bleaching was observed in the other five algae for any treatment. In response to DL + DH and HL + DH, IAA, ABA, GA$_3$, and GA$_4$ levels in the exudates of *M. bisecta* decreased significantly (Fig. 3, a, b, d, and e); *P* < 0.05; Kruskal–Wallis test: chi-squared = 20.07, 18.11, 15.37, 18.32,

Fig. 1. Cumulative growth of free-living and lichen-forming algae. Panels (a)–(c) show the free-living algae *Apatococcus lobatus*, *Chloroidium ellipsoideum*, and *Myrmecia bisecta*, respectively, and panels (d)–(f) the lichen-forming algae *Asterochloris glomerata*, *Trebouxia* sp., and *T. decolorans*, respectively. Algae were grown under DL (20 µmol photons · m$^{-2}$ · s$^{-1}$). Day 0 is the day of inoculation. DM refers to the dry mass of lyophilized algal material at each time interval. Data are means ± SD (*n* = 3 biological replicates). Arrows show the time points at which cultures were taken for use in subsequent experiments.
FIG. 2. Heat map showing abundance and composition of cellular (C) and extracellular (E) phytohormones detected in free-living and lichen-forming algae kept under control conditions in DL (20 μmol photons m⁻² s⁻¹). The scale bar indicates the number of biological replicates in which an individual hormone was detected from a minimum of 0 to a maximum of 6. White fields indicate that an individual hormone was absent or below the limit of detection, and gray fields indicate low frequency.

**TABLE 1.** Cellular and extracellular levels of the phytohormones IAA, ABA, JA, GA3, GA4, IBA, and ZT of algae after 7 d of exposure to dim light.

| Species          | Free-living | Lichen-forming |
|------------------|-------------|----------------|
|                  | A. lobatus  | C. ellipsoideum| M. bisecta    | A. glomerata | T. decolorans | Trebouxia sp. |
| IAA              | DL_C        | ≤LOD           | 0.11 ± 0.06   | 0.21 ± 0.06  | 0.21 ± 0.08  | 0.19 ± 0.05   | 0.20 ± 0.03   |
|                  | DL_E        | 0.33 ± 0.02    | *3.93 ± 0.89  | *3.09 ± 0.59 | *2.67 ± 0.42 | *1.52 ± 0.14  | *3.53 ± 0.61  |
| ABA              | DL_C        | Traces < 0.01  | ≤LOD          | 0.01 ± 0.01  | 0.08 ± 0.01  | 0.01 ± 0.00   | 0.02 ± 0.00   |
|                  | DL_E        | *0.06 ± 0.02   | *Traces < 0.01| *2.25 ± 0.39 | *2.41 ± 0.32 | *1.09 ± 0.29  | *0.76 ± 0.11  |
| JA               | DL_C        | ≤LOD           | ≤LOD          | ≤LOD         | ≤LOD         | ≤LOD          | ≤LOD          |
|                  | DL_E        | 0.14 ± 0.08    | 0.01 ± 0.01   | Traces < 0.01| *0.01 ± 0.00 | ≤LOD          | ≤LOD          |
| GA3              | DL_C        | ≤LOD           | ≤LOD          | *0.16 ± 0.02 | ≤LOD         | ≤LOD          | ≤LOD          |
|                  | DL_E        | ≤LOD           | ≤LOD          | 0.01 ± 0.01  | ≤LOD         | ≤LOD          | ≤LOD          |
| GA4              | DL_C        | ≤LOD           | Traces < 0.01 | *0.51 ± 0.06 | ≤LOD         | ≤LOD          | ≤LOD          |
|                  | DL_E        | ≤LOD           | Traces < 0.01 | ≤LOD         | ≤LOD         | ≤LOD          | ≤LOD          |
| IBA              | DL_C        | ≤LOD           | ≤LOD          | ≤LOD         | ≤LOD         | ≤LOD          | ≤LOD          |
|                  | DL_E        | ≤LOD           | ≤LOD          | ≤LOD         | ≤LOD         | ≤LOD          | ≤LOD          |
| ZT               | DL_C        | ≤LOD           | Traces < 0.01 | ≤LOD         | ≤LOD         | ≤LOD          | ≤LOD          |
|                  | DL_E        | ≤LOD           | Traces < 0.01 | ≤LOD         | ≤LOD         | ≤LOD          | ≤LOD          |

Statistically significant differences (*) between cellular and extracellularly released hormones were assessed by Mann–Whitney U test (P ≤ 0.01; significances in bold font). DL, dim light; subscript letters C and E denote cellular and extracellular phytohormone levels, respectively, normalized to algal dry mass (DM).
FIG. 3. Extracellular phytohormone release of free-living algae in response to different environmental stimuli. These included dim light (DL) used as a control, high light (HL), dehydration followed by rehydration under dim light (DL + DH), dehydration followed by rehydration under high light (HL + DH). Levels of released phytohormones of (a–e) Myrmecia bisecta, (f–h) Apatococcus lobatus, and (i and j) Chloroidium ellipsoideum were normalized to algal dry mass (DM). See Table S1 in the Supporting Information for further details. Boxplots (n = 5 to 6 biological replicates) show median, 25% and 75% percentiles, maximum and minimum values, and outliers (dots). Statistically significant differences (P < 0.05) are marked with different letters (Kruskal–Wallis test followed by Dunn’s post-hoc test).
respectively, df = 3), whereas JA levels significantly increased (Fig. 3c; Kruskal–Wallis test: chi-squared = 19.35, df = 3; P < 0.05). In the lichen-forming algae Trebouxia sp., Asterochloris glomerata (Fig. 4, a and e; Kruskal–Wallis test: chi-squared = 16.99, 14.24, respectively, df = 3, P < 0.05) and in the free-living alga Chloridioidium ellipsoideum (Fig. 3i; Kruskal–Wallis test: chi-squared = 15.01, df = 3, P < 0.05) IAA release was also strongly affected by DL + DH and/or HL + DL. Under DL + DH A. glomerata (Fig. 4c, Kruskal–Wallis test: chi-squared = 16.99, df = 3, P < 0.05) and Trebouxia sp. (Fig. 4a, Kruskal–Wallis test: chi-squared = 14.24, df = 3, P < 0.05) released two to four times more IAA, and C. ellipsoideum released, besides the previously described results for HL, four times more IAA under both, DL + DH and HL + DH (Fig. 3i, Kruskal–Wallis test: chi-squared = 15.01, df = 3, P < 0.05). Furthermore, M. bisecta (Fig. 3b, Kruskal–Wallis test: chi-squared = 18.11, df = 3, P < 0.05), Trebouxia sp. (Fig. 4b, Kruskal–Wallis test: chi-squared = 21.15, df = 3, P < 0.05), and T. decolorans (Fig. 4i, Kruskal–Wallis test: chi-squared = 9.99, df = 3, P < 0.05) showed decreased ABA release under DL + DH, whereas for C. ellipsoideum the ABA release was significantly increased under HL + DH (Fig. 3j, Kruskal–Wallis test: chi-squared = 17.29, df = 3, P < 0.05). A significantly decreased JA release was found exclusively in A. glomerata under DL + DH and HL + DH (Fig. 4g, Kruskal–Wallis test: chi-squared = 13.955, df = 3, P < 0.05). In Trebouxia sp., GA₃ release strongly increased under HL + DH (Fig. 4d, Kruskal–Wallis test: chi-squared = 19.56, df = 3, P < 0.05). No further significant differences (Kruskal–Wallis test: P > 0.05) were observed.

**DISCUSSION**

The present study reports on the composition and levels of phytohormones produced and their extracellular release by free-living and lichen-forming Trebouxia sp. and Asterochloris glomerata. Furthermore, the effects of environmental stimuli, including light and dehydration treatments, on extracellular phytohormone release were tested and a potential role for phytohormones in chemical communication is discussed.

**Cellular phytohormones.** Other authors reported on the occurrence of phytohormones in cells of chlorophytes, for example IAA, ABA, CKs (Lu and Xu 2015) GAs, BRs (Stirk et al. 2013a), JAs (Fujii et al. 1997), SA (Onofrejova et al. 2010), or IBA (Gupta and Bucher 2011). Reports on cellular phytohormones in aero-terrestrial Trebouxia sp. are limited to Chlorella sp. (see Lu and Xu 2015, for review) and Myrmecia bisecta, in which IAA, GAs, CKs, and BRs were found (Stirk et al. 2013a,b). With the exception of the study of Hinojosa-Vidal et al. (2018), who found ABA in the lichen-forming algal strain Trebouxia sp. TR9, we did not find reports on other phytohormones or on phytohormone composition of isolated lichen-forming algae. In this study, we observed cellular phytohormone production (Fig. 2) in the free-living algae Apatococcus lobatus (ABA), M. bisecta (IAA, ABA, and GA₄), Chloridioidium ellipsoideum (IAA and ZT), and in the lichen-forming algae Trebouxia sp. (IAA, ABA, and IBA), Astrerochloris glomerata (IAA and ABA), and T. decolorans (IAA, ABA, and ZT), extending the results of Stirk et al. (2013a,b) and those reviewed by Lu and Xu (2015). IAA is probably the best studied auxin in plants and also in algae, whereas IBA has received much less attention. For example, IBA was found in maize roots (Epstein et al. 1989) and in the thalli of multicellular green algae such as Ulva and Monostroma (Gupta et al. 2011), but not in unicellular free-living or symbiotic microalgae. Epstein et al. (1986) and Ergün et al. (2002) found IAA, ABA, ZT, and GA₃ in various lichens, for example, Ramalina duriae and Xanthoria parietina. In these three studies, it was unclear by which symbiont these phytohormones were produced, but the fungal symbiont (i.e., the “mycobiont”) was assumed to be the main source.

**Extracellular release of phytohormones.** In contrast to the cellular occurrence of phytohormones in green algae, data on the extracellular phytohormone release are scarce and limited to studying phytohormone release into liquid media (Marsálek et al., 1992, Mazur et al. 2001), whereas we used solid media. For aero-terrestrial green algae, culture on solid growth medium is more similar to their natural environmental conditions (Ettl and Gartner 2014). This assumption was supported by Rippin et al. (2017), who showed that physiological and transcriptional responses to dehydration of streptophytic algae grown on solid medium differ from those of algae grown in liquid medium. Phytohormones could be key molecules for inter-kingdom signaling, as reviewed by Spaepen and Vanderleyden (2010) regarding the role of auxin in plant–microbe interactions. Xu et al. (2015) demonstrated for rice and the pathogen Xanthomonas oryzae that SA and ABA are involved in bidirectional cross-communication, orchestrating host immune responses as well as modulating microbial virulence traits. Furthermore, increased extracellular IBA concentrations were proposed to play a role in arbuscular mycorrhiza formation in Zea mays, when co-cultured with Glomus intraradices (Kaldorf and Ludwig-Müller 2000). Hardly any knowledge exists about extracellularly released phytohormones in microalgae, but it is reasonable to assume that phytohormones could be involved in chemical crosstalk with other organisms in their environment. We observed that phytohormones were released extracellularly (Fig. 2) by the free-living microalgae Apatococcus lobatus (IAA, ABA, and JA), Chloridioidium ellipsoideum (IAA, ABA, JA, and GA₄), Myrmecia bisecta (IAA, ABA, JA, GA₄, and GA₃), and by the lichen-forming Asterochloris glomerata (IAA, ABA, and JA), Trebouxia decolorans (IAA
and ABA), and Trebouxia sp. (IAA, ABA, JA, GA₃, and IBA). Interestingly, when normalized to algal DM, the levels of phytohormones accumulated in the growth medium within 7 days were up to 100 times higher in the extracellular space than within cells (Table 1). Some phytohormones were only found in the extracellular exudates. Their rather low cellular levels, below the LOD (Fig. 2) in A. lobatus (IAA, JA), C. ellipsoideum (ABA, JA, GA₃), M. bisecta (JA, GA₃), A. glomerata (JA), and Trebouxia sp. (JA, GA₃; i.e., suggest that these phytohormones were produced and quickly released by cells, and

Fig. 4. Extracellular phytohormone release by lichen-forming algae subjected to diverse environmental stimuli. These included dim light (DL) used as control, high light (HL), dehydration followed by rehydration under dim light (DL + DH), dehydration followed by rehydration under high light (HL + DH). Levels of released phytohormones of (a–d) Trebouxia sp., (e–g) Asterochloris glomerata, and (h and i) T. decolorans were normalized to algal dry mass (DM). See Table S2 in the Supporting Information for further details. Boxplots (n = 6 biological replicates) show median, 25% and 75% percentiles, maximum and minimum values, and outliers (dots). Statistically significant differences (P < 0.05) are marked with different letters (Kruskal–Wallis test followed by Dunn's post-hoc test).
then accumulated in the extracellular environment. Our main interest was in studying the presence and abundance of extracellularly released phytohormones with potential roles in inter-kingdom signaling. We appreciate that intracellularly, each phytohormone can be regulated by many mechanisms including degradation, conjugation, and interaction with other hormones, and that phytohormones present in media can be degraded by exposure to high light intensities, salinity, or pH (Dunlap and Robacker 1988, Nissen and Sutter 1990). We showed that extracellularly released phytohormones were present in the exudates, where they may represent a potential source for inter-kingdom signaling, although it was outside the remits of this study to investigate such interactions, or how the individual phytohormones are regulated intracellularly and how they decompose after release into the extracellular space.

Furthermore, we showed that light and dehydration treatments influenced the levels of the released phytohormones (Figs. 3 and 4). No clear patterns were observed between free-living algae and lichen-forming algae. Instead, phytohormone release in response to environmental stimuli appeared to be species-specific. However, common denominators are that (1) IAA and ABA were released extracellularly by all six species studied; (2) levels of extracellular exudates of all six species; and (3) extracellular levels of IAA, ABA, and (if found above LOD) JA were in the same order of magnitude in the three lichen-forming algae, but strongly varied in the free-living species. In addition, under DL + DH IAA was found in extracellular exudates at the highest levels compared to controls grown under DL in Asterochloris glomerata (Fig. 4e) and Trebouxia sp. (Fig. 4a), and under HL, DL + DH, and HL + DH in Chlororionum ellipsosideum (Fig. 4i). Furthermore, in Myrmezia bisecta, DL + DH and HL + DH treatments led to a decline in the levels of all phytohormones in the exudates, except for JA (Fig. 3c), which was found to be significantly increased by these treatments. The precise role of JA in microalgae is yet to be elucidated, but considering its involvement in programmed cell death, leaf senescence, and pathogen defence in plants (Reinbothe et al. 2009), the JA increase in response to the dehydration treatments could indicate that the viability of M. bisecta cells was declining, in agreement with the visually observed bleaching. By contrast, only GA3 levels were halved in the exudates of M. bisecta by the HL treatment, whereas IAA, ABA, JA, and GA4 did not change significantly, indicating that M. bisecta was likely more tolerant of HL than the two dehydration treatments (Fig. 3, a–e).

Due to the current lack of knowledge, it is difficult to draw conclusions about the potential roles of extracellularly released phytohormones in microalgae. Studies on the effects of exogenously applied phytohormones on chlorophytes may allow to offer some preliminary deductions about their putative roles. For example, Piotrowska-Niczyporuk et al. (2012) suggested that exogenously applied phytohormones, including auxins, CKs, GAs, and JA, could be involved in the regulation of heavy metal biosorption and toxicity in Chlorella vulgaris. Furthermore, Piotrowska-Niczyporuk and Baiguz (2014) showed in the same green alga that growth, pigment, and antioxidant contents can be influenced by exposure to IAA or IBA. Furthermore, exogenously applied ABA induced tolerance against oxidative stress in Chlamydomonas reinhardtii (Yoshida et al. 2003). In the multicellular chlorophyte Codium fragile, exogenous IAA treatment increased growth (Hanisak 1979). Exogenous treatment with CKs also enhanced growth of the lichen photobiont Trebouxia irregularis (Backor and Hudák 1999), and the auxins IAA and IBA increased growth of the mycobionts Nephromopsis ornata, Myelochroa irrugans, and Usnea longissima (Wang et al. 2009, Wang et al. 2010). However, care must be taken in the interpretation of data obtained from exogenously treated organisms as often very high concentrations above the physiological range are used (Bradley 1991).

CONCLUSIONS

We used UHPLC-MS/MS to measure cellular levels of phytohormones of six Trebouxiophyceae grown on solid medium and extracellularly released phytohormones. We found that (1) phytohormones were produced and extracellularly released by all tested algae, (2) levels of extracellular exudates were influenced by environmental stimuli, and (3) no clear patterns, neither in the composition of released phytohormones, nor in the changes of extracellular levels in response to environmental stimuli were observed between free-living and lichen-forming algae. Our study will help defining the physiological range of phytohormone levels to use for exogenous applications, also supporting further studies to identify the effects of phytohormones on microalgae and other organisms. More importantly, we envisage our study to be a useful basis for future studies of microalgae regarding the roles of phytohormones in molecular cross-talk and inter-kingdom communication, particularly with lichenising fungi and bacteria.

ACKNOWLEDGEMENTS

We gratefully acknowledge support by Daniele Armaleo (Duke University), who generously provided cultures of A. glomerata. Furthermore, support by Erwann Arc, Davide Gerna, Thomas Roach, Jean-Nicolas Haas (University of Innsbruck), and Alberto Pallavicini (University of Trieste) for constructive comments on a draft of this paper is much appreciated, as is the help with data analyses by Erwann Arc.
This work was funded by grants of the Austrian Science Fund (FWF), projects P 28902 to IK and I 1951-B16 to AH, and by the Office of the Vice Rector for Research of the University of Innsbruck (Doktoratsstipendium der Nachwuchsförderung; Überbrückungs-Stipendium an österreichische Graduierte) to GP.

Ahmadjian, V. 1975. Resynthesis of lichens. In Ahmadjian, V. & Hale, M. E. [Eds.] The Lichens. Academic Press, New York, USA, pp. 565–79.

Andersen, R. A. 2005. Traditional microalgae isolation techniques. In Andersen, R. A. [Ed.] Algal Culturing Techniques, 1st edn. Academic Press, New York, USA, pp. 83–100.

Armaleo, D. & May, S. 2009. Sizing the fungal and algal genomes of the lichen Cladonia grayi through quantitative PCR. Symbiosis 49:43–51.

Baćtor, M. & Hudák, J. 1999. The effect of cytokinins on the growth of lichen photobiont Trebouxia irregularis cultures. Lichenology 31:207–10.

Bailly, C. & Kranner, I. 2011. Analyses of reactive oxygen species and antioxidants in relation to seed longevity and germination. In Kermode, A. R. [Ed.] Seed dormancy: Methods and protocols, methods in molecular biology. Humana Press, New York, USA, pp. 343–367.

Banchi, E., Candotto Carniel, F., Montagner, A., Petruzzellis, F., Pichler, G., Girola, V., Bartels, D., Pallavicini, A. & Tretiach, M. 2018. Relation between water status and desiccation-affected genes in the lichen photobiont Trebouxia gelatinosa. Plant Physiol. Bioch. 129:189–97.

Bandurski, R. S., Cohen, J. D., Slovin, J. P. & Reinecke, D. M. 1995. Auxin biosynthesis and metabolism. In Davies, P. [Ed.] Plant hormones – physiology, biochemistry & molecular biology. 2nd edn. Springer, Dordrecht, Netherlands, 44 pp.

Bertuzzi, S., Gustavs, L., Pandolfini, G. & Tretiach, M. 2017. Heat shock treatments for the control of liphobionts: a case study with epilithic green microalgae. Int. J. Environ. Bioremediat. Biodegrad. 123:236–45.

Böld, H. C. 1949. The morphology of Chlamydomonas chlamy- dogauma sp. nov. Bull. Torrey. Bot. Club. 76:101–8.

Bradley, P. M. 1991. Plant hormones do have a role in controlling growth and development of algae. J. Phycol. 27:317–21.

Buchner, O., Roach, T., Gertzen, J., Schrenk, S., Karadár, M., Sööggl, W., Miller, R., Bertel, C., Neuner, G. & Kranner, I. 2017. Drought affects the heat-hardening capacity of alpine plants, as indicated by changes in xanthophyll cycle pig- ments, single oxygen scavenging, t-tocopherol and plant hormones. Environ. Explo. Bot. 135:159–75.

Candotto Carniel, F., Gerdol, M., Montagner, A., Banchi, E., De Moro, G., Manfrin, C., Muggia, L., Pallavicini, A. & Tretiach, M. 2016. New features of desiccation tolerance in the lichen photobiont Trebouxia gelatinosa are revealed by a transcriptomic approach. Plant Mol. Biol. 91:319–39.

Candotto Carniel, F., Zanelli, D., Bertuzzi, S. & Tretiach, M. 2015. Desiccation tolerance and lichenization: a case study with the aeroterrestrial microalga Trebouxia sp. (Chlorophyta). Planta 242:493–505.

Costacurta, A. & Vanderleyden, J. 1995. Synthesis of phytohormones by plant-associated bacteria. Crit. Rev. Microbiol. 21:1–18.

Dunlap, J. R. & Robacker, K. M. 1988. Nutrient salts promote light-induced degradation of indole-3-acetic acid in tissue culture media. Plant Physiol. 88:379–82.

Epstein, E., Chen, K. H. & Cohen, J. D. 1989. Identification of indole-3-butyric acid as an endogenous constituent of maize kernels and leaves. Plant Growth Regul. 8:215–23.

Epstein, E., Sagee, O., Cohen, J. D. & Garty, J. 1986. Endogenous auxin and ethylene in the lichen Ramalina duriaeae. Plant Physiol. 82:1122–25.

Ergün, N., Topcuoğlu, Ş. F. & Yıldız, A. 2002. Auxin (indole-3-acetic acid), gibberellic acid (GA3), abscisic acid (ABA) and cytokinin (zeatin) production by some species of mosses and lichens. Turk. J. Bot. 26:13–8.

Ettl, H. & Gärter, G. 2014. Syllabus der Bodew.- Luft- und Flechtenalgen, 2nd edn. Gustav Fischer Verlag, Stuttgart, Germany, pp. 1–12.

Fujii, S., Yamamoto, R., Miyamoto, K. & Ueda, J. 1997. Occurrence of jasmionic acid in Dunaliella (Dunaliellales, Chloro- phyta). Physiol. Res. 45:223–6.

Gazarin, I. G., Laigrimini, L. M., Mellon, F. A., Naldrett, M. J., Ashley, G. A. & Thornley, R. N. 1998. Identification of skatolhydroperoxide and its role in the peroxidase-catalysed oxidation of indol-3-ylactic acid. Biochem. J. 333:223–32.

Grappin, P., Bouinot, D., Sotta, B., Miginiac, E. & Jullien, M. 2000. Control of seed dormancy in Nostocata plumaginifolia: post-inhibition abscisic acid synthesis imposes dormancy maintenance. Planta 210:279–85.

Gray, D. W., Lewis, L. A. & Cardon, Z. G. 2007. Photosynthetic recovery following desiccation of desert green algae (Chloro- phyta) and their aquatic relatives. Plant Cell Environ. 30:1240–55.

Grube, M., Cardinale, M., de Castro, J. V., Muller, H. & Berg, G. 2009. Species-specific structural and functional diversity of bacterial communities in lichen symbioses. ISME J. 3:1105–15.

Gupta, V., Kumar, M., Brahmbhatt, H., Reddy, C. R. K., Seth, A. & Jha, B. 2011. Simultaneous determination of different endogenous plant growth regulators in common green seaweeds using dispersive liquid-liquid microextraction method. Plant Physiol. Bioch. 49:1259–63.

Gustavs, L., Schumann, R., Eggert, A. & Karsten, U. 2009. In vivo growth fluorometry: accuracy and limits of microalgal growth rate measurements in ecophysiological investigations. Aquat. Microb. Ecol. 55:95–104.

Gustavs, L., Schumann, R. & Karsten, U. 2016. Mixotrophy in the terrestrial green alga Apatococcus lobatus (Trebouxiophyceae, Chlorophyta). J. Phycol. 52:311–4.

Haniska, M. D. 1979. Effect of indole-3-acetic acid on growth of Codium fragile subsp. tomentosoides (Chlorophyceae) in culture. J. Phycol. 15:124–7.

Hinajosa-Vidal, E., Marco, F., Martinez-Alberola, F., Escaray, F. J., García-Breijo, F. J., Reig-Armíñana, J., Carrasco, P. & Barreno, E. 2018. Characterization of the responses to saline stress in the symbiotic green microalga Trebouxia sp. TR9. Planta 248:1475–86.

Hirsch, R., Hartung, W. & Gimm, H. 1989. Abscisic-acid content of algae under stress. Bot. Acta. 102:326–34.

Holzinger, A. & Karsten, U. 2013. Desiccation stress and tolerance in green algae: consequences for ultrastructure, physiological and molecular mechanisms. Front. Plant Sci. 4:327.

Hughes, D. T. & Sparrandio, V. 2008. Inter-kingdom signalling: communication between bacteria and their hosts. Nat. Rev. Microbiol. 6:111–20.

Kaldorf, M. & Ludwig-Müller, J. 2000. AM fungi might affect the root morphology of maize by increasing indole-3-butyric acid biosynthesis. Physiol. Plant. 109:58–67.

Kranner, I., Beckett, R., Hochman, A. & Nash, T. H. 2008. Desic- cation-tolerance in lichens: a review. Bryologist 111:576–93.

Kranner, I., Cram, W. J., Zorn, M., Wornik, S., Yoshimura, I., Stabenheiner, E. & Pfiffner, H. W. 2005. Antioxidants and photoprotection in a lichen as compared with its iso- lated symbiotic partners. Proc. Natl. Acad. Sci. USA 102:3141–6.

Kroken, S. & Taylor, J. W. 2000. Phylogenetic species, reproductive mode, and specificity of the green alga Trebouxia forming lichens with the fungal genus Letharia. Bryologist 103:645–60.

Kulichová, J., Škaloud, P. & Neustupa, J. 2014. Molecular diversity of green coralloid microalgae from two sub-Mediterranean European localities. Eur. J. Phycol. 49:345–53.

Liu, L., Pohnert, G. & Wei, D. 2016. Extracellular metabolites from microbial microalgae and their biotechnological potential. Mar. Drugs. 14:91.

Lu, Y. & Xu, J. 2015. Phytohormones in microalgae: a new oppor- tunity for microalgal biotechnology? Trends Plant Sci. 20:273–82.
Maršálek, B., Zahradničková, H. & Hronková, M. 1992. Extracellular production of abscisic acid by soil algae under salt, acid or drought stress. Z. Naturforsch. C 47:701–4.

Mazur, H., Konop, A. & Synak, R. 2001. Indole-3-acetic acid in the culture medium of two axenic green microalgae. J. Appl. Physiol. 13:35–42.

Meeßen, J., Eppenstein, S. & Ott, S. 2013. Recognition mechanisms during the pre-contact state of lichens. II. Influence of algal exudates and ribitol on the response of the mycobiont of Fulgensia braeckei. Symbiosis 59:131–43.

Miransari, M. & Smith, D. L. 2014. Plant hormones and seed germination. Environ. Exp. Biol. 99:110–21.

Muggia, L., Fernandez-Brime, S., Gruber, M. & Wedin, M. 2016. Schizoscyphus as an experimental model for studying interkingdom symbiosis. FEBS Microbiol. Ecol. 92:6165.

Nagpal, P., Ellis, C. M., Weber, H., Ploense, S. E., Barkawi, L. S., Nagpal, P., Ellis, C. M., Weber, H., Ploense, S. E., Barkawi, L. S., Guiffoyle, T. J., Hagen, G., et al. 2005. Auxin response factors ARF6 and ARF8 promote jasmonic acid production and flower maturation. Development 132:1107–18.

Nissen, S. J. & Sutter, E. G. 1990. Stability of IAA and IBA in nutrient medium to several tissue culture procedures. Hot. Science 25:800–9.

Nugari, M. P., Pietrinia, A. M., Caneva, G., Imperi, F. & Visca, P. 2013a. Hormone profiles in Hormonas and cytokinin relationships in Algae. [Ed.] Industrial Applications. The Mycota (A Comprehensive Treatise on Fungi as Experimental Systems for Basic and Applied Research). Springer, Berlin, Heidelberg, Germany, pp. 183–211.

Umehara, M., Gao, M., Akiyama, K., Akatsu, T., Seto, Y., Hanada, A., Li, W., Takeda-Kamiya, N., Morimoto, Y. & Yamaguchi, S. 2013. Structural requirements of strigolactones for shoot branching inhibition in rice and Arabidopsis. Plant Cell Physiol. 50:1059–72.

Verma, V., Ravindran, P. & Kumar, P. P. 2016. Plant hormone-mediated regulation of stress responses. BMC Plant Biol. 16:86.

Wang, X. Y., Wei, X. L., Luo, H., Kim, J. A., Jeon, H. S., Koh, Y. J. & Hur, J. S. 2010. Plant hormones promote growth in lichen-forming fungi. Mycologia 38:176–9.

Wang, Y., Han, K. S., Wang, X. Y., Koh, Y. J. & Hur, J. S. 2009. Effect of ribitol and plant hormones on aposymbiotic growth of the lichen-forming fungi of Ramalina farinacea and Ramalina fastigiata. Mycologia 37:29–30.

Went, F. W. & Thimann, K. V. 1937. Phytohormones. The Macmillan Company, New York, USA, pp. 57–71.

White, T. J., Bruns, T., Lee, S. & Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis, M. A., Gelfand, D. H., Sninsky, J. J., White, T. J. [Eds.] PCR Protocols: A Guide to Methods and Applications, 19 edn. Academic Press, Cambridge, MA, USA, pp. 18:315–22.

Xu, J., Zhou, L., Venturi, V., He, Y. W., Kojima, M., Sakakibara, H., Höfte, M. & De Vleesschauwer, D. 2015. Phytohormone-mediated interkingdom signalling shapes the outcome of rice-Xanthomonas oryzae pv. oryzae interactions. BMC Plant Biol. 15:10.

Yoshida, K., Igarashi, E., Mukai, M., Hirata, K. & Miyamoto, K. 2003. Induction of tolerance to oxidative stress in the green alga, Chlamydomonas reinhardtii, by abscisic acid. Plant Cell Environ. 26:451–7.

Yoshimura, I., Yamamoto, Y., Nakano, T. & Finnie, J. 2002. Isolation and culture of lichen photobionts and mycobionts. In: Kranz, I. C., Beckett, R. F. & Varma, A. K. [Eds.] Protocols in Lichenology, Springer, Berlin, Heidelberg, Germany, pp. 3–33.

Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher’s web site:

Figure S1. Microboxes used to measure cellular and released phytohormone levels.

Table S1. Cellular and extracellular levels of the phytohormones IAA, ABA, JA, GA₃, GA₄, IBA and ZT of the free-living alga, Chlorella ellipsoidea, Apatococcus lobatus and Myrmeica bisecta, after 7 days of exposure to different treatments. DL, dim light; HL, high light; DL + DH, de-rehydration cycle under dim light; HL + DH, de-rehydration cycle under high light; subscript letters C and E denote cellular and extracellular phytohormone levels, respectively, normalized to algal dry mass (DM).

Table S2. Cellular and extracellular levels of the phytohormones IAA, ABA, JA, GA₃, GA₄, IBA and ZT of the lichen-forming alga, Astrochloris
glomerata, Trebouxia decolorans and Trebouxia sp., after 7 days of exposure to different treatments. DL, dim light; HL, high light; DL + DH, de-rehydration cycle under dim light; HL + DH, de-rehydration cycle under high light; subscript letters C and E denote cellular and extracellular phytohormone levels, respectively, normalized to algal dry mass (DM).