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Ecophysiological Response on Dehydration and Temperature in Terrestrial *Klebsormidium* (Streptophyta) Isolated from Biological Soil Crusts in Central European Grasslands and Forests

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Abstract The green algal genus *Klebsormidium* (Klebsormidiophyceae, Streptophyta) is a typical member of biological soil crusts (BSCs) worldwide. Ecophysiological studies focused so far on individual strains and thus gave only limited insight on the plasticity of this genus. In the present study, 21 *Klebsormidium* strains (*K. dissectum*, *K. flaccidum*, *K. nitens*, *K. subtile*) from temperate BSCs in Central European grassland and forest sites were investigated. Photosynthetic performance under desiccation and temperature stress was measured under identical controlled conditions. Photosynthesis decreased during desiccation within 335–505 min. After controlled rehydration, most isolates recovered, but with large variances between single strains and species. However, all *K. dissectum* strains had high recovery rates (>69%). All 21 *Klebsormidium* isolates exhibited the capability to grow under a wide temperature range. Except one strain, all others grew at 8.5 °C and four strains were even able to grow at 6.2 °C. Twenty out of 21 *Klebsormidium* isolates revealed an optimum growth temperature >17 °C, indicating psychrotrophic features. Growth rates at optimal temperatures varied between strains from 0.26 to 0.77 μ day^{-1}. Integrating phylogeny and ecophysiological traits, we found no phylogenetic signal in the traits investigated. However, multivariate statistical analysis indicated an influence of the recovery rate and growth rate. The results demonstrate a high infraspecific and interspecific physiological plasticity, and thus wide ecophysiological ability to cope with strong environmental gradients. This might be the reason why members of the genus *Klebsormidium* successfully colonize terrestrial habitats worldwide.

Keywords *Klebsormidium* · Aeroterrestrial algae · Desiccation · Growth · Ecophysiology · Temperature · Biodiversity Exploratories

Introduction

Members of the filamentous green algal genus *Klebsormidium* (Klebsormidiophyceae, Streptophyta) can be found worldwide in a number of geographical regions ranging from temperate to tropical, and from cold to hot environments [e.g., 1–5]. Like many other green algae, they occur in freshwater habitats, but *Klebsormidium* species are not restricted to aquatic systems, since they colonize plant and rock surfaces, as well as soil [6]. In Europe, *Klebsormidium* has been reported from a number of artificial and natural habitats, including basements of walls [7], golf courses [8], dumps and post-mining areas [9, 10], dunes [11, 12], soil [3], and biological soil crusts (BSCs) [13, 14]. Among these habitats, BSCs have the most complex community structure. These microecosystems consist of cyanobacteria, bacteria, algae, microfungi, lichens, and bryophytes, compromising the topsoil layer bound by organic material by these organisms [15]. Through their ecological important functions such as primary production [16], nutrient cycling [16–19], water retention [17], and soil stabilization [18], BSCs can be characterized as ecosystem engineers. As a member of pioneer habitats and BSCs, *Klebsormidium* has the potential to cope with wide

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changes of temperature, water availability, and solar radiation [e.g., 19–23]. Changes in the environmental conditions are not only a result of the intrinsic characteristics of the habitat, but they could also be a result of mechanical disturbances, nutrient inputs, and land use intensities by agriculture and forestry. Land use is estimated as the major driver for biodiversity changes in the near future [24].

The phylogenetic diversity of *Klebsormidium* was in the past years subject of a number of studies. Molecular methods based on ITS and *rbc*L sequences identified seven superclades A to G [25]. Rindi et al. [24] showed that the genetic diversity is greater than the morphological diversity would suggest [1]. Clade A corresponded with the genus *Interfilum*, and B–G comprised *Klebsormidium* species from various habitats and geographical origin [25, 26]. *Klebsormidium* species, which have been found in Europe, were assigned to superclades B–F [1, 13, 25, 27–30], while members of clade G were so far mainly reported in BSCs of South African drylands [25, 31]. However, some strains seem to prefer certain habitats, such as low or high pH substrates [27, 29]; either humid or xerophytic conditions [13]; aquatic, artificial, or natural habitats [32, 33]; or BSCs in arid regions [25]. These conspicuous preferences point to genetic adaptations in *Klebsormidium* with regard to environmental conditions. In recent years, several studies focused on physiological traits against desiccation, temperature, light, UVR, and pH [19–23, 27, 31, 34–39]. Morphological and structural features, for example, can explain differences in desiccation tolerance between two co-occurring *Klebsormidium* species from high alpine BSCs [37, 40, 41]. *K. crenulatum* forms long, firm filaments, sometimes as rope-like aggregates, which protect against water loss. In contrast, *K. dissectum* exhibits thinner filaments, which easily disintegrate and hence resulte in faster dehydration. The broad ecophysiological response patterns along with specific ultrastructural properties of the cell wall in *Klebsormidium* are supported by some recent transcriptomic and genomic analysis [34, 42]. Both studies demonstrated that within *Klebsormidium* similar plant-like ancestral physiological adaptations for terrestrial environments exist. The genome of *K. flaccidum* (superclade E) shows the presence of a basic system involved in high-light protection, which includes cyclic electron flow activity at photosystem I, and that is activated under radiation stress and desiccation [42]. The cyclic electron flux is assumed to enhance the proton gradient across the thylakoid membrane, which induces nonphotochemical quenching and ATP biosynthesis, followed by the dissipation of excess radiation energy [42].

So far, all ecophysiological studies focused on individual strains representing the existing *Klebsormidium* superclades. Conclusions from single results to whole phylogenetic lineages cannot be generalized [32], as these studies are spotlights and differences might be specific characteristics of individual strains. A comprehensive ecophysiological study on a high number of genetically closely related *Klebsormidium* strains is still missing. Therefore, we characterized for the first time ecophysiological traits in 21 *Klebsormidium* strains from temperate BSCs of the so-called Biodiversity Exploratories in Germany [43]. The Biodiversity Exploratories are a German Science Foundation funded project for large-scale and long-term functional biodiversity research providing a set of standardized field plots with different land use intensities in grassland and forests along a north-south meteorological and soil gradient in Germany [43]. Many of these environmentally well-characterized plots contain BSC communities, and hence provide the unique opportunity to investigate whether and how the ecophysiological response patterns of the studied *Klebsormidium* isolates are influenced along those gradients and land use intensities. A previous study characterized 75 strains genetically and revealed a surprisingly low genetic diversity in each of the prevailing superclades B/C and E [28].

The aim of the present study was to evaluate physiological plasticity among many genetically closely related *Klebsormidium* strains, and to verify whether there are ecophysiological differences that might explain taxa-specific habitat preferences. Desiccation tolerance was investigated by measuring photosynthetic activity under controlled dehydration and recovery conditions. Additionally, the growth response as function of a temperature gradient was determined. Using the data of the ecophysiological traits, we analyzed the phylogenetic signals.

Material and Methods

Strain Origin and Culture Conditions

The strains were originally collected and isolated from BSCs collected in 2011 and 2012 within the DFG priority program Biodiversity Exploratories [44]. Three regions in Germany, Swabian Alb (sub-montane to montane plateaus), Hainich (a low mountain range), and Schorfheide-Chorin (lowland with diverse postglacial geomorphological structures), forest and grassland sites were sampled (Fig. 1). The sampled plots represent different land use systems ranging from near-natural to intensively used plots. Grassland plots were categorized into pastures, mown pastures, and meadows differing in fertilization, grazing, and number of cuts per year. The forest plots were either natural sites or age class forests in different states. Detailed BSC sampling strategy and isolation of the *Klebsormidium* strains via enrichment culturing technique are described by Glaser et al. [28]. Detailed data on habitat, origin, and taxonomic assignment of the 21 strains are summarized in Table 1.

The stock cultures were maintained in Erlenmeyer flasks (volume 100–200 mL) filled with modified Bold's Basal Medium (3NBBM+V; [44]). The algae were kept at 20 °C

Fig. 1 Location of the three sampling sites Swabian Alb, Hainich, and Schorfheide-Chorin in the Biodiversity Exploratories (Fischer et al. [42])

and 30–40 μmol photons m^{-2} s^{-1} under a light/dark cycle of 16:8 h L/D (Osram Daylight Lumilux Cool White lamps L36W/840, Osram, Munich, Germany). The low-light conditions have been chosen on the base of previous experiments, which all indicate optimum photosynthesis at low-light conditions for *Klebsormidium* and closely related *Interfilum* species [33, 41–43]. For all experiments, log-phase cultures were always used.

Phylogenetic Analysis

Twenty-nine strains of *Klebsormidium* and *Interfilum* were used for comparison with the 21 herein ecophysiologically investigated *Klebsormidium* strains (Table 1). The sequences of the 21 *Klebsormidium* strains were obtained as described by Glaser et al. [32]. Multiple alignment of ITS1, 5.8S, and ITS2 rDNA sequences of all strains were assembled using clustalo algorithm implemented in seaview software v4.4.2 [45]. The evolutionary model that fitted the data was chosen on the lowest AIC [46] calculated with the software MEGA v7.0.18 [47]. The unrooted Bayesian tree was constructed in MrBayes v3.2.2 [48] using the GTR+G+I model with 5,000,000 generations. Two runs of four Monte Carlo Markov chains were calculated simultaneously, with trees sampled every 500 generations. Split frequency between the runs was below 0.01 at the end of calculation. The trees sampled before the likelihood scores reached saturation were discarded afterwards.

Desiccation and Rehydration Experiment

For the desiccation experiments, a standardized setup was applied to follow kinetics of controlled dehydration and subsequent rehydration on the effective quantum yield of photosystem II (PSII) using noninvasive pulse amplitude modulation (PAM) fluorometry. The experiment was carried out in a modified desiccation chamber after [39]. All PAM measurements were done on low-light acclimated samples (30–40 μmol photons m^{-2} s^{-1}). Cells of each *Klebsormidium* strain were concentrated on four replicate MGF glass fiber filters (Munktell and Filtrak, Bärenstein, Germany). Onto each filter 200 μL of the fragment suspension (ca. 1–2 mg chlorophyll *a* L^{-1}) were concentrated in the center as a light green spot. These moist filters were positioned on perforated metal grids on top of a glass Petri dish placed on four glass columns inside a transparent 200-mL polystyrol box. Each box was filled with 100 g of freshly activated silica gel (Silica Gel Orange, Carl Roth, Karlsruhe, Germany) in order to create relative air humidity of ~10% and sealed with a transparent top lid. The boxes were kept at 22 ± 2 °C and 30–40 μmol photons m^{-2} s^{-1} (Osram light sources, see above). The methodological details are described in Karsten et al. [39].

The effective quantum yield ($\Delta F/F_m'$) of photosystem II was regularly determined during the dehydration period (up to 505 min depending on the strain) using a pulse-amplitude modulated fluorimeter (PAM 2500, Heinz Walz GmbH, Effeltrich, Germany). $\Delta F/F_m'$ was calculated as $(F_m' - F)/F_m'$ with F as the fluorescence yield of light-treated algal cells and F_m' as the maximum light-adapted fluorescence yield after employing a 800-ms saturation pulse as described by Schreiber and Bilger [49]. The PAM light probe was positioned outside the coverlid of the boxes (always 2-mm distance) to guarantee undisturbed RAH conditions inside, i.e., all fluorescence measurements were done through the polystyrol lids. The distance from the PAM light probe to the algal sample onto the glass fiber filters was always kept constant at 10 mm.

After the dehydration period, the dried glass fiber filters were transferred to a new polystyrol box which was filled with 100 mL tap water instead of silica gel to create a high-humidity atmosphere (>95%). The filters were rehydrated by adding 200 μL of the standard growth medium (3NBBM+V, see above) to each filter and recovery of $(F_m' - F)/F_m'$ was followed with the same methodology as described above in regular intervals with the last measurement 24 h after the experimental begin.

Table 1 Overview of the *Klebsormidium* strains investigated in this study: strain number, assigned species according to Rindi et al. [24], origin, short description of the sampling site, and accession number

Strain	Identification	Clade assignment to Rindi et al. [24]	Origin, habitat	Accession number
AEG12-1	*K.* cf. *dissectum*	B/C	RW 3526015, HW 5361315; fertilized meadow, Swabian Alb	LK392526
AEG18-1	*K.* cf. *dissectum*	B/C	RW 3538700, HW 5360700; fertilized meadow, Swabian Alb	LK392544
AEG42-2	*K.* cf. *dissectum*	B/C	RW 3527920, HW 5362310; fertilized mown pasture, Swabian Alb	LK392539
HEG3-1	*K.* cf. *dissectum*	B/C	RW 4390100, HW 5652600; fertilized meadow, Hainich	LK392527
SEG31-3	*K.* cf. *flaccidum*	B/C	RW 5422220, HW 5891400; unfertilized meadow, Schorfheide-Chorin	LK392537
SEG37-3	*K.* cf. *flaccidum*	B/C	RW 5424911, HW 5889781; unfertilized pasture, Schorfheide-Chorin	LK392519
AEW13-2	*K.* cf. *subtile*	E	RW 3529608, HW 5360197; managed spruce forest, Swabian Alb	LK392554
AEW23-1	*K.* cf. *flaccidum*	B/C	RW 3536219, HW 5361004; managed beech forest, Swabian Alb	LK392531
AEW33-3	*K. nitens*	E	RW 3536400, HW 5361600; managed spruce forest, Swabian Alb	LK392573
AEW35-1	*K. subtile*	E	RW 3530790, HW 5367630; managed beech forest, Swabian Alb	LK392553
AEW35-5	*K. flaccidum*	B/C	RW 3530790, HW 5367630; managed beech forest, Swabian Alb	LK392520
HEW1-2	*K. nitens*	E	RW 4382900, HW 5673600; managed spruce forest, Hainich	LK392582
HEW1-3	*K. nitens*	E	RW 4382900, HW 5673600; managed spruce forest, Hainich	LK392580
HEW36-3	*K.* cf. *dissectum*	B/C	RW 4388700, HW 5664670; natural beech forest, Hainich	LK392541
HEW40-1	*K.* cf. *dissectum*	B/C	RW 4394870, HW 5661610; natural beech forest, Hainich	LK392540
HEW42-5	*K.* cf. *flaccidum*	B/C	RW 4392230, HW 5664203; natural beech forest, Hainich	LK392534
HEW42-7	*K.* cf. *flaccidum*	B/C	RW 4392230, HW 5664203; natural beech forest, Hainich	LK392535
HEW42-9	*K. flaccidum*	E	RW 4392230, HW 5664203; natural beech forest, Hainich	LK392581
SEW25-3	*K.* cf. *subtile*	E	RW 5416808, HW 5866100; managed oak forest, Schorfheide-Chorin	LK392557
SEW29-1	*K. nitens*	E	RW 5423780, HW 5876699; managed pine forest, Schorfheide-Chorin	LK392552
SEW35-1	*K. nitens*	E	RW 5423000, HW 5864916; managed beech forest, Schorfheide-Chorin	LK392585
SEW35-6	*K.* cf. *subtile*	E	RW 5423000, HW 5864916; managed beech forest, Schorfheide-Chorin	LK392591

Geographical position (RW and HW) is given as Gauß-Krüger coordinate system

Growth Experiment Under Temperature Gradient

The growth rate was monitored as an increase of in vivo chl *a* fluorescence F_t over time as an indicator for biomass accumulation [50]. For the in vivo measurement of the effect of different temperatures on the growth, cultures were grown in quadruples in 24-well microplates (Costar, Corning GmbH, Kaiserslautern, Germany) with each well filled with 1.5% agar of modified 3NBBM+V. Ten different temperatures were applied (6.2, 7.0, 8.5, 11.6, 14.4, 17.0, 18.8, 20.2, 21.7, 23.5, 24.5, and 26.9 °C ± 1 °C) at 40 μmol photons m^{-2} s^{-1} and a light/dark cycle of 16:8 h L/D. For the growth experiments, a modified self-constructed algal incubator as described in Woelfel et al. [51] was used. The adjusted incubator was equipped with an LED array (LED neutral white Ediline III 3.5 W COB Modul, Edison Opto Corporation, Taipei, Taiwan). All measurements were done with log-phase cultures, which were adapted for 10 days to the temperature conditions described above. After this preincubation, 200 μL of the fragment suspension (ca. 1–2 mg chlorophyll *a* L^{-1}) was

transferred onto the agar medium in a new 24-well microplate with quadruples for each strain. The chlorophyll *a* fluorescence as proxy for growth was measured every 24 h for 10 days with a SpectraMax M2e multiplate reader (MPR; Molecular Divices, Biberach, Germany). For chlorophyll *a* fluorescence, an excitation wavelength of 480 nm was chosen and the emitted fluorescence was detected at a wavelength of 680 nm, using the top read function. Increasing fluorescence values were measured every 24 h for 10 days as relative fluorescence units (RFUs). The fluorescence measured directly after the inoculation of the 24-well microplates served as a starting value. Before each measurement, a dark incubation of 10 min was performed in order to open all reaction centers of photosystem II. The calculation of the growth rate for each individual replicate was performed according to the well-established protocol of Gustavs et al. [50] which requires a linear relationship between algal biomass and chlorophyll *a* fluorescence as well as exponential growth of the investigated algae. This method offers a simple, rapid, noninvasive, reproducible, and calibration-free measurement of growth rates in unialgal cultures, and the low detection limits avoid self-shading and nutrient limitation during growth rate determination.

During the exponential growth phase of the *Klebsormidium* strains, the fluorescence F_t (RFU) at a given time point t can be calculated as $F_t = F_0\, e^{\mu t}$ with F_0 (RFU) as initial fluorescence and μ (day^{-1}) as growth rate in the respective time interval. For the calculation of μ the measured chlorophyll *a* fluorescence values were fitted with the growth equation mentioned above. The fitting was based on the sum of the mean square error A_{Fl} which was calculated as $(F_t - F_{t,cal})^2$ with F_t (RFU) as measured fluorescence at a given time point t and $F_{t,cal}$ (RFU) as calculated fluorescence at a given time point t with at least three subsequent fluorescence values. A_{Fl} was minimized by the MS Excel 2013 add-in Solver with the model "GRG-nonlinear". A growth rate of 1 means a doubling of biomass within 1 day.

Statistical Analysis

All statistical analyses were done with the statistical software R [52].

Statistical significance of the median of recovery rates were tested with a one-way ANOVA followed by a Tukey's multiple comparison test to find significant differences between species.

For the quantitative traits of dehydration and recovery rate, the existence of phylogenetic signal was calculated using Blomberg's K and Pagel's λ [53, 54]. Since available data are restricted to our analyzed *Klebsormidium* and as outgroup species *Interfilum* sp. SAG36.88 (EU434027), we could only use these two traits. The trait values for *Interfilum* sp. SAG36.88 were taken from Karsten et al. [39]. Further values of *Interfilum* sp. SAG36.88 and other potential strains were based on different methodological approaches and thus not suited for the analysis performed.

Blomberg's K quantifies the phylogenetic signal by estimating the accuracy of the original phylogeny to describe the variance-covariance pattern observed in the data set. K values of 1 imply a strong phylogenetic signal and trait evolution under Brownian motion model. K value >1 imply closer similarity of relatives, than expected under Brownian motion model of trait evolution. The reverse is true for K <1.

Pagel's λ measures correlations relative to the correlation expected under Brownian evolution. Values of $\lambda = 1$ indicates a strong phylogenetic signal in the trait and the evolution under Brownian motion model. Values <1 imply no phylogenetic signal in the trait than expected under Brownian motion model.

The K value and randomization test was calculated by "phylosignal" function in the Picante package [55]. While "phylosignal" accept no polytomies, we transformed all polytomies into series of dichotomies with the function "multi2di" in the Ape package [56]. Values for λ were calculated using "fitContinous" function of the Geiger package [57].

Additionally, in order to visualize the dissimilarities between the *Klebsormidium* strains according to their ecophysiological performances, a redundancy analysis (RDA) was performed using the command rda from the vegan package. The following ecophysiological parameters were included: minimum, maximum and optimum temperature, growth rate, recovery rate, and dehydration time.

Results

Phylogenetic Analysis

The genetic identification of the analyzed strains assigned to the *Klebsormidium* clades B/C and E followed the proposed system of Rindi et al. [25] (Fig. 2). The sequence difference between strains belonging to the clades B and C were low; therefore, we referred to B/C as one clade [25, 27]. All 21 *Klebsormidium* strains used is the present study could unambiguously assigned to the strains responding to *K. dissectum*, *K. flaccidum*, *K. nitens*, and *K. subtile* using ITS1, 5.8S, and ITS2 rDNA sequences.

Dehydration and Recovery

The standardized methodological approach with the dry chamber and PAM measurements from outside allowed comparative effective quantum yield determinations in all samples during the desiccation experiment (for initial values, see Supplement Table 2). The results demonstrated

Fig. 2 Molecular phylogeny of *Interfilum* and *Klebsormidium* based on ITS1, ITS2, and 5.8S rDNA sequence comparison. Bayesian tree showing phylogenetic position of the studied *Klebsormidium* strains (*bold*) within the genus *Klebsormidium*. The clade designation (A–G) followed the suggestion of Rindi et al. [24]. Probability under 0.6 is not shown

that all tested *Klebsormidium* strains gave a positive signal of $\Delta F/F_m'$ for at least 335 min, before a threshold was reached, after which the signal rapidly decreased to a quantum yield of zero or values near to zero (Fig. 3). The strains identified as *K. flaccidum* and *K.* cf. *flaccidum*, and *K. dissectum* and *K.* cf. *dissectum* (both B/C clade) had similar time points until the thresholds of $\Delta F/F_m'$ were reached, 380–505 and 400–505 min, respectively. The *K. nitens* strains showed a decrease of $\Delta F/F_m'$ for 390–450 min. The *K. subtile* and *K.* cf. *subtile* strains exhibited for 335–480 min a positive signal of $\Delta F/F_m'$ (Fig. 3). The time points within each species showed the lowest variation in *K. nitens* and were largest in *K. subtile*.

After dehydration and subsequent rewetting, almost all samples were rehydrated within 90 min (data not shown). Nevertheless, recovery of the effective quantum yield was variable between the *Klebsormidium* strains of one species, as well as between species, and there were conspicuously different responses during the rehydration process (Fig. 4). In general, the recovery after 1440-min rehydration varied between 0.4 and 95.9% (Fig. 4). The *K. dissectum* and *K.* cf. *dissectum* strains (clade B/C) had in comparison with all other *Klebsormidium* strains the highest recovery rates of 68.7–95.9%. The recovery rates for *K. flaccidum* and *K.* cf. *flaccidum* strains varied between 0.4 and 64.5%. Strain *K. flaccidum* AEW35-5 and *K.* cf. *flaccidum* SEG37-3, both B/C clade members, exhibited with 0.4 and 1.5% the overall lowest recovery rates (Fig. 4). Such low recovery rates indicate the non-survival of the experimental desiccation conditions, which is supported by the observation of no further increase in fluorescence signal even after extended recovery intervals (data not shown). *K. nitens* strains showed recovery rates of 20.5–77.7%, whereas only HEW1-3 and SEW29-1 reached more than 50%. Three out of four of *K. subtile* and *K.* cf. *subtile* had high recovery rates (64.0–90.4%; Fig. 4). As we had for *K. subtile* and *K. dissectum* strains quite high recovery rates, there was no significant difference on both species. The same was observed for the low recovery rates of *K. nitens* in comparison with *K. flaccidum*. Conversely, this means that the differences between *K. dissectum* and

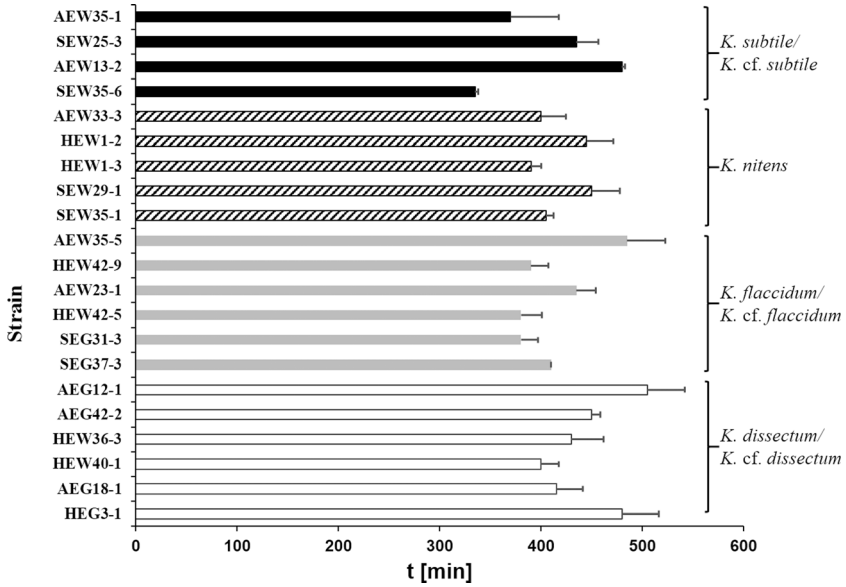

Fig. 3 The effect of desiccation on the chlorophyll *a* fluorescence of photosystem II in 21 strains of *Klebsormidium* isolated from biological soil crusts in the Biodiversity Exploratories (see Fig. 1). Shown are the effect of controlled desiccation on the time point when the effective quantum yield ($\Delta F/F_m'$) reached zero (no fluorescence signal detectable) during the course of the experiment. (n = 4, median value ± SD). Additionally, the phylogenetic assignment towards species, B/C clade and E clade of each strain is given. All measurements were done under 30–40 μmol photons m^{-2} s^{-1}, 22 ± 1 °C and measured with a PAM 2500

K. flaccidum, and between *K. dissectum* and *K. nitens* were highly significant ($p \leq 0.001$). The same was true for the comparison of *K. subtile* and *K. flaccidum*, as well *K. subtile* and *K. nitens* ($p \leq 0.001$ and $p \leq 0.01$, respectively).

The ecophysiological trait dehydration and recovery rate showed no phylogenetic signal in Blomberg's K and Pagel's λ. However, both trait values were lower than expected under Brownian model of evolution ($K < 1$ and $\lambda < 1$). Only the recovery rate has a trend towards statistical significance ($K = 0.109$ and $p = 0.014$).

Some strains showed a fast return of the quantum yield value within the first 90 min after rewetting, e.g., *K.* cf. *subtile* AEW13-2, others had after the same period only weak fluorescence signals, indicating a very low to almost no activity of the photosynthetic apparatus, e.g., *K.* cf. *flaccidum* SEG37-3 (Supplement Fig. 1). However, even longer rehydration periods up to 1500 min did not lead to significantly increased recovery values (Supplement Fig. 1). This observed response pattern further points to increasing mortality of strains with low recovery rates.

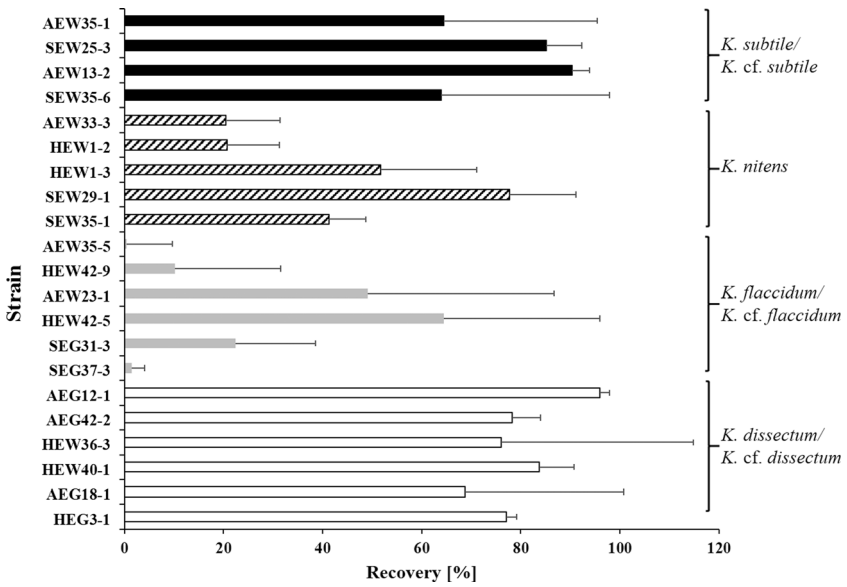

Fig. 4 The effect of controlled rehydration on recovery rates on the effective quantum yield ($\Delta F/F_m'$) of photosystem II in 21 strains of *Klebsormidium* isolated from biological soil crusts in the Biodiversity Exploratories (see Fig. 1) (n = 4, median value ± SD). For better comparison, the highest effective quantum yield value have been standardized to 100%. Additionally, phylogenetic assignment towards species, B/C clade, and E clade of each strain is given. All measurements were done under 30–40 μmol photons m^{-2} s^{-1}, 22 ± 1 °C and measured with a PAM 2500

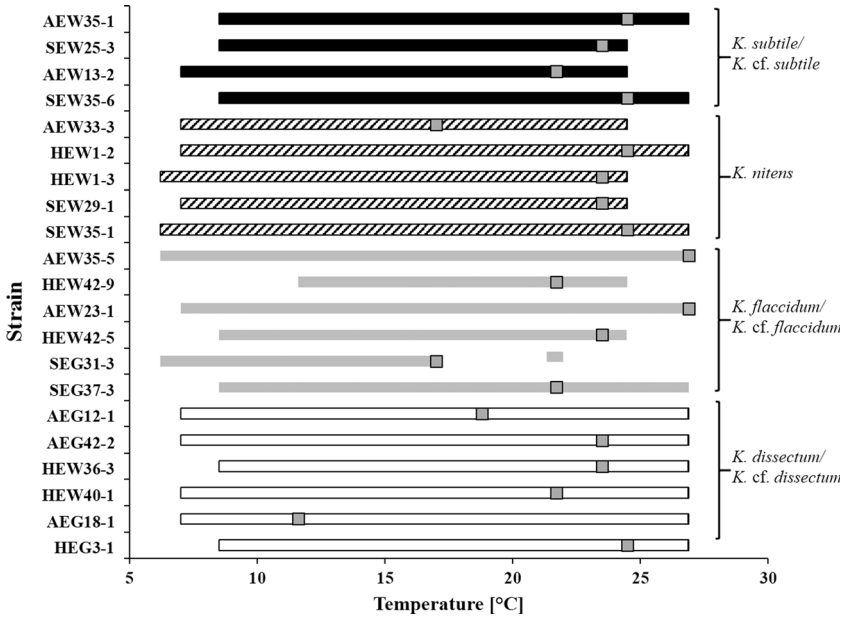

Fig. 5 Temperature range in which the studied 21 *Klebsormidium* strains studied showed growth (*bars*). *Gray boxes* mark the temperature with the highest growth rate (as μ day^{-1}, $n = 4$). *Asterisks* mark strains, which might have higher optimum temperature than tested in the present experiment. Additionally, species identification and phylogenetic assignment towards B/C clade and E clade of each strain is given

Temperature Requirements for Growth

The effect of increasing temperatures showed a wide range in the capability for growth in all investigated *Klebsormidium* strains, whereas only *K. nitens* SEW35-1 and *K. flaccidum* AEW35-5 grew at the complete temperature range tested (6.2–26.9 °C; Fig. 5). *K. dissectum* and *K* cf. *dissectum* strains required a minimum temperature of 8.5 °C for growth; four strains were able to grow at 7.0 °C (AEG12-1, AEG18-1, AEG42-2, and HEW40-1). The maximum growth temperature for all *K. dissectum* and *K* cf. *dissectum* strains were 26.9 °C. *K. flaccidum* and *K.* cf. *flaccidum* strains exhibited the overall most individual growth pattern for each strain. The minimal growth temperatures varied between 6.2 and 11.6 °C. The same was observed for the maximum temperatures, which varied between 21.7 and 26.9 °C. The most striking growth pattern of all tested strains was observed in *K.*cf. *flaccidum* SEG31-3 (Figs. 5 and 6). This strain was able to grow between 6.2 and 17 °C, with optimum growth at 17 °C. Surprisingly, SEG31-3 grew at 23.5 °C, but not between 18.8

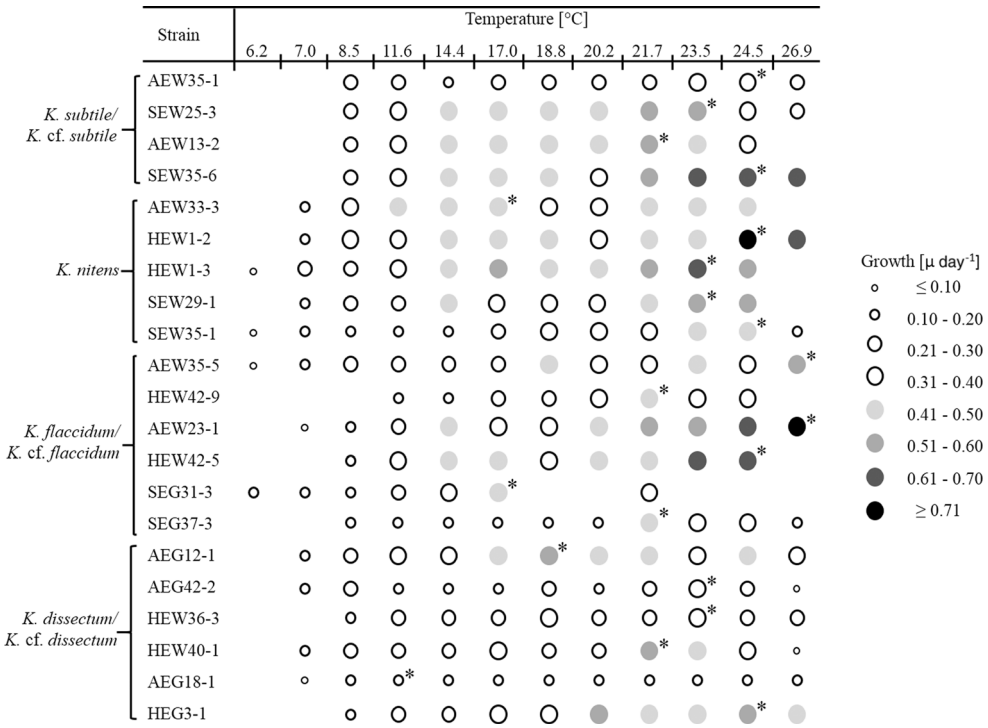

Fig. 6 Growth rate (as μ day^{-1}) of 21 *Klebsormidium* strains under optimum temperature conditions ($n = 4$, median value ± SD). Additionally, species identification of each strain is given

and 21.7 °C (Fig. 6). This response pattern was observed in two independent runs of the experiment (data not shown). The *K. nitens* strains grew at a minimum temperature of 7.0 °C, and two strains were already able to grow at 6.2 °C (HEW1-3 and SEW29-1). The maximum temperature for *K. nitens* were 24.5–26.9 °C. The *K. subtile* and *K.* cf. *subtile* strains required a minimum temperature of 8.5 °C for growth. One *K.* cf. *subtile* strain was already able to grow at 7.0 °C (AEW13-2). The maximum growth temperature for *K. subtile* and *K.* cf. *subtile* strains were 24.5 and 26.9 °C, respectively (Fig. 5).

The B/C clade strains *K. dissectum* and *K. flaccidum* exhibited both a broad distribution in their optimal growth temperature (Fig. 6; Suppl. 1). The *K. subtile* and *K. nitens* strains, members of the E clade, showed in general more coherent optimal growth temperatures within the higher tested temperatures (Fig. 6; Suppl. 1). The six *K. dissectum* strains exhibited optimal growth temperatures ranging from 11.6 to 24.5 °C with growth rates between 0.26 and 0.54 μ day^{-1} (Fig. 6) The *K. dissectum* group showed with 11.6 °C the overall lowest optimal temperature, as well with 0.26 μ day^{-1} the lowest growth rate. Nevertheless, *K. dissectum* strain AEG18-1 was capable to grow between 7.0 and 26.9 °C, at least with low rates. Strains, which were identified as *K. flaccidum* and *K.* cf. *flaccidum*, exhibited a similarly diverse pattern for optimal growth temperatures as the *K. dissectum* isolates. The temperatures ranged between 17.0 and 26.9 °C, with growth rates between 0.42 and 0.77 μ day^{-1} (Fig. 6). The *K. flaccidum* strains AEW35-5 and AEW23-1 showed the highest growth rate at the highest tested temperature of 26.9 °C (Fig. 6). If both strains are capable to grow at even higher temperature remains open. *K. nitens* strains exhibited the highest growth rates at temperatures of 23.5–24.5 °C (0.60–0.74 μ day^{-1}). Only *K. nitens* AEW33-3 had a much lower optimal growth temperature of 17.0 °C with a rate of 0.46 μ day^{-1} (Fig. 6) The optimal growth temperature for *K. subtile* and *K.* cf. *subtile* strains varied between 21.7 and 24.5 °C with growth rates between 0.31 and 0.59 μ day^{-1}. The ecophysiological trait optimal growth temperature had no phylogenetic signal on Blomberg's K (K = 0.553, p = 0.088). However, similar to the traits dehydration and recovery rate, optimal growth temperature value was lower than expected under Brownian model of evolution ($K < 1$ and $\lambda < 1$).

The phylogenetic signal of the traits dehydration, recovery rate, and optimal temperature was not significant. The recovery rate and growth rate captured ~55% of total variance in the RDA (Fig. 7). There is one group of strains with low and another one with high recovery activity. All strains plotted in direction of the arrow "Recovery" showed a high recovery rate (> 69%); all other strains plotted in the opposite direction of this arrow exhibited a low recovery rate (< 25%). The group of strains with high recovery rate consist all *K. dissectum* strains, as well *K. subtile* AEW 13-2 and SEW25-3, and *K. nitens* SEW29-1. The RDA clearly separated *K.* cf. *dissectum* AEG18-1 as the most isolated from all other *Klebsormidium* strains, especially due to the combination of

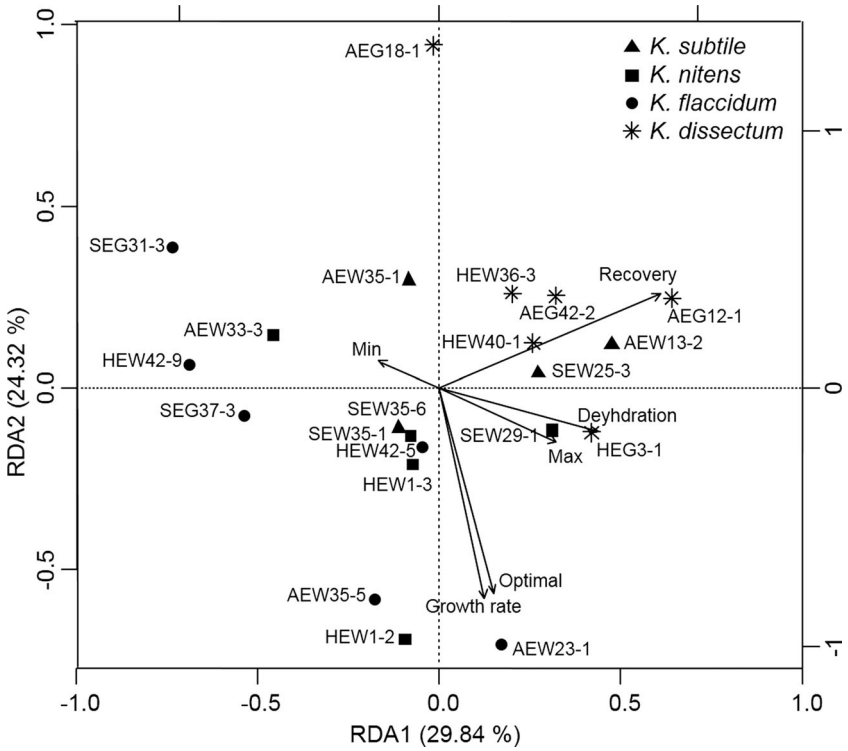

Fig. 7 Redundancy analysis (RDA) diagram showing the response of 21 *Klebsormidium* strains to dehydration time (Dehydration), recovery rate (Recovery), temperature range (Min, Max), optimum temperature (Optimal), and growth rate (Growth rate)

the lowest optimum growth temperature (11.6 °C) in conjunction with the lowest growth rate (0.26 μ day^{-1}; compare Figs. 5 and 6).

Discussion

Dehydration and Rehydration

The standardized approach to investigate desiccation stress in all 21 Central European *Klebsormidium* strains isolated from BSCs revealed a tolerance for at least 335 min. Members of both phylogenetic clades exhibited similar temporal ranges until a zero signal of the effective quantum yield was reached. In B/C-clade (*K. dissectum* and *K. flaccidum*), it ranged from 380 to 505 min, and 350 to 480 min in E clade members (*K. nitens* and *K. subtile*). More specifically, the difference was not only within one species high (*K. subtile* with 335–480 min) but also between both E clade members (*K. nitens* vs. *K. subtile*). In contrast to cyanobacteria, lichens, bryophytes, and angiosperm resurrection plants, only few publications on the ecophysiological responses under changing water potentials exist in terrestrial green algae (Streptophyta and Chlorophyta; [19, 58], and references therein), which makes a comparison challenging. Karsten et al. [31] compared four *Klebsormidium* sp. strains concerning desiccation tolerance, three from African G clade and one Arctic E clade member (according to [25]), and used a comparable methodological approach. The African strains exhibited photosynthetic activity for 330–380 min and the Arctic *Klebsormidium* sp. strain little less (300 min) until the effective quantum yield was completely inhibited [31]. In contrast, *K. dissectum* (E clade) from a European high alpine BSC revealed under ambient experimental desiccation conditions only for 180 min a positive effective quantum yield signal [40]. Arctic and Antarctic strains from B as well E clade showed with ~410 min similar dehydration response curves [59] as in the present study. However, all these studies focused on a low number of individual strains, which makes it difficult to draw for single species or clades conclusions that are more comprehensive. This is even more difficult by the fact of the use of different methodological approaches in the mentioned studies [e.g., 21, 23, 37, 40]. The observation by Mikhailyuk et al. [13] that B/C clade members are more adapted to moderate xerophytic conditions than clade E members is neither supported nor rejected by the data presented in our study.

For the other genera of the Klebsormidiophyceae, *Interfilum*, *Entransia*, and *Hormidiella*, only limited ecophysiological data on the response to desiccation stress exist too. *Interfilum* showed with 330–405 min similar dehydration periods like *Klebsormidium* [39]. Two strains of the sister genus *Entransia* exhibited with 210–230 min a shorter dehydration time and one *Hormidiella* isolate with 370 min a similar temporal interval in comparison to *Klebsormidium* [60]. However, the investigated Central European *Klebsormidium* strains of the present investigation showed a generally slightly lower dehydration capacity and thus higher desiccation tolerance compared with other *Klebsormidium* strains from different phylogenetic clades as well as biogeographic regions. Other streptophyte green algae of the Zygnematophyceae exhibit a much lower tolerance to desiccation. Photosynthetic activity of cells of filamentous *Zygogonium* strains was completely inhibited after 150-min desiccation [61]. Two filamentous *Zygnema* strains from hydroterrestrial habitats dehydrated within 60–120 min [62]. The response to desiccation in four polar *Zygnema* isolates was even faster (< 20 min) when the experimental design caused a very fast dehydration [63]. In contrast, when the design allowed a slow desiccation, the tolerance towards desiccation was higher and resulted in prolonged positive effective quantum yield signals [63, 64]. A higher desiccation tolerance was also observed in older cultures of *Zygnema*, which consisted mainly of pre-akinetes (cells with thickened cell walls and filled with storage compounds; [62]). The higher desiccation tolerance in *Klebsormidium* in comparison to *Zygnema* can be explained by habitat-specific adaptions, as *Zygnema* usually occurs in moist soils or streams. In contrast, *Klebsormidium* occurs in soils and BSCs; hence, *Zygnema* is adapted to habitats with more humid conditions. Furthermore, in comparison with *Zygnema*, *Klebsormidium* cell walls contain a higher callose content, which was even enhanced by desiccation stress [65]. As desiccation stress has been described to cause drastic cell wall deformations and callose is involved in wound responses related to mechanical stress, the latter authors assumed that this glucan is incorporated into deformed areas of the *Klebsormidium* cell wall. This would imply an important contribution to desiccation tolerance, since flexible cell walls are crucial for surviving cellular water loss by allowing regulated shrinkage of the protoplast and thereby preserving the structural integrity of the cell including all organelles [37, 65]. Whether the BSC coccoid *Coccomyxa* (Trebouxiophyceae) has such structural specification in the cell wall remains unknown, but Antarctic strains of this genus tolerated similar dehydration periods like *Klebsormidium* (300–400 min; [66]). The genus *Coccomyxa* belongs to the Trebouxiophyceae, a group of derived Chlorophyta. Together with Chlorophyceae, Trebouxiophyceae switched almost completely from saltwater to freshwater and many terrestrial habitats during the evolution of higher plants and colonization of land [67]. The adaptation to freshwater and moist habitats was a crucial step for the colonization of land by plants. The genome analysis of *K. flaccidum* indicated that terrestrial plant-like physiological adaptations for survival in terrestrial habitats indeed exist ([42]; see "Introduction").

Recovery kinetics of $\Delta F/F_m'$ after dehydration of the desiccated *Klebsormidium* strains exhibited an unexpectedly

large variability. Almost all strains were rehydrated within 90 min after the end of the dehydration period, and according to the response patterns, the 21 isolates can be divided into two groups with strain specific differences (Fig. 6). The first response type is characterized by high recovery rates (> 69%). The second response type exhibited strains, which showed low recovery rates (< 25%). The latter group includes of *K. subtile*, *K. nitens*, and *K. flaccidum* strains. The strains with high recovery rates are more homogenous and include all *K. dissectum*, and only single *K. subtile* and *K. nitens* strains. These results, together with the RDA, show that adaption for xerophytic or humid conditions seems to be heterogeneous distributed within the phylogenetic clades as previously observed by Mikhailyuk et al. [13]. In order to analyze if *Klebsormidium* strains with low and high recovery rates show differences in long-time response, and hence long-time survival patterns, it is necessary to expand the experimental setup to a longer period. High recovery rates of *Klebsormidium* are similar to those of members of the closely related genera *Interfilum* [31, 39], *Entransia*, and *Hormidiella* [65]. Strain-specific differences can be confirmed for *Klebsormidium* isolates from different phylogenetic clades and geographical origin [21, 31, 40]. In contrast to the morphological interpretation (e.g., cell wall properties) of Karsten et al. [31], the specific response differences in the present study cannot be explained by strain morphology and ultrastructure alone, because isolates with disintegrated, as well as with firm filaments showed both low and high recovery rates. These data rather point to physiological traits, especially the recovery rate.

In general, all investigated *K. dissectum* and *K. subtile* strains showed high recovery rates, which indicates that they can at least survive short-term and fast desiccation situations at very low rH conditions of approximately 10%, and that they can be photosynthetically active during desiccation for several hours. Such ecophysiological characteristics are the prerequisite for long-term survival under terrestrial conditions. The other tested *Klebsormidium* strains had lower recovery rates, which makes predictions for long-term survival difficult. Nevertheless, the present results indicated only the survival during short stress intervals (24 h), and hence, in future studies, the long-term ecophysiological performance of various *Klebsormidium* strains of one species, as well of the different phylogenetic lineages, should be tested too. So far, there exists only one publication on long-term desiccation effects on the optimum PSII quantum efficiency in *K. dissectum* [40]. These authors demonstrated that *K. dissectum* exposed for up to 3 weeks to different rH scenarios of 100, 55, and 5%, followed by rehydration for another 2 weeks, fully recovered under all conditions but with different recovery kinetics.

Similar to the mentioned dehydration process, members of the Zygnematophyceae from freshwater habitats exhibited also generally slower and lower recovery rates than those of the Klebsormidiophyceae [61, 63, 64]. In contrast, various coccoid Chlorophyta (among others *Chlorella* sp., members of the Trebouxiophyceae, etc.) from desert BSCs recovered almost completely within 1 h from desiccation [68], while four *Coccomyxa* (Trebouxiophyceae) strains showed a fast, but only partial recovery rate, varying from 34 to 59% [66]. As for the Klebsormidiophyceae and Zygnematophyceae, also members of the Trebouxiophyceae exhibit strain-specific responses to rehydration. The recovery rate can be partly influenced by other abiotic conditions during the desiccation process. Recovery rates of trebouxiophyceaen algae were higher, when the cells desiccated in dark and under a slow dehydration scenario [68, 69]. Fast responses within minutes on rehydration, measured as an increase of the effective quantum yield, are not only detectable for isolated green algal cultures but also for natural green algal biofilms on building surfaces [70]. Such fast responses have been observed in prokaryotes (cyanobacteria) and eukaryotes (bryophytes), which led to the conclusion that not a protein de novo synthesis is involved, but instead it was due to reassemble PSII from intact and active photosystem components [71, 72].

The strain-specific differences in desiccation tolerance as shown in the present study can not only be explained by morphological and ultrastructural features, but rather reflect a spectrum of physiological responses for members of the same lineage identical controlled conditions which is interpreted as plasticity width of similar/identical genotypes. As organisms living in a habitat like BSCs with extreme changing environmental conditions, it may be a great benefit to have a certain plasticity and hence an adaption to a broad spectrum of abiotic conditions.

Temperature and Growth Response

The response of increasing temperatures on growth in the tested *Klebsormidium* strains matches the environmental conditions in the respective sampling sites. Except strain *K. flaccidum* HEW42-9, all other strains grew at the mean annual temperature of the localities (Swabian Alb and Hainich 6.5–8 °C, Schorfheide-Chorin 8.0–8.4 °C; [43]). Additionally, the investigated strains exhibited relatively high optimum growth temperatures (> 17 °C, except strain AEG18-1 *K.* cf. *dissectum* with 11.6 °C), and hence, they can be regarded as psychrotroph to psychrotolerant [73, 74]. In accordance with the definition of psychrotroph, almost all *Klebsormidium* strains were capable to grow at temperatures below 10 °C. Similar temperature requirements for photosynthesis have been described for *K. crenulatum* and *K. dissectum* from a high alpine BSC [40, 41]. In contrast, *Klebsormidium* strains from African and Arctic BSCs had a narrower range for photosynthesis towards higher or lower temperature, respectively [31]. The data of the present study support the existence of different ecophysiological response pattern in

Klebsormidium as function of the temperature conditions in the natural habitat [31]. This is in accordance to certain *Klebsormidium* genotypes preferring acidic habitats [27] or natural versus artificial substrates [32].

Similar to the genus *Klebsormidium*, further data on the temperature requirements for growth in the closely related genera of the Klebsormidiophyceae *Interfilum*, *Entransia*, and *Hormidiella* are missing. Growth-temperature data on other green algae are rare as well, but the reported growth rates well correlate with the temperature conditions of the respective sampling sites, such as in the genera *Cosmarium* (Zygnematophyceae; [75]), *Coccomyxa* (Trebouxiophyceae; [66]), and *Stichococcus* (Chlorophyceae; [76]). Interestingly, the temperature requirements for growth of some of these strains were not affected or changed by artificial long-term cultivation, even the culture conditions did not reflect the original habitat conditions [66, 76], pointing to genetically fixed traits. Whether this is true for the investigated *Klebsormidium* strains remains an open question, as these isolates underwent only short-time cultivation.

Organisms living in BSCs have to cope among other environmental factors with seasonal and diurnal changes of temperature. These fluctuations depend on microclimatic and geographical factors. At the sampling sites of the Biodiversity Exploratories, the temperature in 5-cm soil depth, varied between −10 and −2 °C in January 2011, and in July 2011, it reached values of 17–30 °C (data not shown, BExIS). These natural temperature data of the soil indicate that the tested *Klebsormidium* strains experience in Central European BSCs suitable environmental conditions for growth and biomass production for long periods of the year. This might explain the conspicuously high ecological success of *Klebsormidium* in many BSC communities in this geographic region.

Problems in Estimating Significant Statistic Signals and Future Implications

The statistical analysis of ecophysiological values of the herein investigated *Klebsormidium* strains gave contrasting results. The phylogenetic signal in Blomberg's K and Pagel's λ indicated no significance with each trait value below 1 (dehydration and recovery rate). The significance of the phylogenetic signal of traits is influenced by several factors. The first point is the sample size of the dataset. Blomberg's K has a good power to detect significance with a sample size > 20 [54] and Pagels's λ > 30 [77]. With n = 21 for the number of species we included in each trait, we achieved the prerequisite only for Blomberg's K. This means, even we found no phylogenetic signal, in another analysis based on a bigger dataset, one might find a significant signal. The low number of species is even more intensified by the limited number of strains per species and clade. The phylogenetic signal may vary already between species or clades, but this could be also true for only higher taxonomic scales (more precisely genera). This leads to a further challenge. For the investigated traits, only some data from other studies exists, mostly these data have been generated by other methodological approaches. For example, Karsten and Holzinger [40] measured the dehydration capacity under ambient conditions, while the herein presented data were collected through a desiccation chamber, which simulates defined atmospheric conditions. A further problem is the measurement error, which influences Blomberg's K in at least simulated data [78]. This error can also increase if different studies are combined. Another issue to consider is the low genetic diversity within the herein used *Klebsormidium* strains from BSCs in Central Europe, especially in strains assigned to clade E [28]. The reason for that might be low genetic diversity within the internal transcribed spacer region, which were used for that study [24]. Taken together, future studies should address the following points. First, more strains (> 30) of the different species, clades, and especially different climatic conditions (e.g., tropical vs. polar strains) should be included. Secondly, appropriate genetic maker with high resolution should be considered (e.g., combination of ITS and *rbc*L). Thirdly, ecophysiological parameters should be collected under comparable conditions and with similar methods.

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