Anpassungsmechanismen an extreme Umweltbedingungen in physiologischem und genetischem Kontext antarktischer Flechten und ihrer Photobionten

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Abkürzungsverzeichnis

bp	Basenpaare
cDNA	codierende DNA
DEPS	De-epoxidationsstatus des Xanthophyllpools
F_0	minimale Chlorophyll *a*-Fluoreszenz im dunkelakklimatisierten Zustand
F_v/F_m	maximale Quanteneffizienz des Photosystem II
GABA	γ-Aminobuttersäure
ITS	*internal transcribed spacer*
mRNA	*messenger*-RNA
NPQ	kontrollierte nicht-photochemische Energielöschung
PCR	Polymerase-Kettenreaktion
PPFD	photosynthetisch aktive Photonen-Flussdichte
PS	Photosystem
qPCR	quantitative Polymerase-Kettenreaktion
rbcL	Ribulose-1,5-bisphosphat-carboxylase/-oxygenase, große Untereinheit
ROS	reaktive Sauerstoffspezies
RPM	*reads per million*
RuBisCo	Ribulose-1,5-bisphosphat-carboxylase/-oxygenase
Y(ND)	Donorseiten-Limitierung des Photosystem I

1 Einleitung

1.1 Antarktische Habitate

1.1.1 Klima

Die Antarktis bildet die Region südlich des 60. Breitengrades. Dieser Bereich umfasst den südlichen Ozean, mehrere Inselgruppen, sowie als größte Landmasse den Südkontinent Antarktika. Durch seine isolierte Lage um den geographischen Südpol und durch die zirkumpolare Strömung des Polarmeers ist Antarktika klimatisch und biogeographisch weitgehend isoliert (Barker und Thomas 2004, Clarke *et al.* 2005). Hinzu kommen die monatelangen Polarnächte während des antarktischen Winters, der flache Winkel der Sonneneinstrahlung sowie die hohe Abstrahlung durch von Eis und Schnee bedingte hohe Albedo (Wuttke *et al.* 2006). Antarktika wird als der kälteste, windigste, abgelegenste, und auch trockenste Kontinent der Welt bezeichnet (Broeke *et al.* 2004). Dieser Kontinent ist zu 99,6% mit einer permanenten Eisschicht bedeckt, deren Stärke durchschnittlich zwei Kilometer beträgt (Huiskes *et al.* 2006). Die wenigen eisfreien Habitate stellen die Küstenregionen sowie Nunatakker dar, felsige Bergspitzen, die aus dem umgebenden Eis ragen (Hughes *et al.* 2006).

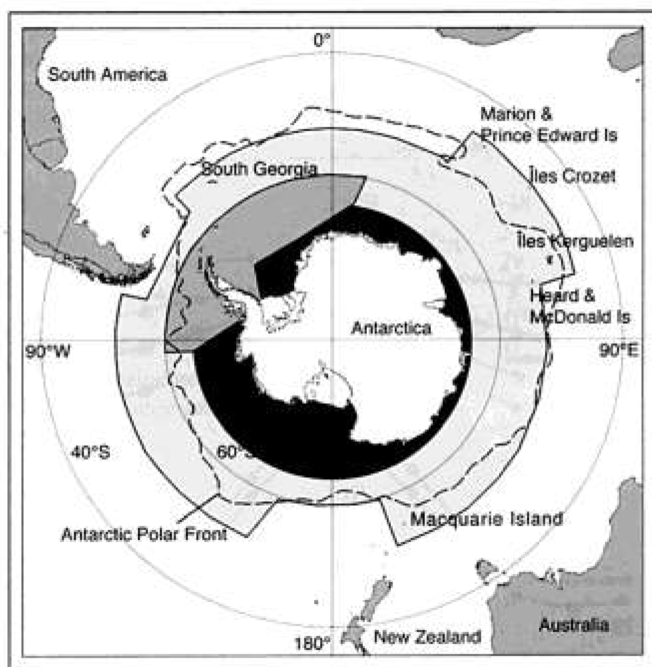

Abbildung 1 Karte der Antarktis. Die gestrichelte Linie gibt die nördliche Grenze der südlichen Polarfront an. Die Subantarktis ist hellgrau unterlegt, die maritime Antarktis dunkelgrau und die kontinentale Antarktis schwarz. Das Kreuz markiert den geographischen Südpol. Aus Huiskes *et al.* (2006)

Antarktika wird nach klimatischen Gesichtspunkten in maritime und kontinentale Regionen eingeteilt (Smith 1984, Longton 1988, Abb. 1), wobei die maritime Region sich über den nördlichen Teil der Antarktischen Halbinsel erstreckt. Hier kann das Klima insgesamt im Verhältnis zur kontinentalen Antarktis als gemäßigter bezeichnet werden. Die Temperaturen, und besonders die Niederschlagsmengen sind höher, sodass auch Regen fällt, während Niederschläge in der

kontinentalen Antarktis ausschließlich als Schnee auftreten. In der nördlichen maritimen Antarktis können bis zu 500 mm Regen pro Jahr fallen (Øvstedal und Smith 2001). Bei ausreichender Bodenbildung können in dieser Region auch Höhere Pflanzen überleben (Cannone *et al.* 2006). In der südlichen maritimen Antarktis herrschen harschere Bedingungen vor. So beträgt der durchschnittliche Niederschlag auf Alexander Island nur 200 mm/Jahr (Smith 1988). Die maritime Antarktis, einschließlich ihrer Inselgruppen, ist besonders von der globalen Erwärmung betroffen (Sancho *et al.* 2007). Neben dem direkten Effekt der Temperaturerhöhung werden maritim-antarktische Biome besonders durch mit steigender Temperatur verbundene höhere Wasserverfügbarkeit beeinflusst (Green *et al.* 2011). Die kontinentale Antarktis zeigt in den letzten Jahren hingegen einen Trend zur Abkühlung und zunehmenden Vergletscherung (Guglielmin und Cannone 2012). Jedoch kann angenommen werden, dass auch kleine Änderungen der klimatischen Bedingungen große Auswirkungen auf die spezialisierten Organismen kontinental-antarktischer extremer Habitate haben können (Robinson *et al.* 2003).

1.1.2 Vegetation

Die im Vergleich zur Größe des Kontinents wenigen zumindest temporär eisfreien Gebiete von Antarktika bieten Lebensraum für eine angepasste Vegetation, welche hauptsächlich aus Mikroorganismen, Bryophyten und Flechten besteht (Øvstedal und Smith 2001). Die Vegetationszeiten sind über den gesamten antarktischen Bereich als sehr kurz zu bezeichnen, an kontinentalen Standorten aber als extrem kurz. So erreicht selbst in Küstengebieten die Temperatur lediglich für sehr kurze Zeit Werte über dem Gefrierpunkt, und positive Durchschnittstemperaturen treten in der kontinentalen Antarktis nur etwa einen Monat lang im antarktischen Sommer auf (Walton 1984, Convey 1996a). Hinzu kommen starke Schwankungen der Luft- und Substrattemperatur innerhalb eines Tages, die sich von +20°C bis -30°C erstrecken können. Im Jahresverlauf liegen maximale und minimale Temperaturen um bis zu über 90°C auseinander (Smith 1988, Bölter 1992, Convey 1996b). Die ausschließlich in Form von Schnee auftretenden Niederschläge sind gering, auch in den Küstenregionen. Sonneneinstrahlung, Temperatur und verfügbare Feuchtigkeit sind die Schlüsselfaktoren für die meisten Prozesse terrestrischer Ökosysteme. Die Variabilität dieser Faktoren, ihre physiologischen Optima und von Organismen tolerierbaren Extreme formen die Struktur und Funktion der Ökosysteme. Sowohl für die kontinentale, als auch für die maritime Antarktis gilt, dass

Wasserverfügbarkeit den bedeutsamsten limitierenden Faktor terrestrischer Vegetation darstellt (Kennedy 1993, Block 1996).

In der nördlichen maritimen Antarktis sind zwei Samenpflanzen (Phanerogamen) heimisch, die Antarktische Perlwurz (*Colobanthus quitensis*) und die Antarktische Schmiele (*Deschampsia antarctica*). Hinzu kommen wenige eingeschleppte Neophyten, wie das Einjährige Rispengras (*Poa annua*) in der nördlichen maritimen Antarktis. Ansonsten werden terrestrische Habitate, abgesehen von als biologische Krusten wachsenden Mikroorganismen, von Bryophyten und Flechten (Kryptogamen) dominiert. In südlicheren Regionen der maritimen Antarktis und in der kontinentalen Antarktis kommen schließlich keine Phanerogamen mehr vor (Øvstedal und Smith 2001). Die antarktische Kryptogamenvegetation zeichnet sich im Vergleich zu den Phanerogamen u.a. dadurch aus, dass sie längere Zeiten von Trockenheit tolerieren kann und nicht auf eine Humusschicht angewiesen ist. Somit besiedeln vor allem Flechten auch exponierte Standorte direkt auf den Felsen, wobei sie primär Mikronischen besiedeln, die sich durch erhöhte Wasserverfügbarkeit auszeichnen (Engelen *et al.* 2010). Relevant für eine erfolgreiche Besiedlung sind die mikroklimatischen Bedingungen dieser Nischen. Das Meso- oder Makroklima der Habitate ist von geringerer Bedeutung und sekundär für den Besiedlungsprozess. Die durch diese klimatischen Bedingungen beeinflussten Umweltparameter wirken sich direkt auf physiologische Prozesse der Kryptogamen aus.

1.1 Flechtensymbiose

1.2.1 Allgemeines

Flechten sind keine einzelnen Organismen, sondern symbiotische Assoziationen. In dieser Exosymbiose leben ein Pilz (Mycobiont) und eine Grünalge und/oder ein Cyanobakterium (Photobiont) zusammen, wobei eine einzigartige thallöse Struktur gebildet wird (Honegger 1991). Die Mycobionten stammen hauptsächlich aus der Gruppe der Ascomyceten, wenige sind Basidiomyceten. Die Taxonomie der Flechten beruht auf den lichenisierten (Flechten bildenden) Pilzen und zeigt einen polyphyletischen Ursprung der Flechtensymbiose, d.h. sie ist mehrfach unabhängig voneinander entstanden (dePriest *et al.* 1997). Bei den Grünalgenflechten (Chlorolichenen) werden die Struktur des Thallus sowie die meisten sekundären Metabolite zudem durch Hyphen des Mycobionten gebildet (Jahns 1988). Der Photobiont ist für die Versorgung der gesamten symbiotischen Assoziation mit assimiliertem Kohlenstoff, und, im Fall zusätzlich beteiligter

Cyanobakterien, auch assimiliertem Stickstoff zuständig (Feige und Jensen 1992). Beide Symbionten können einzeln kultiviert werden, jedoch ist der Prozess der sexuellen Vermehrung des Mycobionten nur in der Symbiose möglich. Die sexuellen Stadien des Photobionten hingegen sind im Flechtenthallus unterdrückt. Der Photobiont vermehrt sich in der symbiotischen Assoziation nur mitotisch, wobei die Zellteilung durch den Mycobionten kontrolliert wird (Henssen und Jahns 1974). Dies kann als Koordinierung der symbiotischen Organismen gewertet werden (Hill 1993). Durch die Struktur des Thallus wird eine Umgebung geschaffen, in der auch bei für isolierte Grünalgen lebensfeindlichen Außenbedingungen physiologische Aktivität möglich ist (Honegger 2009). Ansonsten unzugängliche Habitate werden so für Photobionten kolonisierbar. Auch auf physiologischer Ebene sind Vorteile der Symbiose für beide Symbionten zu beobachten. So konnten synergistische Effekte der antioxidativen (Kranner *et al.* 2005, Kranner und Birtic 2005) und photoprotektiven Mechanismen nachgewiesen werden (Kosugi *et al.* 2013). Die Flechtensymbiose kann demnach als mutualistisch beschrieben werden.

1.2.2 Anpassungen der Flechten an extreme Standorte

Flechten sind auf jedem Erdteil und in jedem Ökosystem zu finden. Etwa 8% der weltweiten terrestrischen Vegetation wird von Flechten dominiert (Ahmadjian 1995). Auch vollständig oder zeitweise submerse Standorte werden besiedelt (Hawksworth 2000, Sadowsky *et al.* 2012). Flechtendominierte Standorte sind häufig sogenannte Extremstandorte, die für Höhere Pflanzen nicht besiedelbar sind. Somit bilden Flechten häufig die Pioniervegetation wie etwa in Gebieten rezenten Gletscherrückzuges (Sancho und Valladares 1993). Die harschen Umweltbedingungen arider, alpiner und polarer Habitate werden von vielen Flechtenarten toleriert (Seymour *et al.* 2005). Dazu gehören Kälte und Schneebedeckung in Polarregionen (Kappen 1993, Dyer und Crittenden 2008), aber auch intensive Sonneneinstrahlung (Barták *et al.* 2004, Nybakken *et al.* 2004, Fernández-Marín *et al.* 2009, 2010). Extrem trockene Kältewüsten des antarktischen Inlandes, wie die McMurdo-Trockentäler, werden von Flechten bevorzugt in Mikronischen besiedelt (Kappen *et al.* 1981, Marchant und Head 2007, Onofri *et al.* 2007).

Da Flechten eine wechselfeuchte (poikilohydre) Lebensform sind und über keinerlei aktive Regulationsmöglichkeiten ihres Wasserhaushaltes verfügen, sind sie vollständig von der Wasserverfügbarkeit ihrer Umgebung und deren Schwankungen abhängig

(Larson 1979). Der Flechtenthallus kann, je nach Wachstumsform, Anatomie und Standort, innerhalb von Minuten austrocknen (Lange *et al.* 1998). Luftgetrocknete Flechtenthalli können einen Restwassergehalt von unter 5% haben. Als Folge kommen die physiologischen Vorgänge zum Erliegen, und es wird ein Zustand latenten Lebens, die Anabiose, erreicht (Kranner *et al.* 2008). Anabiotische Flechten zeigen erhöhte Resistenz gegen eine Vielzahl lebensfeindlicher abiotischer Umweltbedingungen. Starke Strahlung verschiedener Qualitäten, extreme Temperaturen und sogar Weltraumbedingungen können toleriert werden (Kappen 2000, de Vera *et al.* 2002, Sancho *et al.* 2007).

Durch Wiederbefeuchtung können anabiotische Flechten reaktiviert werden. Zyklen von Dehydrierung und Rehydrierung sind, mit Ausnahme einiger tropischer und aquatischer Flechten (Hawksworth 2000), ein im Lebenszyklus normaler Vorgang, der unzählige Male während der Lebensdauer der Symbiose stattfinden kann, ohne sie zu zerstören (Ahmadjian 1965, Lange *et al.* 2001). Ebenso können Flechtenthalli nach Jahre andauernder Anabiose durch Wiederbefeuchtung reaktiviert werden (Honegger 2003, Sadowsky und Ott 2015a). Insbesondere die Fähigkeit zur schnellen Reaktivierung des Flechtenthallus übertrifft die Fähigkeit sogenannter „Wiederauferstehungspflanzen“ wie *Craterostigma plantagineum* (Scrophulariaceae). Diese Phanerogamen heißer arider Gebiete benötigen Stunden bis Tage, um ihre vollständige photosynthetische Aktivität wiederzuerlangen (Bernacchia *et al.* 1996, Bartels *et al.* 2001). Viele Chlorolichenen können zudem allein durch Aufnahme von Luftfeuchtigkeit reaktiviert werden, wodurch sie unabhängig von flüssig verfügbarem Wasser sind (deVries *et al.* 2008).

Schäden durch zu hohe Lichtintensität entstehen, wenn in der photosynthetischen Lichtreaktion absorbierte Lichtenergie nicht mehr effektiv in nachgeschaltete Prozesse, wie die Assimilation von Kohlendioxid, abgeleitet werden kann. Die überschüssige Energie kann dann auf Sauerstoffverbindungen übertragen werden, die als reaktive Sauerstoffspezies (ROS) Schäden an Biomolekülen verursachen können (Krause und Jahns 2004). Mit hoher Sonneneinstrahlung geht eine Erwärmung von Substrat und Flechtenthallus einher, was die Austrocknung beschleunigen kann. Gerade an maritim-antarktischen Standorten kann durch die bei Austrocknung eintretende Anabiose hohe Lichtintensität toleriert werden (Kappen und Valladares 1999; Schlensog und Schroeter 2000). Die Rindenschicht ausgetrockneter Flechtenthalli ist oftmals optisch dichter als im feuchten Zustand, was zusätzlichen Lichtschutz gewährt (Gauslaa und Solhaug 2004).

Zudem können Elemente der Lichtreaktion bei Erreichen der Anabiose entkoppelt werden (Sigfridsson und Öquist 1980). Physiologisch aktive Photobionten verfügen zudem über eigene Schutzmechanismen. So kann überschüssige Energie kontrolliert in Wärme umgewandelt werden. Diese Nicht-photochemische Energielöschung (NPQ) ist keine flechten- oder algenspezifische Eigenschaft, sondern wurde vor allem in Samenpflanzen erforscht (Demmig-Adams und Adams 1996). NPQ korreliert mit der Umwandlung des Xanthophylls Violaxanthin über Antheraxanthin zu Zeaxanthin, welches Energie vom Photosystem (PS) II in Wärme umwandeln kann. Diese kontrollierte Reaktion wird nach den beteiligten Pigmenten Xanthophyll- oder VAZ-Zyklus genannt. Die Absorptionsfähigkeit des PS II kann durch Zerstörung und Abbau seiner Proteine, besonders des im Reaktionszentrum gelegenen D1-Proteins, herabgesetzt werden (Krause und Jahns 2004). Die Resynthese abgebauter PS II-Proteine dauert länger als die Entspannung des VAZ-Zyklus und wird deshalb als chronische Photoinhibition bezeichnet (Schlensog *et al.* 2003). An der Lungenflechte *Lobaria pulmonaria* wurde eine Aktivierung des VAZ-Zyklus bei Austrocknung im Dunkeln nachgewiesen, was als Anpassung an mit austrocknenden Bedingungen verbundene hohe Lichtintensitäten am Standort der Flechte interpretiert werden kann (Fernández-Marín *et al.* 2009, 2010).

Niedrige Temperaturen bis weit unterhalb des Gefrierpunktes sind eine offensichtliche Eigenschaft polarer und besonders antarktischer Ökosysteme. Gerade polare Flechten zeigen herausragende Kälte- und Gefriertoleranz, ihre Temperaturoptima sind niedrig (Kappen und Breuer 1991; Kappen *et al.* 1995; Kappen 2000; Pannewitz *et al.* 2003). Auch extreme künstliche Gefrierbehandlungen von Flechtenthalli wurden toleriert, sodass aus für insgesamt 80 Stunden tiefgefrorenen Thalli isolierte Photobionten ihre Vitalität aufrechterhalten konnten (Kappen und Lange 1970). Am natürlichen Standort wurde Photosyntheseaktivität intakter Flechtenthalli bei -17°C Lufttemperatur gemessen (Kappen *et al.* 1996). An einigen Standorten ist somit Photosynthese unter einer Schneedecke möglich, da geringe Mengen Wasser auf der Thallusoberfläche zur Aktivierung ausreichen und die durch eine Schneedecke verfügbare Lichtintensität photosynthetisch genutzt werden kann (Kappen und Breuer 1991, Kappen 1993, 2000, Schroeter *et al.* 1994, 1997). Allerdings kann die Photosynthese schneebedeckter Flechten verhindert werden, wenn sehr niedrige Temperaturen und eine intensive Schneedecke vorherrschen (Pannewitz *et al.* 2003).

1.2.3 Photobionten

Flechten-Photobionten sind Grünalgen oder Cyanobakterien. Sowohl Grünalgen als auch Cyanobakterien können als Smybionten im selben Thallus vorkommen. So bezeichnet man die Strukturen in Chlorolichenen inkorporierter Cyanobakterien als Cephalodien (Henssen und Jahns 1974). Einige Mycobionten bilden mit Cyano- oder Chlorobionten unterschiedliche Wuchsformen. Diese werden als Photosymbiodeme bezeichnet (Jahns 1988). Während Cyanobakterien in Cephalodien der Versorgung der Symbiose mit fixiertem Luftstickstoff dienen, sorgt die Grünalge als primärer Photobiont für die Ernährung aller Bionten mit Kohlenstoff (Feige und Jensen 1992). Cyanobakterien transportieren assimilierten Kohlenstoff in Form von Glucose an den Mycobionten, während Grünalgen-Photobionten den Zuckeralkohol Ribitol ausscheiden. Ribitol wird dann in den Hyphen des Mycobionten in Mannitol umgewandelt (MacFarlane und Kershaw 1985). An Standorten der kontinentalen Antarktis dominieren Grünalgenflechten, was als Folge niedriger Temperaturen und geringer Verfügbarkeit flüssigen Wassers interpretiert werden kann (Schroeter *et al.* 1997). Die Grünalgengattung *Trebouxia* ist die weltweit am weitesten verbreitete Gattung von Flechten-Photobionten (Henssen und Jahns 1974). Ob die Flechtensymbiose für Photobionten der Gattung *Trebouxia* obligat ist, ist Gegenstand von Diskussion (Wornik und Grube 2010). Ihre Isolierung und Kultivierung unter Laborbedingungen ist jedoch möglich und wird seit vielen Jahren erfolgreich angewendet (Yoshimura *et al.* 2002, Schaper und Ott 2003, Sadowsky und Ott 2012).

Obwohl Algen der Gattung *Trebouxia* als Photobionten die verbreitetsten terrestrischen Grünalgen darstellen, ist über ihre physiologischen Leistungen wenig bekannt. Kappen und Lange (1970) zeigten, dass auch extreme Kältebehandlungen Photobionten innerhalb des Thallus nicht schädigen. Bei isolierten Photobionten der Gattung *Trebouxia* zeigte sich artspezifisch verschiedene Gefriertoleranz (Hájek *et al.* 2012). Über die Reaktion isolierter Photobionten auf Trockenheit und Starklicht ist ebenfalls wenig bekannt. Jedoch wurde in Bezug auf die Osmolarität und Austrocknung von Grünalgen- und Cyanobakterien-Photobionten gezeigt, dass Charakteristika der isolierten Photobionten auch in ihrer symbiotischen Konstitution erhalten bleiben (Kosugi *et al.* 2014).

1.2.4 Antarktische Photobionten

Die Diversität der Mycobionten nimmt an extremen antarktischen Standorten im Vergleich zu gemäßigteren ab (Pointing *et al.* 2015). Dieser Trend kann für terrestrische

Vegetation der Antarktis allgemein ausgemacht werden. Dabei wird keinem strikten Nord-Süd-Gefälle gefolgt, sondern den konkreten mikroklimatischen Bedingungen an Mikrostandorten (Pointing *et al.* 2015). Auf einem Transekt von der nördlichen maritimen Antarktis bis zur Terra-Nova-Bucht in der kontinentalen Antarktis wurde die Diversität verschiedener Photobionten untersucht (Romeike *et al.* 2002, Romeike 2002, Brinkmann 2002, Langohr 2004, Siegesmund 2005, Neuburg 2007, Engelen 2008, Holzwig 2009, Engelen *et al.* 2010, Brandt 2011). Über den gesamten Transekt hinweg dominierten Photobionten aus der Gruppe *Trebouxia jamesii/ Trebouxia simplex*, insbesondere am kontinentalen Standort gehörten die Photobionten von Blatt- und Strauchflechten zum *clade* S der Gattung *Trebouxia* (nach Helms 2003, Brandt 2011). Der *Trebouxia-clade* S wird von einigen Autoren als *T. jamesii* angesprochen (Fernández-Mendoza *et al.* 2011), was jedoch strittig ist. So bilden nach Brandt (2011) ITS-Sequenzen des *clade* S Antarktischer Photobionten eine Schwestergruppe zu Sequenzen aus *Trebouxia simplex* nicht-antarktischen Ursprungs. Die Dominanz von Photobionten der Gattung *Trebouxia* an alpinen und kalten Standorten ist bekannt (Honegger 1998). Dem gegenüber gilt für *Trebouxia* wie für Flechten-Photobionten generell, dass wenig über ihr physiologisches Potential bekannt ist.

1.2.5 Fragestellung und Ziele

Die Dominanz von *Trebouxia*-Photobionten einer begrenzten Verwandtschaftsgruppe in Makroflechten (nicht krustig wachsenden Arten) lässt die Vermutung zu, dass es sich um eine physiologisch begründete Verteilung der symbiotischen Algen handelt. Die folgende Hypothese wird aufgestellt:

> Das physiologische Potential von Photobionten trägt substanziell zur Physiologie der Flechtensymbiose bei. Antarktische Photobionten des *clade* S aus der Gattung *Trebouxia* zeigen Anpassungsmechanismen, welche ihren Erfolg in Makroflechten der kontinentalen Antarktis begründen.

Die abiotischen Umweltfaktoren Starklicht, Trockenheit und Kälte werden untersucht. Dabei wird ein vergleichender Ansatz verfolgt, der Photobionten unterschiedlicher Herkunft gegenüberstellt. Der für das poikilohydre Leben von Flechten-Photobionten zentrale Faktor Trockenheit wird mittels Metabolom- und Transkriptomanalyse genauerer Betrachtung unterzogen. Dabei wird folgenden Fragen nachgegangen:

- Wie verhalten sich isolierte Photobionten unter Stressbedingungen?
- Gibt es ökophysiologische Ähnlichkeiten innerhalb von Verwandtschaftsgruppen der Gattung *Trebouxia*, am Beispiel des *clade* S?
- Welche Metabolite sind an Stressantworten beteiligt?
- Welche Gene werden unter Stressbedingungen exprimiert?
- Wie beeinflusst die ökologische Plastizität der untersuchten Photobionten ihre Fähigkeit, bei klimatischen Veränderungen zu bestehen?

Die ökophysiologischen Experimente schließen an die Vielzahl ökophysiologischer Studien an, welche antarktische Flechtenthalli zum Gegenstand haben, wie sie von Kappen, Lange, Schroeter und anderen durchgeführt wurden (eine Übersicht bietet Kappen 2000). Erkenntnisse zur Physiologie isolierter Photobionten erweitern das Wissen über den Beitrag des photosynthetischen Partners zum Erfolg von Flechten auf Antarktika. Die Analyse des Metaboloms und Transkriptoms antarktischer Photobionten wird zum ersten Mal durchgeführt und eröffnet einen genaueren Blick auf grundlegende Prozesse der Umweltanpassung dieser Organismen, als dies bisher möglich war.

2. Ergebnisse und Diskussion

2.1 Photobionten

Die Photobionten sechs verschiedener Flechten von drei antarktischen und einem europäischen Standort (Gotland, Schweden) wurden untersucht. Sie gehören sämtlich zur Gattung *Trebouxia.* Es sind neben dem *clade* S (S für *T. simplex*, Phylogenie nach Helms *et al.* 2001, Brandt 2011) zwei weitere Gruppen der Gattung vertreten. Der Photobiont der endemischen antarktischen Krustenflechte *Buellia frigida* gehört zum *clade* A (für *T. asymmetrica*). Der Photobiont von *Fulgensia bracteata* aus Schweden gehört zu *clade* I (für *T. impressa*). Die gezeigte Phylogenie beruht auf Sequenzähnlichkeiten in den *internal transcribed spacer* (ITS)-Regionen der ribosomalen DNA (rDNA). *Trebouxia* aus den antarktischen Flechten *Usnea lambii* und *Umbilicaria decussata* wurde metabolomisch und transkriptomisch untersucht. Zuvor wurden weitere genetische Marker der beiden Photobionten verglichen. Sequenzvergleiche von Actin 1 (Teil des Cytoskeletts, nukleare DNA), rbcL (Große Untereinheit der Ribose 1,5-Bisphosphat-Carboxylase/Oxygenase, Rubisco, plastidäre DNA) sowie ITS wurden verglichen. Auch auf dieser erweiterten Basis konnten die Photobionten nicht in unterschiedliche Gruppen einsortiert werden. Die verwendeten Marker sind geeignet, Photobionten auf Artebene zu unterscheiden (Sadowsky *et al.* 2015a).

Tabelle 1 Arten und Ursprünge der untersuchten Photobionten. Verändert aus Sadowsky und Ott (2012)

Mycobiont	Photobiont	Literatur	Standort
Buellia frigida	*Trebouxia* sp., *clade* A, NCBI AY667580.1)	Brandt 2011	Terra-Nova-Bucht, Nord Victoria-land, kontinentale Antarktis
Fulgensia bracteata	*Trebouxia* sp., *clade* I, sub-group 1	Schaper *et al.* 2003	Gotland, Schweden
Pleopsidium chlorophanum	*Trebouxia jamesii, clade* S	Brandt 2011	Terra-Nova-Bucht
Umbilicaria antarctica	*Trebouxia jamesii*, *clade* S, NCBI AJ431575.1	Romeike *et al.* 2002	Rothera Point, Adelaide Island, Antarktische Halbinsel
Umbilicaria decussata	*Trebouxia jamesii*, *clade* S	Brandt 2011	Terra-Nova-Bucht
Usnea lambii	*Trebouxia jamesii*, *clade* S	Brandt 2011	Coal Nunatak, Alexander Island, südliche maritime Antarktis

2.2 Reaktion auf Kälte

2.2.1 Einführung

Die hohe Kältetoleranz polarer Flechten wurde mehrfach und an verschiedenen Arten nachgewiesen (Kappen und Lange 1972, Kappen und Breuer 1991, Kappen *et al.* 1995, Kappen 2000, Pannewitz *et al.* 2003). Auch mehrjährige Lagerung von Flechtenthalli im getrockneten Zustand bei -20°C setzte die Regenerationsfähigkeit antarktischer Flechten nicht herab. Nach einer zweitägigen Behandlung, die auf Befeuchtung bei niedriger Temperatur, zwei-schrittiger Erwärmung und Beleuchtung mit Schwachlicht (20 µmol Photonen $m^{-2}s^{-1}$) basierte, konnten Photosyntheseaktivitäten gemessen werden, die ungestressten aktiven Flechtenthalli entsprechen (Sadowsky und Ott 2015a). Es konnte erstmals gezeigt werden, dass auch isolierte antarktische Photobionten keine Schädigung durch Gefrierbehandlungen über mehrere Tage erleiden (Sadowsky und Ott 2012) und selbst die Schock-Gefrierung durch flüssigen Stickstoff toleriert werden kann (Sadowsky und Meeßen 2015, Sadowsky und Ott 2015b, Abbildung 2).

2.2.2 Kältestress bei isolierten Photobionten

Isolierte, physiologisch aktive Photobionten verschiedener Flechten (Tab. 1) wurden bei -25°C im Dunklen gelagert, ihr photosynthetisches Potential wurde per Chlorophyllfluoreszenz als maximale Quanteneffizienz des Photosystem (PS) II (F_v/F_m) bestimmt (Sadowsky und Ott 2012). Eine Abnahme von F_v/F_m gegenüber dem Ausgangswert deutet dabei auf Verminderung der Fähigkeit zur Ladungstrennung im PS II hin (Jensen 2002). Es konnte gezeigt werden, dass zwar bei allen Photobionten während der Gefrierbehandlung das photosynthetische Potential abnahm, jedoch in unterschiedlicher Geschwindigkeit. So zeigten nur die Photobionten von *Umbilicaria decussata*, *Pleopsidium chlorophanum* und *Fulgensia bracteata* bereits nach einer Stunde bei -25°C eine signifikante Reduktion von F_v/F_m. Während der 48-stündigen Behandlung sank der Wert nicht signifikant weiter ab. Die Photobionten von *Umbilicaria antarctica* und *Usnea lambii* zeigten eine Reduktion von F_v/F_m erst nach 24 Stunden Gefrierbehandlung, längere Zeit bei -25°C führte zu keinem weiteren Effekt. Besondere Toleranz gegen die Behandlung war beim Photobiont der endemischen Krustenflechte *Buellia frigida* festzustellen. Diese zeigte F_v/F_m-Reduktion erst nach 24 Stunden, jedoch in geringerem Maße als alle anderen untersuchten Photobionten. Erst nach 48 Stunden wurde die geringste photosynthetische Aktivität von *B. frigida* gemessen. Wenn an Photobionten nach dieser Behandlung noch

photosynthetische Aktivität gemessen werden kann, deutet dies darauf hin, dass die Zellen nicht vollständig gefroren sind, weil funktionale Proteine und Membransysteme für die Funktion des PS II notwendig sind. Diese Funktionen bei niedrigen Temperaturen aufrechtzuerhalten erfordert Anpassungsmechanismen auf zellulärer Ebene. Gefriertoleranz in intakten Flechtenthalli kann auf die gezielte Bildung von Eiskeimen außerhalb von Zellen ausgerichtet sein, dies erfordert jedoch die durch einen Mycobionten gewährte Struktur (Harańczyk *et al.* 2012). Eiskristallbildung muss im Zellinneren verhindert werden, damit keine bleibenden mechanischen Schäden an zellulären Strukturen auftreten. Solche Schäden sind etwa bei gefrierbrandgeschädigten Blättern Höherer Pflanzen zu sehen (Pearce 2001).

Terrestrische Grünalgen der Antarktis zeigen eine gute Gefriertoleranz, abhängig von den Umweltbedingungen. So übertrifft die Gefriertoleranz der Günalge *Prasiola sp*. die eines Cyanobakteriums (Davey 1989). Auch die hier untersuchten Grünalgen als Photobionten aus Flechten zeigten abgestufte Gefrierresistenz, weshalb darauf geschlossen werden kann, dass unterschiedlich ausgeprägte Anpassungsmechanismen vorliegen. Die jeweils gleiche Kultivierung der Organismen lässt ausschließen, dass gemessene Unterschiede auf Akklimatisierungen an unterschiedliche Ausgangsbedingungen zurückzuführen sind. Es ist also anzunehmen, dass es sich um den irreversible Anpassungen handelt. Mechanismen der Kältetoleranz antarktischer terrestrischer Grünalgen wurden am Beispiel der coccalen Alge *Coccomyxa subellipsoidea* gefunden. Das sequenzierte Genom dieser Alge zeigt mehrere Funktionen, die entsprechende Stresstoleranz vermitteln können (Blanc *et al.* 2012).

Es ist auffällig, dass die beobachtete Kältetoleranz nicht gleichmäßig auf phylogenetische Gruppen der Gattung *Trebouxia* verteilt ist. Stattdessen zeigen die Photobionten der endemischen Arten *Buellia frigida*, *Umbilicaria antarctica* (Øvstedal und Smith 2001), und *Usnea lambii* (Wirtz *et al.* 2008) länger anhaltende Aktivität bei -25°C. Aus den kosmopolitischen Arten *Umbilicaria decussata* und *Pleopsidium chlorophanum* sowie aus der europäische Art *Fulgensia bracteata* hingegen wurden Photobionten isoliert, die schneller an Photosyntheseaktivität verloren. Photobionten des *clade* S (Brandt 2011) sind in beiden Gruppen zu finden. Dies kann als Hinweis für versteckte Diversität innerhalb dieser *Trebouxia*-Gruppe gewertet werden. Auch die Reaktion auf Austrocknung und Wiederbefeuchtung zeigte Unterschiede zwischen nahe verwandten isolierten Photobionten. Die Trockentoleranz der Photobionten von *U. decussata* und *U.*

lambii wurde molekular und biochemisch untersucht und verglichen (Sadowsky *et al.* 2015a, b). Die Ergebnisse liefern auch Hinweise auf unterschiedliche konstitutive Kältetoleranz und Gefrierresistenz durch hohe zelluläre Konzentrationen von Zuckern und Polyolen (siehe 2.4.3 Zusammensetzung und Dynamik des Metaboloms). Diese gelösten Stoffe können den Gefrierpunkt im Zellinneren heruntersetzen.

2.2.3 Reaktivierung gefrorener Photobionten

Die Reaktivierung nach 48-stündiger Lagerung isolierter Photobionten bei -25°C erfolgte bei 20°C und Dunkelheit. Das photosynthetische Potential, gemessen als F_v/F_m, stieg während des Auftauprozesses auf einen stabilen Maximalwert an, der innerhalb weniger Sekunden bis Minuten erreicht wurde. Lediglich die Photobionten von *Usnea lambii*, *Buellia frigida* und *Umbilicaria antarctica* zeigten leichte Reduktion der Quanteneffizienz des Photosystem II. Es ist möglich, dass die länger anhaltende physiologische Aktivität dieser Photobionten im Vergleich zu den anderen untersuchten Arten zu Stress im PS II geführt hat. Die Photobionten der kosmopolitischen bzw. europäischen Flechten *Umbilicaria decussata* und *Fulgensia bracteata* sind nicht nur durch schnelle Inaktivierung bei -25°C, sondern auch im Vergleich mit den anderen Arten durch langsame Reaktivierung gekennzeichnet. Das Verbreitungsmuster der jeweiligen Flechten könnte sich auch hier auf die Resistenzmechanismen der Photobionten ausgewirkt haben.

Auch die Immersion in flüssigen Stickstoff (-196°C) führte nicht zum Absterben der Photobionten (Abbildung 2). Allerdings konnte hier eine geringe Reduktion von F_v/F_m festgestellt werden, was möglicherweise auf Eiskristallbildung bei dieser extremen und schnellen Abkühlung zurückzuführen ist.

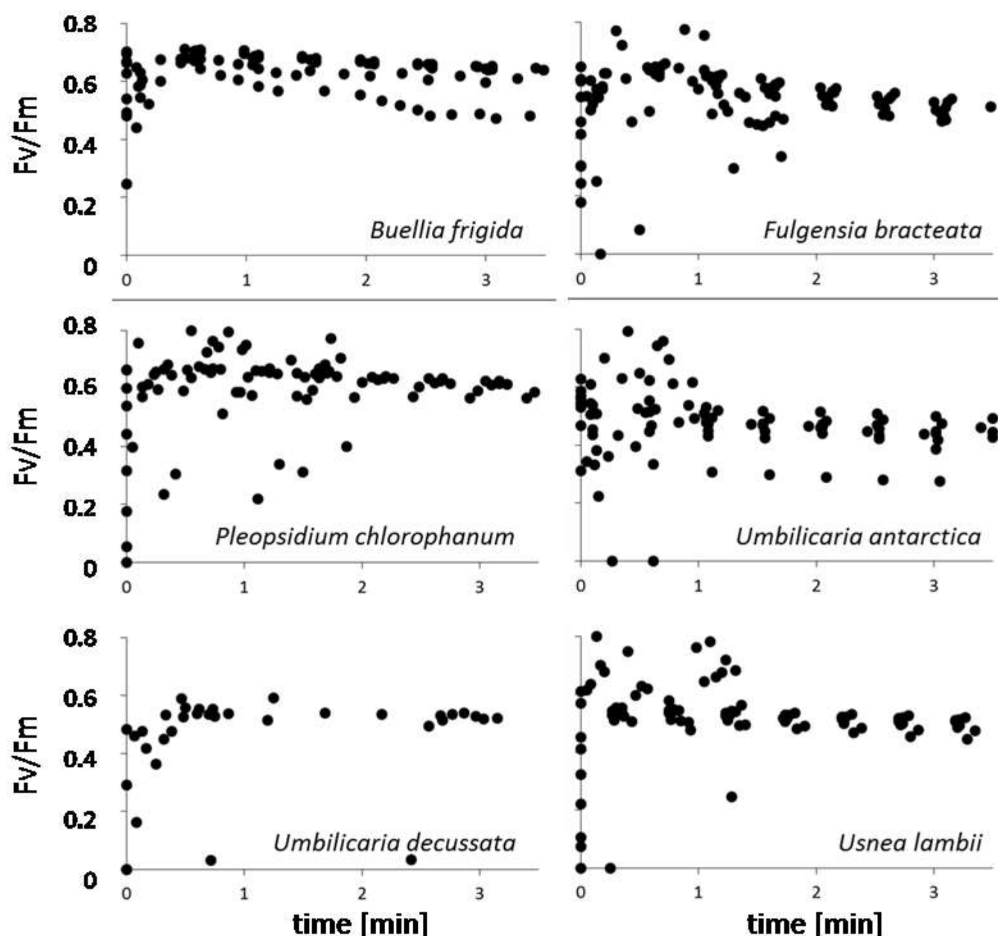

Abbildung 2 Reaktivierung isolierter Photobionten nach Immersion in flüssigen Stickstoff, n = 9. Daten zum *Buellia frigida*-Photobiont aus Sadowsky und Ott (2015b), Daten zum Umbilicaria decussata-Photobiont aus Sadowsky und Meeßen (2015)

2.3 Reaktion auf Starklicht

2.3.1 Einführung

Überschüssige Lichtenergie, welche in der photosynthetischen Lichtreaktion absorbiert, aber nicht für Kohlenstoffassimilation genutzt wird, kann durch die Bildung reaktiver Sauerstoffspezies (ROS) zu Photoinhibition führen (Krause und Jahns 2004). Die untersuchten Photobionten wurden bei niedriger Lichtintensität (20 µmol Photonen $m^{-2}s^{-1}$) kultiviert, da höhere Lichtintensitäten zu Chlorophyllverlust und schließlich zum Absterben der Kulturen führen (Ott, *pers. comm.*). Die Standorte der jeweiligen Flechten können jedoch durch Starklicht gekennzeichnet sein, die photosynthetisch aktive Photonenflussdichte (PPFD) beträgt bis zu c. 2000 µmol Photonen $m^{-2}s^{-1}$ (Sadowsky und Ott 2015). Flechtenthalli können aufgrund von Erwärmung bei steigender

Sonneneinstrahlung während Perioden hoher PPFD austrocknen. Insbesondere während des Austrocknungsprozesses sind die noch aktiven Photobionten in Gefahr, durch Starklicht geschädigt zu werden (Gauslaa *et al.* 2012). An Standorten, deren Wasserverfügbarkeit allein auf Schmelzwasser beruht, wie dies in der niederschlagsarmen kontinentalen Antarktis der Fall ist, kann eine Erwärmung des Substrats jedoch zu verbesserter Wasserversorgung von Flechtenthalli und somit zu anhaltender Aktivität bei Starklicht führen (Schroeter *et al.* 2011). Passive Photoprotektion kann durch optisch dichte Cortices (Rindenschichten) der Flechtenthalli gewährleistet werden (Ertl 1951, Büdel und Lange 1994). Die Schutzleistung kann durch Pigmentierung insbesondere im Cortex verstärkt werden, oder auch durch Reflexion (Dietz *et al.* 2000, Gauslaa und Solhaug 2001, Solhaug *et al.* 2003, Schlensog *et al.* 2003). Der Lichtschutz durch Pigmente wirkt sowohl im aktiven, als auch im anabiotischen Zustand, allerdings können trockene Cortices optisch dichter sein als ihre durchfeuchteten Pendants. Weiterhin kann bei einigen Flechten während der Austrocknung der Xanthophyllzyklus aktiviert (Fernández-Marín *et al.* 2009, 2010) und Komponenten der photosynthetischen Lichtreaktion voneinander entkoppelt werden (Heber 2008). Aktive Mechanismen der Photoprotektion können die Schutzwirkung eines Cortex unterstützen. So wurde Anpassung an hohe PPFD bei der Antarktischen Flechte *Umbilicaria aprina* unter feuchten Bedingungen nachgewiesen (Kappen *et al.* 1998).

2.3.2 Starklichtantwort bei *Umbilicaria decussata*

Am Standort von *Umbilicaria decussata* in der Terra-Nova-Bucht wurden während des antarktischen Sommers starke Schwankungen der Luft-, Substrat- und Thallustemperatur, sowie der Lichtintensität gemessen. Die kosmopolitische Flechte *U. decussata* könnte über eine breite ökologische Amplitude verfügen, was bei anderen antarktischen Flechten mit kosmopolitischer Verbreitung im Vergleich mit Endemiten bereits festgestellt wurde (Romeike *et al.* 2002). Die Flechte *Stereocaulon alpinum* kommt in kalten, alpinen Habitaten beider Hemisphären vor, was auch bei *U. decussata* der Fall ist (Øvstedal und Smith 2001). *S. alpinum* zeigt eine besonders breite ökologische Amplitude und toleriert hohe Lichtintensität bei feuchtem aktiven Thallus (Romeike *et al.* 2002).

Hohe Lichtintensität trat während der Messperiode an Standorten der kontinentalen Antarktis von *U. decussata* nur für kurze Zeit auf. Die Laboruntersuchungen an intakten Thalli der Flechte zeigten, dass kurzzeitige Starklichtbehandlungen bis zu über 1000 µmol Photonen $m^{-2}s^{-1}$ nicht zu Photoinhibition führen. Dies galt auch bei 4°C in

Kombination mit 200 µmol Photonen $m^{-2}s^{-1}$. Exponierte Flechten sind am Standort während der Messperiode nicht der Kombination niedriger Temperatur und relativ hoher PPFD ausgesetzt.

Zwei Schutzmechanismen, die in der Symbiose wirksam sind, konnten festgestellt werden. Zum einen sind die Thalli von *U. decussata* tief schwarz gefärbt. Die Färbung geht auf Melanin zurück, was in dieser Studie erstmals nachgewiesen wurde. Melanine werden von lichenisierten und nichtlichenisierten Pilzen in großer Variation produziert (Beckett *et al.* 2012). Sie absorbieren im ultravioletten, aber auch im photosynthetisch aktiven Bereich und bieten somit effektiven Schutz gegen verschiedene schädliche Effekte der Sonneneinstrahlung (Nybakken *et al.* 2004, Meeßen *et al.* 2013). Die Photobionten, welche in einer definierten Schicht unterhalb des mit Melanin pigmentierten Cortex liegen, können von dieser Schutzfunktion profitieren. Zum anderen wurde die nicht-photochemische Energielöschung (NPQ) aktiviert. Höhere Lichtintensität und höhere Temperatur führten zu höheren NPQ-Werten. Die Kombination von Starklichtvermeidung durch Pigmentierung und Löschung überschüssiger Energie reichte in jedem untersuchten Fall aus, Stress auf Ebene des Photosystem II zu verhindern. Die eingesetzten Lichtintensitäten konnten effektiv genutzt werden.

Isolierte Photobionten hingegen zeigten bei 4°C Anzeichen von Photoinhibition auch bei den Kulturbedingungen entsprechender Lichtintensität (20 µmol Photonen $m^{-2}s^{-1}$). Höhere Temperaturen (12 und 20°C) führten zu verbesserter Starklichttoleranz. Dies korreliert mit erhöhter Aktivität des NPQ, hierbei wurde Violaxanthin zu Antheraxanthin und Zeaxanthin umgewandelt (Sadowsky und Ott 2015).

2.3.3 Austrocknung und Starklicht

Die Europäische Lungenflechte *Lobaria pulmonaria* zeigte Aktivierung des Xanthophyllzyklus bei Austrocknung ohne zusätzlichen Lichtstress, was als Anpassung an mit Austrocknung *in situ* verbundene Starklichtbedingungen, oder an möglicherweise hohe PPFD bei Wiederbefeuchtung gedeutet wurde (Fernández-Marín *et al.* 2010). Die untersuchten isolierten Photobionten (Tabelle 1) zeigten allerdings keine Umwandlung von Violaxanthin in seine de-epoxidierten Derivate. Auch konnte keine verstärkte Exprimierung NPQ-relevanter Gene im Transkriptom des *Usnea lambii*- und des *Umbilicaria decussata*-Photobionten festgestellt werden (siehe 2.4.4). Stattdessen zeigten wiederbefeuchtete Photobionten nach Reaktivierung erhöhte Sensibilität gegenüber

Starklicht. Dies konnte anhand der Donor-Limitierung des Photosystem (PS) I im *U. decussata*-Photobiont gezeigt werden (Abbildung), was auf gestörte Versorgung des PS I mit vom PS II gelieferten Elektronen hinweist.

2.4. Dehydrierung

2.4.1 Einführung

Austrocknung von Flechtenthalli führt zu einer Verminderung der photosynthetischen Leistung (Calatayud *et al.* 1997, Hájek *et al.* 2001) und schließlich zur Anabiose. Dies ist normaler Ausdruck ihrer poikilohydren Lebensweise (Larson 1979) und kann, je nach Flechtenart und Habitat, bis zu mehrmals am Tage erfolgen (Ahmadjian 1965, Kappen *et al.* 1988). Antarktische Habitate sind durch geringe Wasserverfügbarkeit charakterisiert, was besonders für die kontinentale und südliche maritime Antarktis gilt (Øvstedal und Smith 2001). Somit müssen antarktische Flechten über Austrocknungstoleranz verfügen. Die Myco- und Photobionten müssen Mechanismen aufweisen, welche eine bleibende Schädigung ihrer Zellen verhindern (Kranner und Lutzoni 1999). In der Symbiose können synergistische Effekte, welche die Stresstoleranz gegenüber den isolierten Symbionten verstärken, auftreten (Kranner und Birtic 2005, Kosugi *et al.* 2009). Über die physiologische Charakterisierung unterschiedlicher Trockentoleranz beteiligter Photobionten ist jedoch bislang wenig bekannt. Um den Beitrag zur Stresstoleranz und das physiologische Potential einiger antarktischer Photobionten zu untersuchen, wurde die Reaktion auf Austrocknung auf verschiedenen Ebenen untersucht. Diese sind Funktion und Schutz der photosynthetischen Lichtreaktion als Schlüsselfunktion des Photobionten-Metabolismus, die Bildung von und Ausstattung mit protektiven Metaboliten. Die genetische Basis wurde anhand der Transkriptome zweier ausgewählter Photobionten untersucht. Diese spiegeln eine frühe Stufe der Genexpression und wichtigen Ansatzpunkt für relevante Erkenntnisse zu den regulatorischen Prozessen wieder.

2.4.2 Reaktion des Photosystem II

Alle untersuchten Photobionten (Tabelle 1) wurden durch Dehydrierung photosynthetisch inaktiviert. Es konnte jedoch ein Photobionten-spezifisches Verhalten gezeigt werden, wobei sich die Geschwindigkeit der Inaktivierung deutlich unterschied. Die Photobionten der endemisch antarktischen Flechte *Usnea lambii* und von *Pleopsidium chlorophanum*

hielten hohe Werte der maximalen Quanteneffizienz (F_v/F_m) länger aufrecht als andere untersuchte Photobionten (Sadowsky und Ott 2012). Wie in Bezug auf die Gefrierresistenz zeigte sich bei der Inaktivierung durch Trockenheit keine an den phylogenetischen Gruppen ausgerichtete Verteilung der unterschiedlichen Austrocknungstoleranz. Dies ist besonders auffällig im Vergleich der Photobionten von *U. lambii* und *Umbilicaria decussata*, welche zum *clade* S der Gattung *Trebouxia* gehören. Bei langsamerer Austrocknung tritt noch deutlicher zutage, dass der *U. decussata*-Photobiont deutlich schneller inaktiviert wird (Sadowsky und Meeßen 2015).

Die Reaktivierungskinetik zeigte dem gegenüber ein verbindendes Element innerhalb des *clade* S. Bei diesen Photobionten war ein Anstieg der Minimalfluoreszenz des Chlorophyll *a* im PSII (F_0) in den ersten Minuten der Wiederbefeuchtung zu beobachten. Es könnte sich um den Effekt eines langsam regenerierenden Schutzmechanismus handeln, der unter Stressbedingungen die Stabilität des PSII unterstützt. Hájek *et al.* (2006) beobachteten eine Löschung von F_0 in osmotisch gestressten Thalli von *Lasallia pustulata* und *Umbilicaria hirsuta*. Ein Energielöschungsmechanismus der an den Antennen des PS II angreift (Jensen *et al.* 1999, Chakir und Jensen 1999) wurde postuliert. Auch an der Flechte *Parmelia sulcata* wurde ein Anstieg von F_0 während der Reaktivierung gemessen, dieser erfolgte jedoch deutlich schneller (Veerman *et al.* 2007). Ein schneller F_0-Anstieg konnte auch beim Photobiont der Flechte *Umbilicaria antarctica* beobachtet werden. Innerhalb weniger Sekunden wurde ein stabiles Level erreicht (Abbildung 3). Nach der raschen Austrocknung im experimentellen Aufbau zeigten alle reaktivierten Photobionten eine Reduktion ihrer photosynthetischen Kapazität gegenüber der Kontrollgruppe. Eine solche Reduktion, gemessen als verringerte F_v/F_m, ist ein Anzeichen für Degradation des D1-Proteins im Reaktionszentrum des Photosystem II (Richter *et al.* 1990). Ein weiterer Hinweis auf Beeinträchtigung des PSII liefert die Aktivität des in der Elektronentransportkette nachgeschalteten PS I reaktivierter Photobionten. Dessen früher auftretende Donor-Limitierung (siehe 2.3.2) ist ein Hinweis auf verminderte Fähigkeit zur Ladungstrennung im Photosystem II.

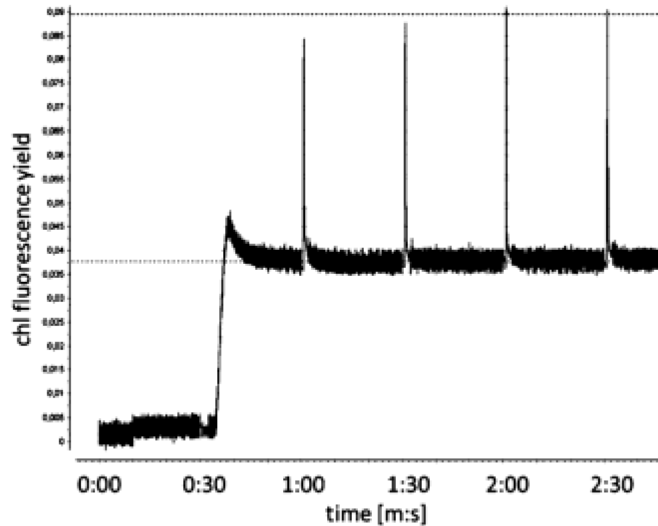

Abbildung 3 Chlorophyll *a*-Fluoreszenz des isolierten Photobionten von *Umbilicaria antarctica* während der Rehydrierung. Messlicht eingeschaltet bei 0:10, Wasser hinzugefügt bei 0:30. Peaks ab 1:00 spiegeln Sättigungspulse zur Messung der Quanteneffizienz wieder

2.4.3 Zusammensetzung und Dynamik des Metaboloms

Die Photobionten von *Umbilicaria decussata* und *Usnea lambii*, welche beide zum *clade* S der Gattung *Trebouxia* gehören, wurden aufgrund ihrer physiologischen Differenzen für eine Analyse der Metabolitzusammensetzung unter Kultur- und Austrocknungsbedingungen herangezogen. Dies stellt die erste Metabolomstudie isolierter Flechten-Photobionten dar. Während der Austrocknung über Silicagel wurden Proben zu drei Zeitpunkten genommen: nach 15 und 30 Minuten sowie am Ende der Dehydrierung. Letzterer Zeitpunkt wurde definiert durch die Reduktion der maximalen Quanteneffizienz des PS II auf 0. Im Fall des *U. lambii*-Photobionten trat dies nach 60 Minuten ein, während am *U. decussata*-Photobiont bereits nach 45 Minuten keine F_v/F_m gemessen werden konnte. Der Fokus der Analyse wurde auf hydrophile Substanzen gelegt, unter denen sich viele osmotisch wirksame Metabolite wie Zuckeralkohole oder Aminosäuren finden. Auch Aminosäuren mit potentiell regulativer Funktion sollten so identifiziert werden. Diese Stoffklassen sind in Flechten, Grünalgen und Pflanzen verbreitete Bestandteile der Antwort auf Trockenstress (Holzinger und Karsten 2013, Kranner *et al.* 2008).

Einige freie Aminosäuren, welche eine bekannte Rolle in der Antwort auf osmotischen und Trockenstress haben, wurden untersucht und in den Photobionten gefunden. Hier sind insbesondere Valin, Prolin und γ-Aminobuttersäure (GABA) zu nennen. Valin und Prolin

akkumulierten während der Austrocknung im *U. decussata*-Photobiont. Diese beiden Aminosäuren vermitteln Trockentoleranz in Höheren Pflanzen (Mattioli *et al.* 2008, Sharma *et al.* 2014) und Grünalgen (Delauney und Verma 1993). Die Konzentration von GABA erhöhte sich in beiden Photobionten. GABA kann direkt als Osmolyt wirken, Membranen und Proteinkonformation unter Austrocknungsbedingungen schützen (Sharma *et al.* 2014), oder möglicherweise ein Signalmolekül der Stressantwort sein. Bislang wurde ein dafür nötiger GABA-Rezeptor jedoch nur in einer Samenpflanze nachgewiesen (Žárský 2015).

In beiden Photobionten fiel ein hoher Gehalt an Ribitol und Sucrose auf. Beide Stoffe waren im *Usnea lambii*-Photobiont um ein Vielfaches höher konzentriert als im *Umbilicaria decussata*-Photobiont. Der Zucker Sucrose ist ein Osmolyt, kann also das Wasserpotential gegenüber einer Sucrose-freien Lösung herabsetzen, was Wasserverlust in die Umgebung einschränkt. Hohe Osmolyt-Konzentrationen in der Zelle können also konstitutive Mechanismen der Austrocknungsresistenz vermitteln. Besonders Trehalose kann zudem bei hoher Konzentration zu einer Verdichtung des Cytoplasmas führen. In diesem glasartigen Zustand sind Proteine und Membranen vor Denaturierung geschützt, während der Metabolismus zum Erliegen kommt (Holzinger und Karsten 2013). Trockentolerante Embryos Höherer Pflanzen enthalten ebenfalls hohe Konzentrationen von Sucrose, wodurch Proteine und Membranen bei Trockenheit geschützt werden. Allerdings hemmen hohe Sucrosekonzentrationen im Cytoplasma den Zellmetabolismus (Yancey 2005). Der Photobiont von *U. lambii* zeigte eine Reduktion der Sucrosekonzentration um etwa ¾ der Ausgangsmenge während der Austrocknung. Es ist möglich, dass der Zucker als Energiequelle genutzt wurde, um andere Mechanismen der Trockenheitsantwort zu stützen. Dazu kann die Synthese von Proteinen zählen. Der *U. lambii* Photobiont zeigte während der Austrocknung eine Überexpression von Proteinen der photosynthetischen Lichtreaktion sowie des Proteinsyntheseapparates im Allgemeinen (siehe 2.4.4).

Ribitol stellt die Transportform assimilierten Kohlenstoffs in der Flechtensymbiose dar. Mycobiontenzellen wandeln Ribitol, welches vom Photobionten ausgeschieden wird, in Mannitol um. Sowohl Ribitol als auch Mannitol sind Zuckeralkohole, also Polyole. Mannitol konnte in den untersuchten Photobionten nicht nachgewiesen werden. Im Gegensatz zu Sucrose zeigen Zuckeralkohole wie Ribitol keine hemmende Wirkung auf den Zellmetabolismus. Sie können als Energielieferant abgebaut werden, was auch unter

Stressbedingungen nachgewiesen werden kann (Karsten 2007). Als Charakteristikum terrestrischer Grünalgen und Pflanzen ist Ribitol nicht in aquatischen Algen wie *Chlamydomonas reinhardtii* zu finden (Roser *et al.* 1992, Holzinger und Karsten 2013). Diese Metabolite können die unter beständig aquatischen Bedingungen unnötige Trockenheitstoleranz terrestrischer Algen vermitteln, indem sie als Osmolyt wirken oder direkt die Struktur von Biomolekülen stabilisieren. Beide untersuchten Photobionten zeigten eine Reduktion der Konzentration einiger gemessener Polyole (Ribitol, Myoinositol und Sorbitol) während der Austrocknung. Dies kann auf Abbau der Polyole zur Energiegewinnung zurückzuführen sein. Glycerol, ein auch bei marinen Algen an der Stressantwort beteiligtes Polyol (Rathinasabapathi 2000), akkumulierte im *U. lambii* Photobiont und zeigte keine Konzentrationsänderungen im *U. decussata*-Photobiont. Im Metabolom des *U. lambii*-Photobionten konnte am Ende der Austrocknungsphase Regeneration der initialen Konzentrationen vormals abgebauter Polyole festgestellt werden. Dieser Photobiont zeigte auch unter Kulturbedingungen höhere Konzentrationen aller gemessenen Polyole. Insgesamt kann angenommen werden, dass Polyol-basierte Mechanismen der Trockenheitstoleranz im *U. lambii*-Photobiont effektiver sind als im *U. decussata*-Photobiont. Dies gilt sowohl für konstitutiven Schutz durch höhere Konzentrationen, als auch für stressinduzierte Akkumulation von Glycerol.

Die Ergebnisse zeigen, dass die Ausstattung und Dynamik hydrophiler Metabolite eine höhere Trockentoleranz des *Usnea lambii*-Photobionten bedingen können. Diese basiert insbesondere auf konstitutiv in höherem Maße verfügbaren Zuckern und Polyolen, sowie auf deren stressinduziertem Abbau. Die langsamere Inaktivierung und bessere Reaktivierungsfähigkeit gegenüber dem *U. decussata*-Photobiont kann von diesen Mechanismen gestützt werden. *U. lambii*-Thalli sind an ihren Standorten häufig besonderer Trockenheit ausgesetzt (Øvstedal und Smith 2001). Auf Mars Oasis, Alexander Island, wurde *U. lambii* („*U. sphacelata*", umbenannt nach Wirtz 2008) meist exponiert auf porösem Gestein gefunden, welches ansonsten nur von Krustenflechten besiedelt werden konnte (Romeike 2002). Die mittlere Thallusfeuchtigkeit an diesen Mikrostandorten betrug unter 30% des Trockengewichtes der Flechte. Höhere Wassergehalte der *U. lambii*-Thalli wurden nur sehr selten gemessen, vor allem an einem durch eine Schmelzwasserrinne gespeisten Mikrostandort. Zudem können die Flechten durch ihre Wuchsform schneller austrocknen und weniger von an der Thallusbasis entlangfließendem Wasser profitieren als *Umbilicaria*-Arten (Romeike 2002). Somit kann

die gesteigerte Toleranz des Photobionten gegen Austrocknung ein wichtiger Faktor für den Erfolg an antarktischen Standorten von *U. lambii* sein.

2.4.4 Zusammensetzung und Dynamik des Transkriptoms

Zum ersten Mal konnten die Transkriptome antarktischer Flechten-Photobionten sequenziert und analysiert werden. Auch komplette antarktische Flechten wurden noch nie in ähnlicher Weise untersucht. Die Ergebnisse liefern neue Einblicke, wie sie bislang noch nicht möglich waren. Die Transkriptome der Photobionten von *Usnea lambii* und *Umbilicaria decussata* wurden sowohl unter Bedingungen einer nicht gestressten Kultur als auch während des Austrocknungsprozesses analysiert, um die Antwort der Organismen auf den Stressor zu charakterisieren und zu quantifizieren.

Für die Experimente wurde die gesamte messenger-RNA (mRNA) der isolierten Photobionten in cDNA-Bibliotheken umgesetzt. Diese wurden per *next generation sequencing* sequenziert. Die einzelnen sequenzierten Fragmente (*reads*) wurden assembliert, d.h. anhand von Überlappungen zu zusammenhängenden *contigs* zusammengefasst. Zusammengefasst bilden diese eine Gesamtgröße des Transkriptoms von 43,5 Mbp (*U. decussata*-Photobiont) bzw. 47,1 Mbp (*U. lambii*-Photobiont) ab. Diese Länge ähnelt der Genomgröße verwandter freilebender Grünalgen wie *Coccomyxa subellipsoidea* (39,0 Mbp, Blanc *et al.* 2012) und *Chlorella variabilis* (46,2 MbP, Blanc *et al.* 2010). Ein größeres Genom wurde für *Asterochloris* sp., den Photobionten der Flechte *Cladonia grayi*, per qPCR geschätzt (Armaleo *et al.* 2009). Diese Erkenntnisse über *Trebouxia* und *Asterochloris*-Arten können als Hinweis darauf gewertet werden, dass die symbiotischen Algen keine Verkleinerung ihres Genoms erfahren haben, wie es bei einigen symbiotischen Bakterien der Fall ist (McCutcheon und Moran 2012). Besonders hohe Sequenzähnlichkeit der *contigs* wurde zu *C. subellipsoidea* festgestellt. Die Quantifizierung der *reads* wurde per heterologem *mapping* auf Basis des publizierten *C. subellipsoidea*-Transkriptoms durchgeführt.

Die *reads* konnten jeweils c. 6000 Genen quantitativ zugeordnet werden. Wenige Gene, die allerdings nur geringe Expression zeigten, waren nur in einem der beiden Transkriptome zu finden. Bedeutsamer waren die quantitativen Unterschiede im Expressionsmuster der beiden Photobionten. Aufgrund ihrer Transkriptome konnten die Photobionten scharf voneinander abgegrenzt werden. Bereits im ungestressten Zustand der Kontrollgruppe zeigten sich signifikante Unterschiede betreffend 2196 Genen, also

über einem Drittel der quantifizierten Sequenzen. Darunter befanden sich Gene, welche möglicherweise einen Einfluss auf die Trockenheitstoleranz der Photobionten haben.

Mehrere Hinweise auf eine gegenüber dem *U. decussata*-Photobiont höhere Trockentoleranz des *U. lambii* Photobionten (Sadowsky und Ott 2012, Sadowsky *et al.* 2015) konnten mit erhöhter Expression bestimmter Gene unter Kontrollbedingungen in Verbindung gebracht werden:

- Eine Ascorbat-Peroxidase (APX) zeigte höhere Expressionslevel im *U. lambii*-Photobiont. Enzyme aus der Klasse der APX sind an der Antwort auf oxidativen Stress beteiligt, wie er bei Austrocknung und durch überschüssige photosynthetisch absorbierte Lichtenergie auftritt. (Shigeoka *et al.* 2001).
- *Late embryogenesis abundant* (LEA)-Protein-Gene zeigten leicht höhere Expression. Die Produkte können in verschiedenen pflanzlichen Organismen Trockentoleranz durch Schutz von Proteinen und Membranen vermitteln (Moore *et al.* 2009).
- *Heat shock*-Proteine (Hsp) sind sowohl für die Neusynthese von Proteinen, als auch für die Reparatur fehlgefalteter Peptidketten notwendig, wie sie unter Stressbedingungen auftreten kann (Wang *et al.* 2004). Betreffende Transkripte zeigten höhere Abundanz im Transkriptom des *U. lambii* Photobionten, wobei besonders solche Sequenzen vertreten waren, die eine Chaperonfunktion an plastidären Membranen ausüben.

An den austrocknenden Photobionten war ein genereller Trend zur Herunterregulation in verschiedenen Funktionen des Aufbaustoffwechsels (Anabolismus) zu verzeichnen. Dies betraf die Zellteilung, den Zellwandaufbau, die Zellteilung und Chromatin-Organisation. Besonders im *U. lambii*-Photobiont wurde die Synthese von Polyaminen herunterreguliert, was möglicherweise einen negativen Einfluss auf Teilungsaktivität hat (Paschalidis *et al.* 2005, Theiss *et al.* 2002). Diese Entwicklungen deuten auf einen kontrollierten Übergang in die metabolische Inaktivität hin. Gleichzeitig wurden in beiden Photobionten die Respirationsketten der Mitochondrien hochreguliert. Es kann geschlossen werden, dass der Stoffwechsel insgesamt sich von Anabolismus zu Katabolismus (Energiestoffwechsel) hin verschob.

Da der Abbau von Stärke oder Lipiden nicht hochreguliert wurde, ist es möglich, dass Proteine als Energiequelle genutzt wurden. Die starke Hochregulation von Protein abbauenden Genen, besonders im *U. lambii*-Photobiont, könnte zum Teil so begründet werden. Der Proteinabbau könnte außerdem zum Abbau beschädigter Proteine dienen. Der *U. lambii*-Photobiont zeigte gleichzeitig eine erhöhte Expression von Genen, die für Proteine der photosynthetischen Lichtreaktion codieren. Ribosomale Proteine, die für die Proteinbiosynthese benötigt werden, wurden ebenfalls verstärkt exprimiert. Chaperone, welche an den Membransystemen des Chloroplasten angreifen, können den Prozess unterstützen. Somit kann aus einem Zusammenspiel von Abbau und Neusynthese ein verstärktes *turn over* der photosynthetisch bedeutsamen Proteine gewährleistet werden. Da somit intakte Photosysteme während der Trockenbehandlung aufrecht erhalten werden können, kann hierin ein Grund für die gegenüber dem *U. decussata*-Photobionten längere photosynthetische Aktivität des *U. lambii*-Photobionten begründet sein.

3. Zusammenfassung

Die vorliegende Studie belegt Anpassungen auf unterschiedlicher Ebene von Flechten-Photobionten an extreme Standorte der Antarktis. Photobionten der Gattung *Trebouxia* wurden aus fünf antarktischen Flechten sowie einer europäischen Flechte isoliert, kultiviert und auf ihre Reaktion auf extreme Trockenheit, Kälte und Lichtintensität hin geprüft. Dabei zeigten sich deutliche Unterschiede des physiologischen Potentials im Vergleich verschiedener Photobionten der jeweiligen Flechtenarten.

Die dominante Gruppe von *Trebouxia*-Photobionten in antarktischen Makroflechten ist der *clade* S. Innerhalb dieser Gruppe variierten die physiologischen Reaktionen auf potentielle abiotische Stressoren signifikant. Es konnte gezeigt werden, dass hinsichtlich Gefriertoleranz und Trockenheitstoleranz jene Photobionten ein höheres Potential besitzen, welche aus endemischen antarktischen Flechten isoliert wurden. Somit übertraf die Stresstoleranz der Photobionten von *Usnea lambii*, *Pleopsidium chlorophanum* und *Umbilicaria antarctica* jene des Photobionten von *Umbilicaria decussata*. Die *clade* S-Photobionten der Flechten endemischen Ursprungs ähnelten insofern dem Photobionten der antarktischen endemischen Krustenflechte *Buellia frigida* (*clade* A). Die geringere Stresstoleranz des Photobionten der kosmopolitischen Flechte *Umbilicaria decussata* hingegen war eher vergleichbar mit derjenigen des Photobionten der schwedischen Flechte *Fulgensia bracteata* (*clade* I). Zusätzlich ist die Wuchsform der betreffenden Flechten zu beachten. So kann eine erhöhte Trockenheitstoleranz für den Photobionten von *U. lambii* von Vorteil sein, da die Aufrechte, verzweigte Wuchsform eine langfristige Durchfeuchtung mit Schmelzwasser erschwert.

Die *clade* S-Photobionten von *Usnea lambii* und *Umbilicaria decussata* wurden aufgrund ihrer signifikanten stressphysiologischen Differenzen unter Austrocknungsbedingungen mit Methoden der Metabolitanalyse und Molekularbiologie genauer untersucht. Die so erreichte Tiefe der Analyse ist für antarktische Flechten-Photobionten sowie für antarktische Flechten überhaupt eine Neuheit. Die Ergebnisse lieferten wertvolle Erkenntnisse über zu Grunde liegende Mechanismen der Stressantwort.

Metabolite, vor allem Zucker, Polyole und Aminosäuren, zeigten hohe Dynamik während des Austrocknungsprozesses. Hinweise auf den Abbau schnell verfügbarer energiereicher Moleküle sowie die Akkumulation möglicherweise Trockentoleranz vermittelnder

Aminosäuren wiesen auf eine kontrollierte Einstellung des Metabolismus auf die rasche Austrocknung hin. Besonders hinsichtlich konstitutiv, das heißt nicht stressinduziert vorhandener Osmotika wie Ribitol und Sucrose, wurde die höhere Trockentoleranz des *U. lambii*-Photobionten widergespiegelt.

In der Genexpression waren Unterschiede der beiden Photobionten ebenfalls signifikant und betrafen verschiedene Funktionen des Metabolismus. Wie auch betreffend der Ausstattung mit Metaboliten, zeigten sich bedeutsame Unterschiede konstitutiv vorhandener Schutzmechanismen. Diese konnten mit höherer Stresstoleranz im *U. lambii*-Photobiont in Verbindung gebracht werden. Ein weiterer als wichtig einzuschätzender Faktor, welcher besonders die verlängerte Aktivität des Photosystem II des *U. lambii*-Photobionten während der Austrocknung erklären kann, war in der Änderung des Expressionsmusters während des Prozesses auszumachen. Eine Überexpression von Proteinen der photosynthetischen Lichtreaktion sowie Hochregulation im Proteinstoffwechsel und in Funktionen zur Sicherstellung korrekter Proteinfaltung speziell an den Membranen der Chloroplasten waren zu verzeichnen. Dies kann als Strategie interpretiert werden, welche sowohl während der Austrocknung die Funktion der Photosynthese gewährleistet, als auch während einer auf Austrocknung folgenden Reaktivierung intakte Mechanismen zur Verfügung stellt. Bei beiden Photobionten konnten Hinweise auf einen Umschlag von aktivem Aufbaustoffwechsel zu Energiestoffwechsel festgestellt werden. Es kann geschlossen werden, dass ein kontrollierter Übergang in die Anabiose (latentes Leben nach Austrocknung) im Metabolismus der Photobionten stattfindet.

Die Hypothese, dass antarktische Photobionten des *Trebouxia-clade* S einheitliche Anpassungen an Extrembedingungen zeigen, muss zurückgewiesen werden. Unterschiede innerhalb dieser Gruppe, die auch bei identischen Kulturbedingungen evident sind, zeigen, dass die Zusammenhänge komplexer sein müssen. Es kann also von verdeckter ökologisch bedeutsamer Diversität innerhalb dieser Gruppe von Photobionten gesprochen werden. Stattdessen können unterschiedliche Anpassungsmechanismen in den untersuchten Photobionten festgestellt werden. Diese sind in Verbindungen zu bringen mit der geographischen Verbreitung und Wuchsform der betreffenden Flechten.

Die Ergebnisse zeigen ein bemerkenswertes physiologisches Potential der untersuchten Photobionten. Es konnte zwischen generalistischen Mechanismen, wie bei der

kosmopolitischen Flechte *Umbilicaria decussata* und ihrem Photobiont, und hoch spezialisierten Anpassungsmechanismen bei antarktischen Endemiten unterschieden werden. Aufgrund dieser Ergebnisse kann postuliert werden, dass bei einer möglichen Erwärmung antarktischer Flechten-Habitate, welche mit verbesserter Wasserverfügbarkeit einhergehen kann, spezialisierte Endemiten gegenüber Arten mit breiterer ökologischer Amplitude benachteiligt werden.

Auf der Basis der Erkenntnisse dieser Studie wird die aktuelle Situation charakterisiert. Diese Charakterisierung ist relevant für die Vorhersage zukünftiger Entwicklungen im terrestrischen antarktischen Ökosystem.

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5. Publikationen

5.1 Photosynthetic symbionts in Antarctic terrestrial ecosystems: the physiological response of lichen photobionts to drought and cold

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Photosynthetic symbionts in Antarctic terrestrial ecosystems: the physiological response of lichen photobionts to drought and cold

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Abstract Lichens form an important part of the biodiversity in terrestrial ecosystems of Antarctica where they represent the dominant vegetation. Previous studies on the genetic diversity of photobionts of lichens have indicated that clade S *Trebouxia* photobionts are the most widespread in continental Antarctica, predominantly in macrolichens. For the first time, a comparative study of the physiology of a variety of isolated Antarctic lichen photobionts (genus *Trebouxia*) was performed. Photosynthetic activity was examined by chlorophyll *a* fluorescence and correlated with freezing and desiccation under laboratory conditions and photosynthetic pigments were quantified in response to desiccation. Data were obtained from photobionts collected from the Antarctic regions of North Victoria Land, Coal Nunatak and Rothera Point, as well as from a European site (Gotland, Sweden). While the isolated algae reacted individually to stress treatments, they were highly susceptible to desiccation stress but could rapidly recover from freezing. Photobiont-specific physiological adaptations are considered to explain the dominance of clade S *Trebouxia* photobionts.

Keywords Rehydration · Desiccation · Freezing · Xanthophyll cycle · Photosystem II · Isolated photobionts

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Abbreviations

A	Antheraxanthin
chl FY	chlorophyll fluorescence yield
DEPS	de-epoxidation status of the xanthophyll pool
F_0	minimum chl FY in the dark-acclimatized state
F_M	maximum chl FY in the dark-acclimatized state
F_V/F_M	maximum quantum yield of PS II
HPLC	high performance liquid chromatography
NPQ	non-photochemical quenching
PAM	pulse-amplitude modulation
PS II	photosystem II
ROS	reactive oxygen species
SE	standard error of the mean
TOM	*Trebouxia* organic medium
V	violaxanthin
Z	zeaxanthin

1 Introduction

Lichens dominate the macroflora of terrestrial habitats in continental Antarctica (Kappen 2000). The region is characterized by drought, low temperatures and high insolation. These give rise to extreme conditions for vegetation (Huiskes et al. 2006; Block 1996). For poikilohydric lichens, water availability is especially important in maritime as well as in continental Antarctica (Kennedy 1993). Uptake of water from snow on the thallus surface of lichens, even at subzero temperatures, can be adequate for rehydration (Kappen and Breuer 1991; Kappen 1993, 2000; Schroeter et al. 1994, 1997; Pannewitz et al. 2003). As described by Lange and Kappen (1972) and Walton

(1982), lichens receive sufficient light to photosynthesize even under several centimeters of snow. Especially, in continental Antarctica, the insulating effects of snow cover may maintain temperatures below the minimum for photosynthesis (Pannewitz et al. 2003). The negative effects of prolonged snow cover may also include a combination of high thallus humidity and low light during winter. Therefore, lichens may experience a net carbon deficiency as a result of respiration under snow (Kappen 2000, Pannewitz et al. 2003).

In order to establish and thrive under this harsh environment, lichens must have physiological characteristics that are highly adapted to the conditions experienced. When there is a lack of water both bryophytes and lichens can enter a state of anabiosis and are then capable of resisting temperature extremes (Kappen and Lange 1972; Kappen 1973; Harańczyk et al. 2012) and high levels of irradiance (Kappen and Valladares 1999; Schlensog and Schroeter 2000). This feature contributes substantially to the success of lichens in these habitats (Schlensog et al. 2003). Kappen and Lange (1970) investigated the cold tolerance of photobionts from different temperate region lichens. The photosynthetic physiology and energy balance of well-developed lichen thalli in the maritime as well as in continental Antarctica has been addressed in ecophysiological field studies (e.g. Kappen et al. 1995; Schroeter et al. 1994, 2000; Schlensog et al. 2003).

The mycobiont provides a "growth chamber" (Honegger 2009) with suitable conditions for physiological activity of the photosynthetic partner. However, special adaptations in the physiology of the photobiont are postulated to explain the ability of the photobiont to cope with severe environmental conditions.

High radiation and desiccation can be important sources of oxidative stress for the lichen photobionts (Kranner et al. 2005). To avoid the formation of reactive oxygen species (ROS), the non-photochemical quenching (NPQ) of chloroplasts drains excess excitation energy from PS II (Fernández-Marín et al. 2010). The different mechanisms involved include photoinhibition (degradation of the D1 protein in the reaction center of PS II) and thermic energy dissipation by the xanthophyll cycle (Demmig-Adams and Adams 1996; Krause and Jahns 2004). The poikilohydric lifestyle of lichens includes repeated and long-term desiccation of the thallus. Therefore, desiccation stress in the photobionts is induced which leads to the formation of ROS (Kosugi et al. 2009). In addition to the photoprotective function, the formation of zeaxanthin via the xanthophyll cycle can be induced by dehydration of a lichen thallus in the dark, as demonstrated for *Lobaria pulmonaria* from temperate regions in Spain (Fernández-Marín et al. 2009, 2010).

Knowledge of adaptation mechanisms at the photobiont level is inadequate (Kranner et al. 2005; Kranner and Birtic 2005), especially with respect to photobionts from Antarctica (Barták et al. 2007). Using isolated symbionts, it has been shown that the photobionts are more susceptible to extreme conditions than the mycobiont in an astrobiological context (de Vera and Ott 2010).

In genetic studies based on non-coding internally transcribed spacer (ITS) sequences (Brandt 2011), photobionts of the *Trebouxia* clade S (phylogeny after Helms et al. 2001) were identified as the dominant photobionts in continental Antarctic macrolichens. These photobionts are currently named *Trebouxia jamesii* (Friedl 1989; Beck 2002; Helms 2003; Brandt 2011).

It was hypothesized that the observed dominance of clade S *Trebouxia* photobionts (Brandt 2011) in continental Antarctica was the result of common physiological features, which contributed positively to the performance of lichen species colonizing this extreme environment. The performance of the photosynthetic light reaction of isolated photobionts of different lichen species from different sites was therefore compared with their reaction to low temperatures and desiccation.

2 Material and methods

2.1 Lichen collection and biont isolation

The investigations were carried out with photobionts isolated from *Buellia frigida* Darb., *Umbilicaria decussata* (Vill.) Zahlenbr. and *Pleopsidium chlorophanum* (Wahlenb.) Zopf, *Usnea lambii* R. Br., *Umbilicaria antarctica* (Frey & I.M. Lamb) and *Fulgensia bracteata* (Hoffm.) Räs.. The lichen thalli were collected dry, air-dried, transported at −20 °C and stored at −20 °C until photobiont isolation. The identity and geographic origin of the respective photobionts is shown in Table 1.

Photobionts were isolated from thallus fragments according to Yoshimura et al. (2002) and cultivated on *Trebouxia* organic medium agar (TOM) with 1 % glucose according to Ahmadjian (1967). The axenic cultures were kept at low light intensity (20 μmol photons m^2s^{-1}; diurnal cycle with 10 h of darkness) and 12 °C in a growth chamber (Rubarth Apparate GmbH, Germany). For physiological experiments, the photobionts were transferred to 1 cm^2 nitrocellulose filter discs on TOM-agar growing in the growth chamber for 4 weeks.

2.2 Chlorophyll *a* fluorescence

Chlorophyll *a* fluorescence was determined by a Mini-PAM (pulse-amplitude modulated) fluorimeter (Walz Mess- und Regeltechnik, Germany) according to Maxwell and Johnson (2000). The parameter $(F_M\text{-}F_0)/F_M = F_V/F_M$ was measured after 20 min of dark acclimation of the photobiont samples

Table 1 Photobiont species and origin. Clade definitions according to Helms et al. 2001

Mycobiont	Photobiont	Literature	Origin
Buellia frigida	*Trebouxia* sp., clade A (identical to NCBI AY667580.1)	Brandt 2011	Gondwana station, North Victoria Land, continental Antarctica
Fulgensia bracteata	*Trebouxia* sp., clade I, subgroup 1	Schaper and Ott 2003	Gotland, Sweden
Pleopsidium chlorophanum	*Trebouxia jamesii*, clade S	Brandt 2011	Gondwana station
Umbilicaria antarctica	*Trebouxia jamesii*, clade S, NCBI AJ431575.1	Romeike et al. 2002	Rothera Point, Adelaide Island, Antarctic Peninsula
Umbilicaria decussata	*Trebouxia jamesii*, clade S	Brandt 2011	Gondwana station
Usnea lambii	*Trebouxia jamesii*, clade S	Brandt 2011	Coal Nunatak, Alexander Island, southern maritime Antarctica

by application of a saturating light pulse (c. 5,000 μmol photons $m^{-2}s^{-1}$).

For dehydration and rehydration, filter discs carrying photobionts were placed into 50 ml glass flasks. The Mini-PAM sensor was attached at a distance of 0.5 cm to the sample and the flask was sealed. The time course of F_V/F_M was measured for 15 or 60 min. Dehydration was performed in a dry atmosphere (RH<5 %) over silica gel. To avoid dehydration in control and rehydration experiments, a water-saturated atmosphere was established in the flask by spraying with deionized water. Additionally, the filter discs carrying photobionts were placed on TOM-moistened cotton to avoid dehydration and osmotic stress. This treatment, in preliminary tests, proved to be a way to obtain unstressed F_V/F_M values. The water-saturated atmosphere method was applied to rehydrate desiccated samples which had been stored in a dry atmosphere for seven days in the dark.

Dark-acclimated photobionts were exposed to −25 °C in a freezer (Liebherr, Germany) in the dark for 1, 24 and 48 h. After 48 h of freezing, F_V/F_M was measured at −25 °C and during the thawing process at 20 °C.

2.3 Pigment analysis

Unstressed and stressed photobiont samples on filter discs were used for acetonic pigment extraction according to Pfeifhofer et al. (2002). The samples were placed in a 1.5 ml reaction tube and frozen in liquid nitrogen immediately. A spatula tip of $MgCO_3$ was added to prevent acidic pigment degradation and the cells were ground with a small pestle in 0.5 ml cold 100 % acetone. After extraction in 100 % acetone, the extract was placed in an HPLC system (Schambeck/Hitachi/Merck, Germany; injection of 20 μl per sample; Solvents: acetonitrile : methanol : tris acetate buffer 87:10:3 and methanol : n hexan 4:1) for pigment determination. The de-epoxidation status of the xanthophyll pool (DEPS) was calculated as DEPS = (Z + A)/(V + A + Z) (Vráblíková et al. 2004). High values of the DEPS indicate activation of the photoprotective xanthophyll cycle.

2.4 Data analysis

Analyses of pigment and chlorophyll fluorescence data were performed in MS Excel 2010 and GraphPad Prism 6. Significant differences between data sets were detected by two-sided t-tests; level of significance α=5 %.

3 Results

3.1 Effects of dehydration

Fast dehydration over silica gel caused a decline of photosynthetic capacity. The maximum quantum yield of photosystem (PS) II (F_V/F_M) dropped to near-zero values in all examined photobionts. This indicates a complete loss of photosynthetic activity and the decline can be represented by sigmoidal kinetics (Fig. 1). The unstressed levels and the rate of photosynthetic inactivation F_V/F_M differed among the photobionts (Table 2). In the clade S *Trebouxia* photobionts of *Usnea lambii*, *Pleopsidium chlorophanum* and *Umbilicaria decussata* (Fig. 1a, b, d), a slow initial decrease was followed by very fast reduction of photosynthetic capacity, expressed by the reduction of F_V/F_M to half the unstressed level within c. 7–17 min, depending on the photobiont isolate. The photosynthetic capacity of the photobionts of *Buellia frigida* and *Umbilicaria antarctica* (Fig. 1c, e) showed a faster initial decrease. The clade I photobiont from the Swedish lichen *Fulgensia bracteata*, which originated from a temperate environment, showed an intermediate reaction with F_V/F_M reduced to half the unstressed level after c. 11 min and the initial reduction of F_V/F_M was similar to that in the *Buellia frigida* and *Umbilicaria decussata* photobionts (Fig. 1f, Table 2). The photobionts from the two *Umbilicaria* species experienced reduction of F_V/F_M to half the unstressed value after short desiccation time compared with the other photobionts in this study (*U. decussata*: after c. 7 min; *U. antarctica*: after c. 10 min). The clade S *Trebouxia* photobionts of *Usnea lambii*, *Pleopsidium*

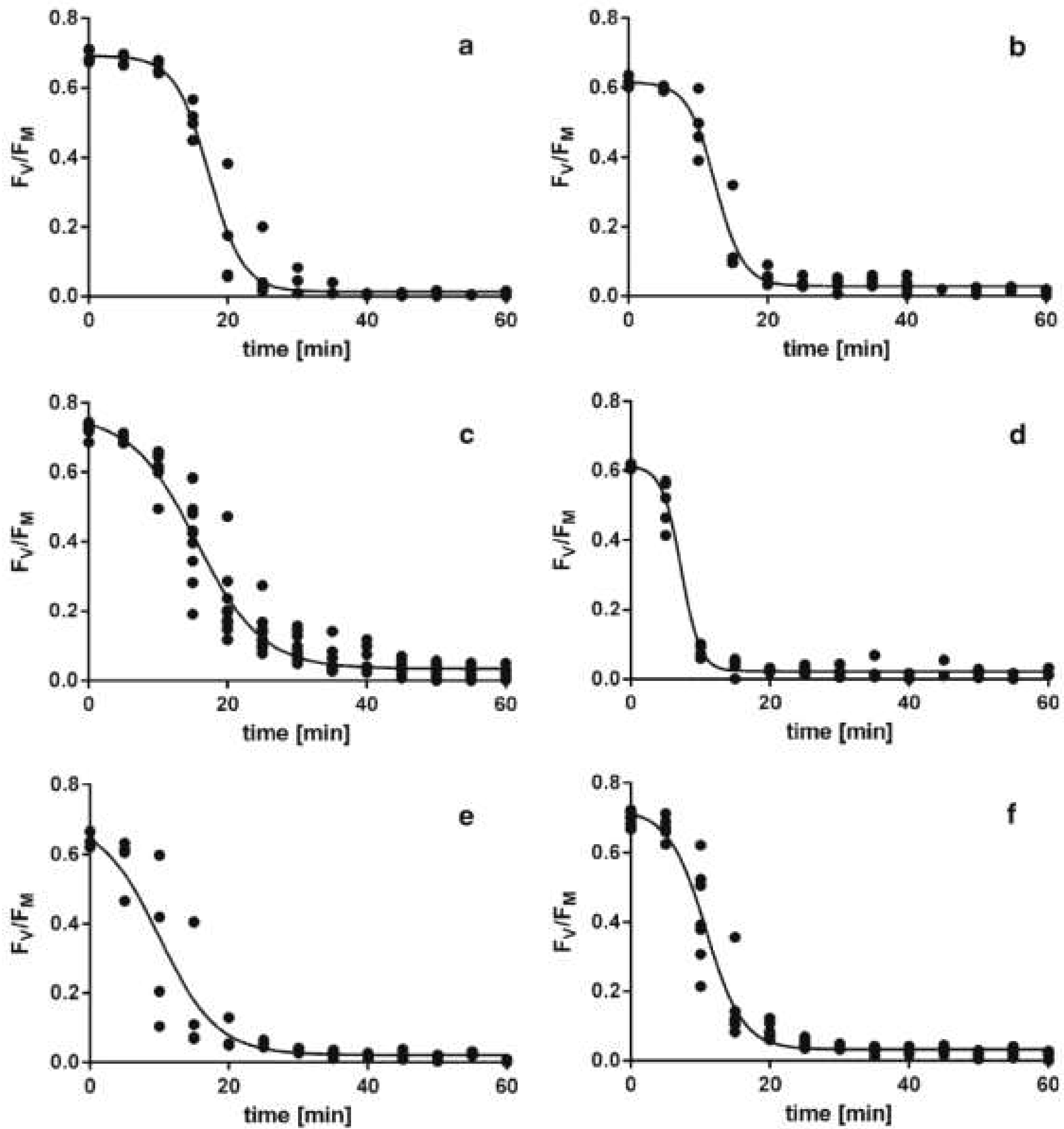

Fig. 1 Kinetics of F_V/F_M during dehydration. Dots indicate the values of single replicates, the line represents a sigmoidal data regression. Photobionts isolated from **a** *Usnea lambii*; **b** *Pleopsidium chlorophanum*; **c** *Buellia frigida*; **d** *Umbilicaria decussata*; **e** *Umbilicaria antarctica*; **f** *Fulgensia bracteata*

Table 2 Statistical analysis of the photosynthetic deactivation during dehydration and reactivation after freezing. Data presented are based on the regression analysis

Lichen species Photobiont clade	U. lam. S	P. chl. S	B. fri. A	U. dec. S	U. ant. S	F. bra. I
Unstressed F_V/F_M: best fit ± SE	0.69±0.02	0.62±0.02	0.77±0,02	0.61±0.01	0.70±0.07	0,72±0.02
Desiccation time [minutes] to reach ½ F_V/F_M	17.32	12.50	15.55	7.22	9.99	10.81
Reactivation after freezing						
Final F_V/F_M: best fit ± SE	0.68±0.01	0.64±0.02	0.69±0.01	0.66±0.03	0.60 ±0.01	0.73±0.02
Thawing time [seconds] to reach ½ final F_V/F_M	3.49	0.68	7.81	21.19	11.35	22.13

U. lam. Usnea lambii; *P. chl. Pleopsidium chlorophanum*; *B. fri. Buellia frigida*; *U. dec. Umbilicaria decussata*; *U. ant. Umbilicaria antarctica*; *F. bra. Fulgensia bracteata*; *SE* standard error of the mean

chlorophanum and the clade A photobiont of *B. frigida* showed slower reduction of F_V/F_M (Table 2).

During dehydration of the photobionts, no significant change in their photosynthetically important pigment composition could be observed (Fig. 2). The results indicate that there is no evidence for pigment conversion during desiccation.

The de-epoxidation status of the xanthophyll pool (DEPS) was low in all examined samples, but small amounts of antheraxanthin and zeaxanthin were present. The clade S *Trebouxia* photobionts from *Usnea lambii*, *Pleopsidium chlorophanum* and *Umbilicaria decussata* showed a generally higher DEPS compared with the other tested photobionts.

3.2 Effects of rehydration

When rehydrated after 1 week of drought, the maximum quantum yield could only be partly regenerated (Fig. 3). In all photobionts investigated, F_V/F_M rose to a maximum level within about 5 min. The level was always below the unstressed activity that had been reached before the treatment. The increase of F_V/F_M was mainly due to increasing F_M, while F_0 of the photobionts of *Buellia frigida*, *Umbilicaria antarctica* and *Fulgensia bracteata* remained nearly unchanged during rehydration. The photobionts of *Usnea lambii*, *Pleopsidium chlorophanum* and *Umbilicaria decussata* (Fig. 3a, b, d; all are clade S *Trebouxia*) showed increasing F_0 values within the first minutes. This was followed by a decline of F_M. *Usnea lambii* and *Umbilicaria decussata* photobionts showed a subsequent decline of F_0. As a consequence, F_V/F_M of the *Pleopsidium chlorophanum* and *Usnea lambii* photobionts was reduced during rehydration, while the *U. decussata* photobiont showed constantly low F_V/F_M (0.15±0.03) in the steady state of rehydration. The reduction of photosynthetic capacity of the *P. chlorophanum* photobionts during rehydration needed longer than in the other tested clade S photobionts (Fig. 3b).

3.3 Effects of freezing

Freezing temperatures of −25 °C caused a reduction of the maximum quantum yield F_V/F_M in all tested photobionts, but with a photobiont-specific time dependency (Fig. 4). For the photobionts of the continental Antarctic lichens *Pleopsidium chlorophanum* and *Umbilicaria decussata* and of the European lichen *Fulgensia bracteata*, 1 h of freezing was sufficient for a significant reduction of PS II activity. Prolonged freezing did not have any further significant effect. The photobionts of *Buellia frigida*, *Usnea lambii* and *Umbilicaria antarctica* showed no significant reduction of F_V/F_M after 1 h of freezing. After 24 h, F_V/F_M was significantly reduced, and this effect was stronger in *Usnea lambii* and *Umbilicaria antarctica*. Prolonged freezing led to

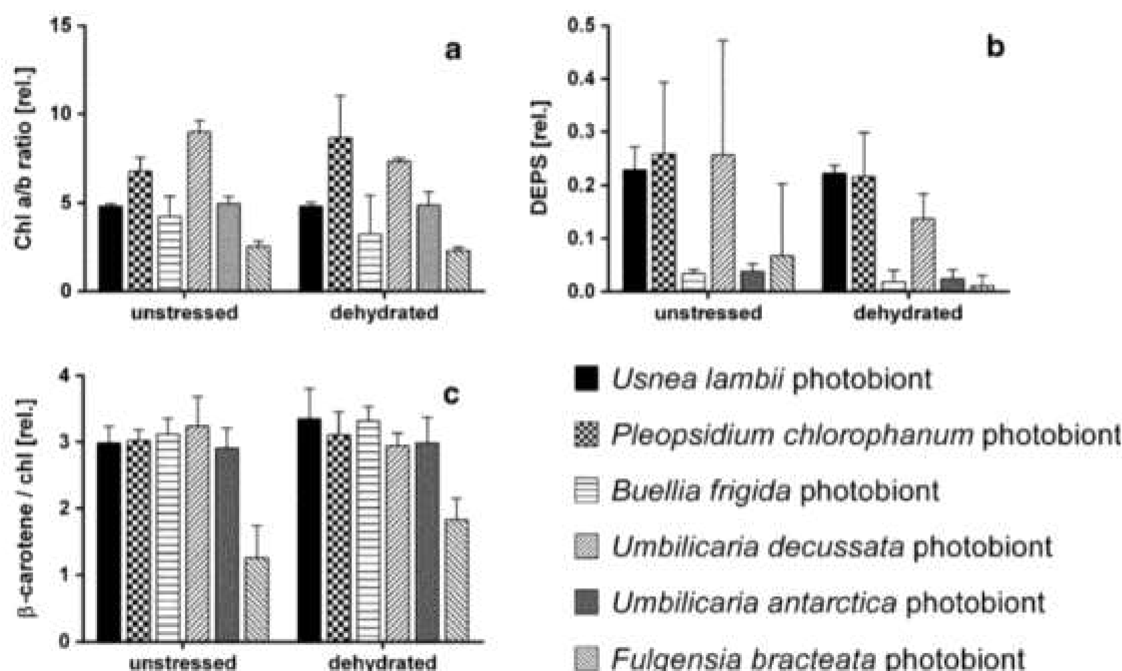

Fig. 2 The effect of desiccation on the pigment composition. Dehydration was performed for 30 min in the dark. All data are relative values, presented as mean + standard deviation. a ratio chlorophyll *a*/chlorophyll *b*; b de-epoxidation state of the xanthophyll pool; c β-carotene/total chlorophyll ratio

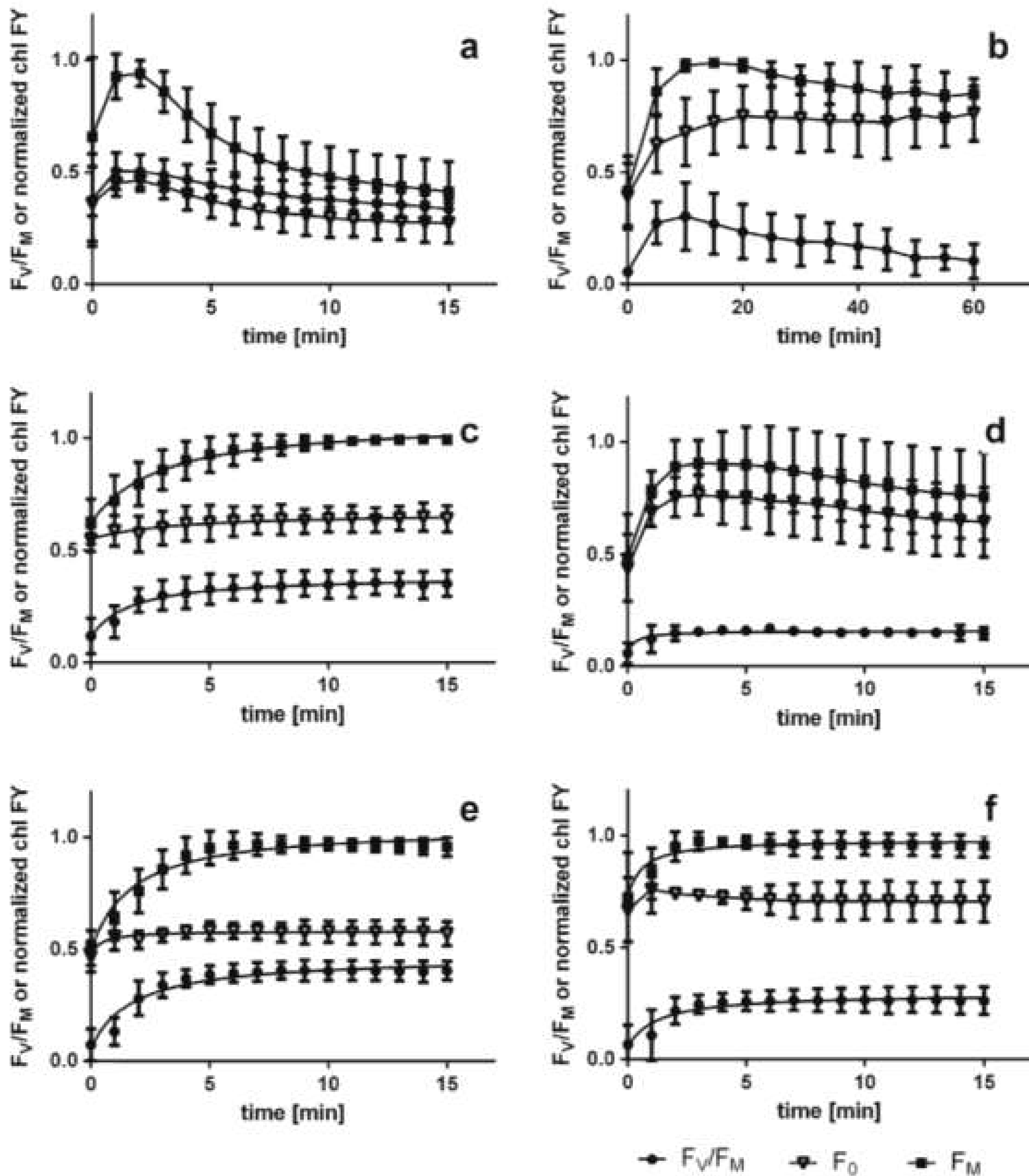

Fig. 3 Effects of rehydration of previously dehydrated photobionts. Values are presented as means with standard deviation (different time scale in b). In c, e and f, the F_M and F_V/F_M data could be fitted by saturation kinetics. F_V/F_M, but not F_M, could be fit in the same way in d. Chl FY values are normalized as maximal $F_M=1$. Photobionts examined from **a** *Usnea lambii*; **b** *Pleopsidium chlorophanum*; **c** *Buellia frigida*; **d** *Umbilicaria decussata*; **e** *Umbilicaria antarctica*; **f** *Fulgensia bracteata*

significantly lower values of F_V/F_M in the *Buellia frigida* photobionts, but not in those of *Usnea lambii* and *Umbilicaria antarctica*.

During thawing, F_V/F_M could be regenerated within seconds to minutes in the photobionts examined (Fig. 5, Table 2). The final photosynthetic capacity of the *Usnea lambii*, *Buellia frigida* and *Umbilicaria antarctica* photobionts after thawing was slightly, but significantly, lower than the unstressed capacity. Though regeneration of F_V/F_M was completed after a short time in the photobionts tested, there were differences in the regeneration rates (Table 2). The fastest regeneration was observed in the clade S photobionts of *Usnea lambii* and *Pleopsidium chlorophanum*, contrasted with the relatively slow regeneration of the *Fulgensia bracteata* and *Umbilicaria decussata* photobionts.

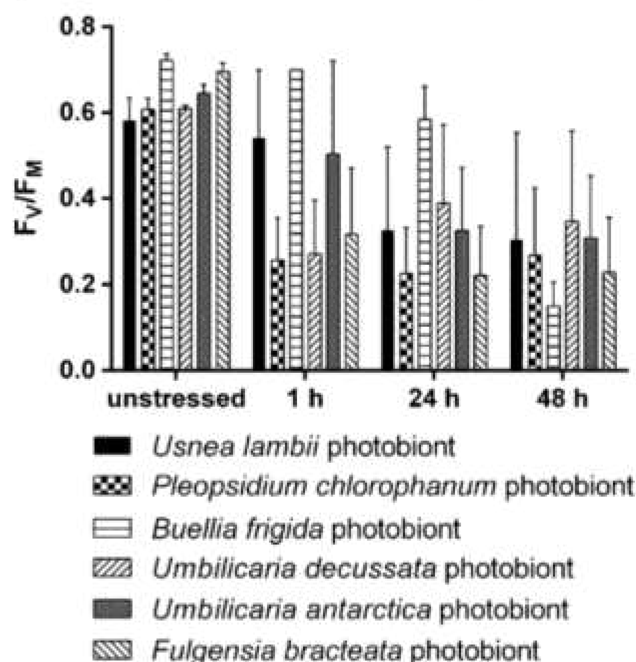

Fig. 4 Effects of freezing at −25 °C for 1, 24 and 48 h of the photobionts investigated. F_V/F_M values are presented as mean + standard deviation

4 Discussion

4.1 Dehydration

Water availability is a key factor in the dry continental environment of North Victoria Land (Huiskes et al. 2006). Air humidity is generally low and precipitation exclusively occurs as snow (Kennedy 1993). Lichen hydration depends mainly on meltwater and snow on thallus surfaces (Pannewitz et al. 2003). Mechanisms for water retention such as greater thallus thickness or dense up and lower cortices, are provided by the symbiotic state (Valladares et al. 1997), but photobionts may profit from their ability to perform photosynthesis under desiccating conditions. In continental Antarctica, lichens hydrated by meltwater can thus be active at high solar irradiation. In maritime Antarctica and also in more temperate regions, lichens depend more on air humidity as a water source and high insolation causes desiccation of lichen thalli. Excess light may also lead to oxidative stress, so it may be of great advantage for symbiotic organisms to adopt an anabiotic state to resist the prevailing hostile conditions (Ertl 1951; Schlensog and Schroeter 2000; Schlensog et al. 2003).

During the desiccation process, the photobionts examined lost the ability to photosynthesize. The Antarctic clade S photobionts of *Usnea lambii* and *Pleopsidium chlorophanum* and also the clade A photobiont of the endemic continental Antarctic lichen *Buellia frigida* showed a higher potential drought resistance compared with the other photobionts. *Umbilicaria decussata* from continental Antarctica hosts a clade S *Trebouxia* photobiont (Brandt 2011) which was also isolated from *Usnea lambii* and the continental *Pleopsidium chlorophanum*. Unlike the endemic *Buellia frigida*, *Umbilicaria decussata* is a cosmopolitan lichen which also occurs in alpine regions across the world (Øvstedal and Lewis Smith 2001). This may explain the resemblance between the results obtained from its photobiont and those of *Umbilicaria antarctica* and *Fulgensia bracteata*.

4.2 Rehydration

The photobionts investigated showed incomplete regeneration of F_V/F_M, which indicates D1 protein degradation (Richter et al. 1990). Photoinhibition caused by D1 degradation (Fig. 3) reduces the potential of light absorption and subsequently the production of reactive oxygen species under stress conditions (Krause and Jahns 2004). If the photobionts cannot fully regenerate their photosynthetic capacity quickly, as found in the tested algae, additional protection against drought stress may be provided in the lichen symbiosis (Valladares et al 1997; Schlensog et al. 2003; Kranner and Birtic 2005; Kranner et al. 2005; Kranner et al. 2008; Kosugi et al. 2009) which may enable the algae to survive cycles of desiccation and rehydration.

Within the first minutes of rehydration, the clade S photobionts of *Pleopsidium chlorophanum*, *Usnea lambii* and *Umbilicaria decussata* were characterized by increasing F_0 values. This increase was slow compared with the almost immediate F_0 increase measured in intact *Parmelia sulcata* thalli during rehydration (Veerman et al. 2007). This may be due to slow relaxation of F_0 quenching based on the antennae of PS II, a mechanism which can protect the stability of PS II under stress conditions (Hájek et al. 2006).

When the photosynthetic apparatus of a non-shaded alga is reactivated during rehydration, it can be exposed to excess light (Schlensog et al. 2003). Slow relaxation of photoprotective mechanisms which are induced during dehydration may lead to retained stress tolerance in the initial phase of reactivation (Fernández-Marín et al. 2009). Accumulation of photoprotective de-epoxidized xanthophylls may contribute to this strategy and has been found in the symbiotic algae of intact *Lobaria pulmonaria* from Spain after slow desiccation (Fernández-Marín et al. 2010). During fast desiccation, the xanthophyll cycle was not activated in the photobionts investigated. The clade S photobionts of *Usnea lambii*, *Pleopsidium chlorophanum* and *Umbilicaria decussata* contained a higher amount of de-epoxidized xanthophylls in the unstressed state, compared with the other photobionts. This could be interpreted as constitutive photoprotection which would be an advantage when there is only a limited

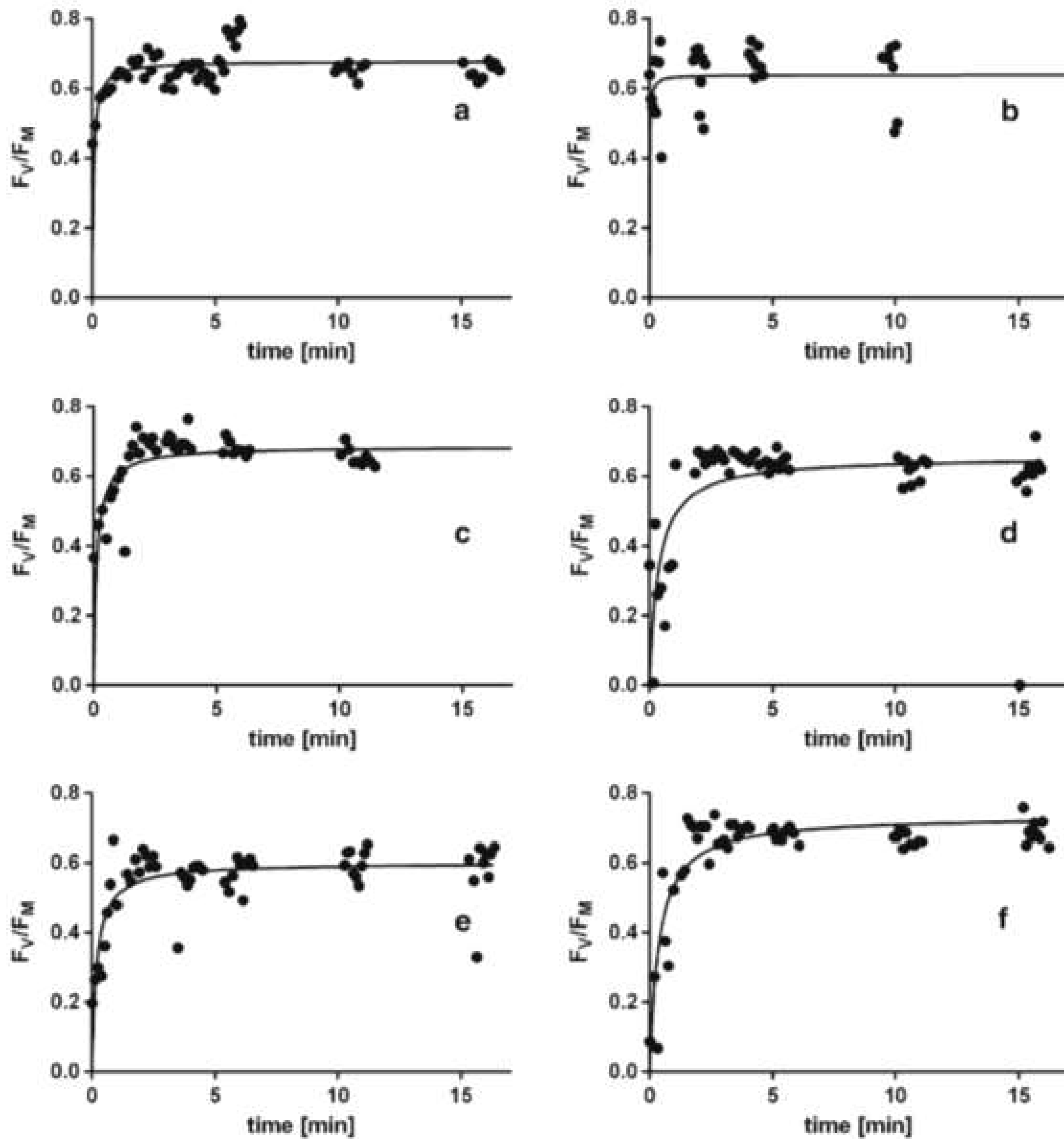

Fig. 5 Development of F_V/F_M during thawing. Dots indicate the values of single replicates, the line represents a regression based on saturation kinetics. Photobionts examined from **a** *Usnea lambii*; **b** *Pleopsidium chlorophanum*; **c** *Buellia frigida*; **d** *Umbilicaria decussata*; **e** *Umbilicaria antarctica*; **f** *Fulgensia bracteata*

time for the induction of a protective mechanism (Kranner et al. 2008).

4.3 Cold

Several studies with polar lichens indicate that photosynthesis can occur at low temperatures, even when snow covered, and that the temperature optima of lichens is low (Kappen and Breuer 1991; Kappen et al. 1995; Kappen 2000; Pannewitz et al. 2003). Green algal photobionts isolated from deep-frozen lichen thalli of temperate regions exhibit considerable vitality (Kappen and Lange 1970). In this study, we demonstrated that freezing temperatures do not cause a severe long-term stress reaction in *Trebouxia* photobionts isolated from different taxonomic groups of this genus.

Recovery of F_V/F_M was nearly immediate in the *Pleopsidium chlorophanum* photobiont, but with respect to retention and recovery of photosynthetic ability, the continental Antarctic photobiont of *Buellia frigida* and the southern maritime Antarctic *Usnea lambii* photobiont exhibited a higher cold resistance potential than the other photobionts investigated. The photobionts of *Umbilicaria decussata* and *Fulgensia bracteata* regenerated F_V/F_M especially slowly.

Umbilicaria decussata has been described as a cosmopolitan lichen (Øvstedal and Lewis Smith 2001) and *Fulgensia bracteata* occurs in habitats which are less extreme than those found in continental and southern maritime Antarctica. This might be the reason for the similar cold resistance of the respective photobionts.

In the photobionts of *Usnea lambii*, *Buellia frigida* and *Umbilicaria antarctica*, the F_V/F_M after reactivation was still slightly lower than that at the unstressed level of F_V/F_M. These photobionts also showed a relatively long retention of the photosynthetic capacity during exposure to freezing temperatures. An interpretation is that the D1 protein was damaged during the slower freezing process, resulting in the lower F_V/F_M values after reactivation.

5 Conclusions

The photobiont has often been suggested to be the more sensitive partner of the lichen symbiosis (de Vera and Ott 2010). The study presented here demonstrates that this general conclusion must be modified. While drought causes substantial reductions in the vitality of isolated photobionts, and the rehydration process provides hints with respect to reasons for long-term damage of the photosynthetic apparatus, sub-zero temperatures up to −25 °C can obviously be tolerated. Particular photobionts react differently to drought and cold as indicated by the comparison between lichens from the Antarctic and those from more temperate regions. The photobionts of the endemic Antarctic lichens *Usnea lambii* and *Buellia frigida* showed a considerable resistance to cold and drought. Not all clade S photobionts exhibited a similar high potential stress resistance as was found in the *Usnea lambii* photobiont. The results point to habitat-specific adaptations which lead to similar behaviour in photobionts that are not closely related. The physiological adaptations of lichen photobionts may substantially contribute to the stress resistance strategy and the colonization capacity of lichens in the Antarctic biome. Remarkably, the differences in stress tolerance were maintained despite the standardized cultivation process under conditions that were similar to those experienced by lichens growing in temperate climates. This indicates a genetic basis for tolerance to extreme conditions in the Antarctic photobionts examined.

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5 Conclusions

The photobiont has often been suggested to be the more sensitive partner of the lichen symbiosis (de Vera and Ott 2010). The study presented here demonstrates that this general conclusion must be modified. While drought causes substantial reductions in the vitality of isolated photobionts, and the rehydration process provides hints with respect to reasons for long-term damage of the photosynthetic apparatus, sub-zero temperatures up to −25 °C can obviously be tolerated. Particular photobionts react differently to drought and cold as indicated by the comparison between lichens from the Antarctic and those from more temperate regions. The photobionts of the endemic Antarctic lichens *Usnea lambii* and *Buellia frigida* showed a considerable resistance to cold and drought. Not all clade S photobionts exhibited a similar high potential stress resistance as was found in the *Usnea lambii* photobiont. The results point to habitat-specific adaptations which lead to similar behaviour in photobionts that are not closely related. The physiological adaptations of lichen photobionts may substantially contribute to the stress resistance strategy and the colonization capacity of lichens in the Antarctic biome. Remarkably, the differences in stress tolerance were maintained despite the standardized cultivation process under conditions that were similar to those experienced by lichens growing in temperate climates. This indicates a genetic basis for tolerance to extreme conditions in the Antarctic photobionts examined.

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5.2 Symbiosis as a successful strategy in continental Antarctica: performance and protection of *Trebouxia* Photosystem II in relation to lichen pigmentation

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Symbiosis as a successful strategy in continental Antarctica: performance and protection of *Trebouxia* photosystem II in relation to lichen pigmentation

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Abstract Lichens as symbiotic associations consisting of a fungus (the mycobiont) and a photosynthetic partner (the photobiont) dominate the terrestrial vegetation of continental Antarctica. The photobiont provides carbon nutrition for the fungus. Therefore, performance and protection of photosystem II is a key factor of lichen survival. Potentials and limitations of photobiont physiology require intense investigation to extend the knowledge on adaptation mechanisms in the lichen symbiosis and to clarify to which extent photobionts benefit from symbiosis. Isolated photobionts and entire lichen thalli have been examined. The contribution of the photobiont concerning adaptation mechanisms to the light regime and temperature conditions was examined by chlorophyll *a* fluorescence and pigment analysis focusing on the foliose lichen *Umbilicaria decussata* from North Victoria Land, continental Antarctica. No photoinhibition has been observed in the entire lichen thallus. In the isolated photobionts, photoinhibition was clearly temperature dependent. For the first time, melanin in *U. decussata* thalli has been proved. Though the isolated photobiont is capable of excess light protection, the results clearly show that photoprotection is significantly increased in the symbiotic state. The closely related photobiont of *Pleopsidium chlorophanum*, a lichen lacking melanin, showed a higher potential of carotenoid-based excess light tolerance. This fact discriminates the two photobionts of the same *Trebouxia* clade. Based on the results, it can be concluded that the successful adaptation of lichens to continental Antarctic conditions is in part based on the physiological potential of the photobionts. The findings provide information on the success of symbiotic life in extreme environments.

Keywords Terrestrial ecosystems · Isolated photobionts · Photoprotection · Secondary metabolites · Photosynthesis

Abbreviations

ANOVA	Analysis of variance
chl *f*	Chlorophyll fluorescence
DEPS	De-epoxidation state of the xanthophyll pool
DMSO	Dimethyl sulfoxide
GANOVEX	German Antarctic North Victoria Land Expedition
HPLC	High-performance liquid chromatography
MY	Malt–yeast
NPQ	Non-photochemical quenching
PAM	Pulse-amplitude modulation
PAR	Photosynthetically active radiation
PPFD	Photosynthetically active photon flux density
PS	Photosystem
ROS	Reactive oxygen species
SLC	Secondary lichen compound
TOM	*Trebouxia* organic medium
UVR	Ultraviolet radiation

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Introduction

The vegetation of continental Antarctica is sparse and consists of highly specialized organisms (Robinson et al. 2003; Hughes et al. 2006; Convey 2010). In this harsh environment, lichens dominate the terrestrial macrovegetation (Kappen 2000). These symbiotic consortia of a fungus (mycobiont) and a photosynthetic partner (photobiont) can, in general, be described as primary colonizers of extreme environments (Chen et al. 2000). The poikilohydric lifestyle of lichens is characterized by anabiosis, the physiologically inactivated state of dehydrated lichen thalli. In particular, in the state of anabiosis, many lichen species show considerable tolerance against abiotic stressors, enabling them to survive under environmental conditions which might be lethal to other organisms (Meeßen et al. 2013). The conditions consist of sustainable life in hot and cold deserts, in alpine regions, but also survival under most extreme parameters such as ultraviolet (UV) irradiation (Sánchez et al. 2014), freezing (Kappen and Lange 1972; Harańczyk et al. 2012), space exposure (Onofri et al. 2012) and even acetone rinsing (Gauslaa and Solhaug 2004).

Lichens are highly resistant to extreme temperatures, a feature that substantially supports the success of these organisms in cold environments such as the ice-free areas of the Antarctic continent and the adjacent islands (Kappen and Lange 1970, 1972; Kappen 1973; Schlensog et al. 2003). While lichens are often highly resistant to temperature extremes in the anabiotic state, photosynthetic activity of polar lichens has been detected at subzero temperatures (Kappen et al. 1996).

Lichen colonization occurs in microniches on the Antarctic continent. The microclimatical conditions of these sites are determined by abiotic factors such as rock topography (Jahns and Fritzler 1982). Such microniches for colonization by various lichen species can be minimum sized rock surface fissures. Microniches in rock fissures are characterized by meltwater supply and therefore, at least temporarily improve water availability. The occurrence of meltwater depends on topography of the respective site, substrate structure and the amount of snow and ice close by. Meltwater availability may be erratic compared to the diurnal change of air humidity (Schroeter et al. 2011). In general, the lichen *Umbilicaria decussata* colonizes rock sites of different size characterized by fissures and especially at continental sites supported by meltwater. Water availability strictly controls lichen activity due to the poikilohydric nature of these organisms (Green and Lange 1995). At continental Antarctic sites, meltwater supply is especially crucial for lichen activity (Kappen et al. 1998). Metabolic activity and especially photosynthetic primary production in continental Antarctica are primarily limited by water availability (Kappen 1985; Gjessing and Øvstedal 1989; Kappen and Breuer 1991; Schroeter et al. 1992).

At habitats of continental Antarctica, lichens live under the conditions of a frigid desert (Huiskes et al. 2006). The physiologically active period of lichens can be characterized by high light intensity (Kappen et al. 1998; Schroeter et al. 2010). Photoprotective mechanisms of the lichen symbiosis are crucial to avoid photodamage which would reduce photosynthetic productivity in the short vegetation period. Lichens of maritime Antarctica mainly depend on air humidity and dehydrate during times of excess insolation (Schlensog et al. 2003). Photoprotection in the hydrated state seems to be a prerequisite for the meltwater-activated lichens at continental sites.

For metabolically active, moist thalli of the continental Antarctic lichen species *Umbilicaria aprina*, considerable tolerance to high light intensities was detected by Kappen et al. (1998). Schlensog et al. (2003) proposed that the reflectance and absorbance of the *U. aprina* thallus cortex are the key factors of irradiation tolerance in this lichen. The shading capacity of lichen cortices reduces the light intensity experienced by the symbiotic photobionts substantially, compared to the ambient light (Ertl 1951; Büdel and Lange 1994).

Green algae of the genus *Trebouxia*, which are the most common among lichen photobionts, are specialized to a lichenized lifestyle. It has still not been clarified to which extent they can survive aposymbiotically under natural conditions (Wornik and Grube 2010). Meanwhile, there is a lot of experience in cultivation of lichen symbionts, especially *Trebouxia* photobionts (Schaper and Ott 2003; Sadowsky and Ott 2012). To compare the performance of photobionts in the lichen thallus to their isolated cultured state is a prerequisite for the knowledge on special adaptation mechanisms of the photobiont. Photobiont adaptations are especially interesting when low photobiont diversity is detected in a lichen community of a definite environment. Special characteristics of photobionts might be based on adaptation mechanisms to the respective environment (Sadowsky and Ott 2012; Domaschke et al. 2013). Clade S *Trebouxia* photobionts (Helms et al. 2001) dominate in macrolichens throughout Antarctica (Romeike et al. 2002).

The photosynthetic response of various isolated Antarctic photobionts to drought and cold showed different patterns. These differences between photobionts could be correlated with the environmental conditions of the corresponding lichen species rather than to their phylogenetic position in the genus *Trebouxia* (Sadowsky and Ott 2012). The environmental conditions inside the thallus normally differ considerably from those outside the thallus. In particular, hydration and light regime for the photobiont are determined by thallus structure and secondary lichen compounds (SLCs) inside the thallus (Sadowsky et al.

2012; Meeßen et al. 2013). Environmental adaptations of lichen symbionts can be regarded as adaptations toward the symbiotic lifestyle. Besides other factors, especially the physiological potential of the isolated symbionts determines the prerequisites for a successful symbiotic lifestyle in respective environments. Therefore, to clarify possible benefits of lichen symbiosis, the performance of symbiotic and aposymbiotic forms should be compared with experiments, as it has been done in the present study.

Materials and methods

Lichen material

Samples of *Umbilicaria decussata* (Vill.) Zahlenbr. (1942) and *Pleopsidium chlorophanum* (Wahlenb.) Zopf (1855) were collected during the 2009/2010 German Antarctic North Victoria Land Expedition (GANOVEX) X in Terra Nova Bay, North Victoria Land, continental Antarctica. Both lichens grew on a sun-exposed rock surface.

All lichen thalli were air-dried and deep-frozen (−25 °C) until photobiont isolation. Both photobionts belong to clade S *Trebouxia* (Brandt 2011; taxonomy after Helms et al. 2001).

Frozen lichen thalli were reactivated prior to the chlorophyll fluorescence analysis. The reactivation procedure was as follows: Entire lichen thalli were sprayed with water and stored at 12 °C in sealed vials (to prevent dehydration) in the dark for 24 h, followed by 14 h of light (20 µmol photons m^2 s^{-1}, fluorescent light source) at the same temperature.

Symbiont isolation and cultivation

Photobionts were isolated from lichen thalli according to Yoshimura et al. (2002) from thallus fragments. Axenic photobiont cultures on *Trebouxia* organic medium agar (TOM) with 1 % glucose (Ahmadjian 1967) were kept at low light intensity (20 µmol photons m^2 s^{-1}; diurnal cycle with 10 h of darkness) and 12 °C in a growth chamber (Rubarth Apparate GmbH, Germany). For pigment isolation and chlorophyll fluorescence experiments, the photobionts were transferred to 1 cm^2 nitrocellulose filter disks on TOM-agar growing in the growth chamber for 4 weeks. Mycobionts were isolated from ascospores and cultivated on malt–yeast (MY) medium under the same conditions as the photobionts.

Chlorophyll fluorescence

Chlorophyll *a* fluorescence (chl *f*) was determined by using a Mini-PAM (pulse-amplitude modulated) fluorimeter (Walz Mess-und Regeltechnik, Germany) according to Maxwell and Johnson (2000). The parameter $(F_m - F_0)/F_m = F_v/F_m$ (maximum quantum yield of photosystem II) was measured in the dark after 20 min of dark acclimation of the samples by applying a saturating light pulse. Quantum yield of photosystem II (dF/F'_m) in the light-acclimatized state was calculated as $(F'_m - F_t)/F'_m$, and non-photochemical quenching (NPQ) of maximum fluorescence was calculated as $(F_m - F'_m)/F'_m$. Slow chl *f* induction was performed by applying constant illumination on the dark-acclimatized samples for 12 min, followed by 20 min of dark relaxation. Eight samples of photobionts and, due to material limitations, four samples of lichen thalli were used per treatment. The effects of temperature and light intensity on quantum yield and NPQ were analyzed by two-way ANOVA ($\alpha = 0.05$). A post hoc test (Tukey–Kramer multiple comparisons) was applied to detect significant reduction in photosystem II quantum yield after illumination and recovery.

Maximum quantum yield of freshly reactivated lichens was compared between the two species *Umbilicaria decussata* and *Pleopsidium chlorophanum* by an unpaired two-sided *t* test ($\alpha = 0.05$). The checked null hypothesis H_0 was that there were no differences between the lichen species.

Pigment analysis

Photobiont pigments were determined from acetone extracts by HPLC as described in Sadowsky and Ott (2012). The de-epoxidation status of the xanthophyll pool (DEPS) was calculated from the xanthophyll concentrations (zeaxanthin + antheraxanthin)/(violaxanthin + antheraxanthin + zeaxanthin) according to Vráblíková et al. (2004). The effects of temperature and photobiont origin on DEPS were analyzed by two-way ANOVA ($\alpha = 0.05$; $n = 4$). A Tukey–Kramer multiple comparisons post hoc test was applied to detect pairs of significantly differing values.

Photospectrometric determination of *Umbilicaria decussata* thallus and mycobiont pigments from acetone, methanol and dimethyl sulfoxide (DMSO) extracts as well as testing on melanin was performed according to Meeßen et al. (2013). Synthetic melanin (>97 %, Sigma-Aldrich) was used as a reference. Despite the test on acetone solubility, the homogenized samples were pre-rinsed with acetone prior to the melanin testing. Mycobiont pigment extraction in DMSO was performed with native and acetone pre-rinsed mycobiont material.

Microclimate at lichen sites

Air, substrate and thallus temperature as well as relative air humidity and light intensity (photosynthetically active photon flux density, PPFD) were measured with sensors

attached closely to lichen thalli near the Gondwana station at Terra Nova Bay, North Victoria Land. Air humidity and temperature were measured with combined temperature/humidity probes (HMP 35 A/TH, Vaisala, Finland). Substrate temperature was measured by thermistors (FF-U-V5-0 and FM-SU-VS5-0, Grant, UK). PPFD was measured using a quantum Sensor (Li-190SA, Licor, USA). Data were recorded on loggers (Grant and Eltek, 1000 series). The measurement was taken from January 4 to February 3 at a site of *Umbilicaria decussata*.

Results

Microclimate

Air, substrate and thallus temperature (Fig. 1) at the site of *Umbilicaria decussata* followed the same time course and showed similar values. At this site, the air temperature slightly exceeded the substrate and thallus temperature. Thallus temperature exceeded the substrate temperature during the daily temperature maximum. The diurnal rhythm was naturally correlated with the changing insolation (Fig. 2). Maximum photosynthetic photon flux density (PPFD) of c. 1600 μmol photons $m^{-2} s^{-1}$ from sunlight was reached at c. 1 p.m. during the time when the measurement site was snow free. Maximum temperature >20 °C was reached about 2 h later. In the middle of the night, when PPFD was as low as c. 50 μmol photons $m^{-2} s^{-1}$, temperatures of air, substrate and *U. decussata* thalli dropped to 0 °C. Air humidity was generally too low for lichen rehydration but reached c. 60 % relative humidity during the night. Air humidity during daytime was generally <20 % (Fig. 2). The measurement site was snow covered from January 22 until the end of the measurement. During snow cover, thallus and substrate temperatures were around or slightly below 0 °C (Fig. 1a). PPFD was reduced to a daily maximum of c. 200–400 μmol photons $m^{-2} s^{-1}$. Relative air humidity (measured above the snow) rose up to c. 90 % (Fig. 2a).

Chlorophyll fluorescence

Entire lichen thalli ($n = 4$) were successfully reactivated and showed maximum quantum efficiencies (F_v/F_m) of 0.61 ± 0.04 (*Umbilicaria decussata*) and 0.66 ± 0.07 (*Pleopsidium chlorophanum*), respectively. No significant difference of the F_v/F_m values of these two lichens could

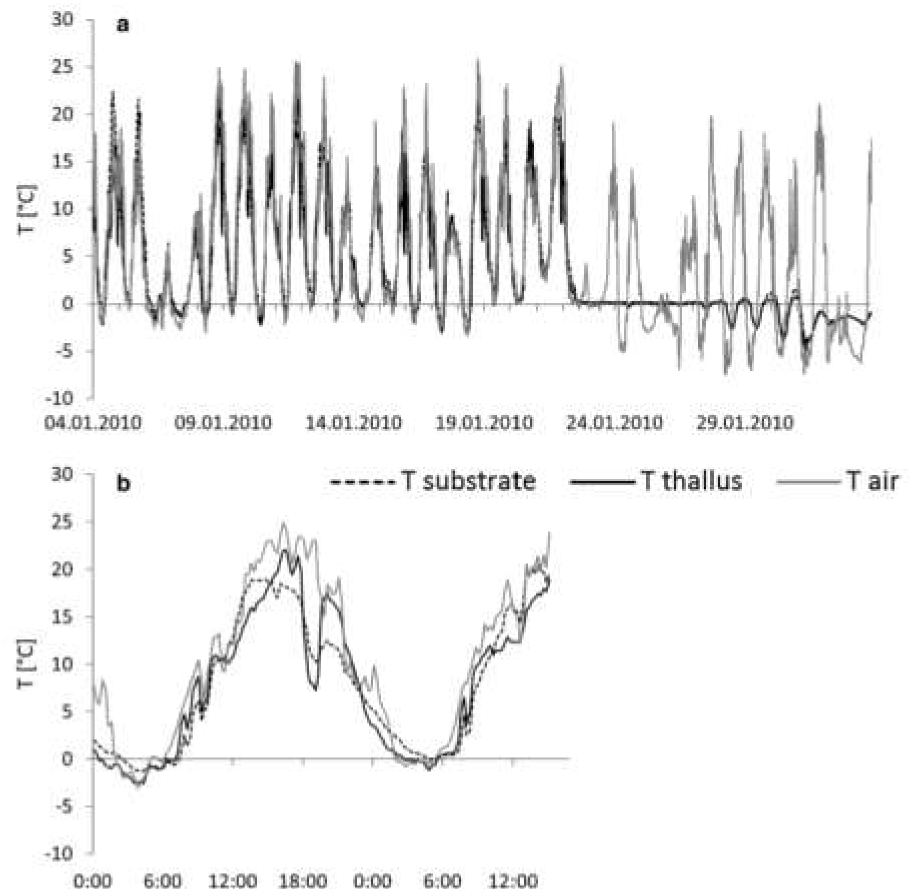

Fig. 1 Microclimate of an *Umbilicaria decussata* site: temperatures. *X* axis represents the time; **a** whole measurement period from January 4 to February 3, 2010; **b** detail (January 16/17). Snow coverage of the site occurred from January 22 to the end of the measurement. The sensor for air temperature remained snow free

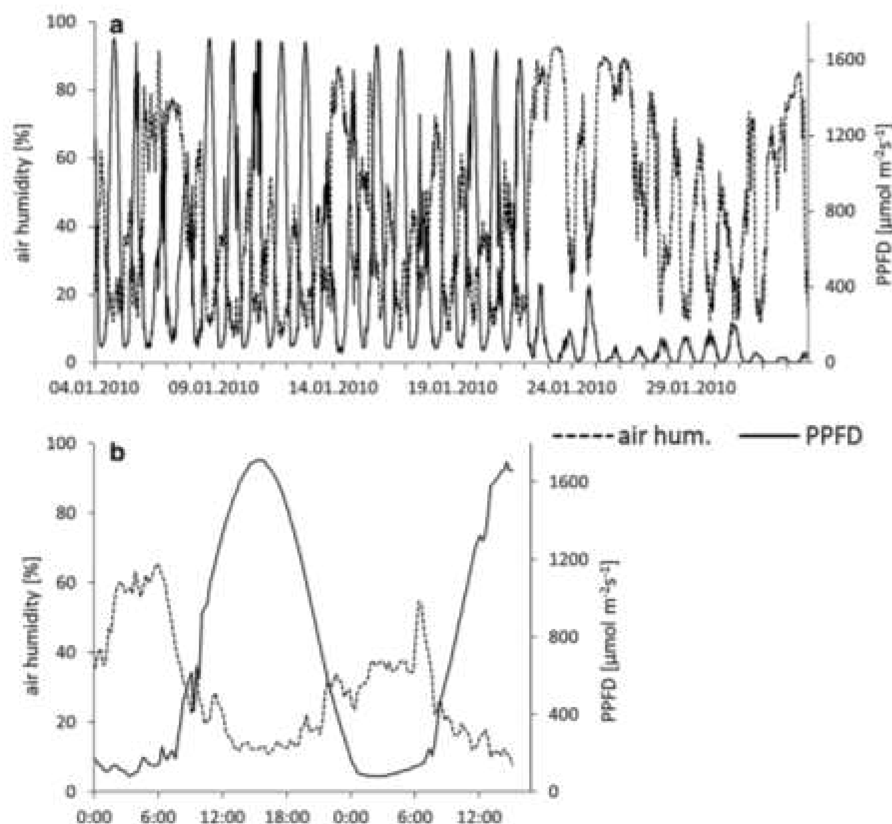

Fig. 2 Microclimate of an *Umbilicaria decussata* site: relative air humidity and photosynthetically active photon flux density (PPFD). *X* axis represents the time; **a** whole measurement period from January 4 to February 3, 2010; **b** detail (January 16/17). Snow coverage of the site occurred from January 22 to the end of the measurement

be detected directly after the reactivation procedure (unpaired two-sided *t* test, $p = 0.19$, $\alpha = 0.05$).

The reaction of reactivated *U. decussata* thalli to continuous illumination at 4, 12 and 20 °C is shown in Fig. 3, Tables 3 and 4. Actual quantum yield dF/F'_m reached a steady state after <5 min at any applied light intensity (20, 200 and 1000 µmol photons $m^{-2}\ s^{-1}$) or temperature except the combination of low temperature and low light intensity (4 °C and 20 µmol photons $m^{-2}\ s^{-1}$, Fig. 3a). Saturation of NPQ occurred more slowly. In particular, at 4 °C and 200 µmol photons $m^{-2}\ s^{-1}$ (Fig. 3b), NPQ did not reach a steady state within the illumination time. By two-factor ANOVA, a significant effect of light intensity and temperature on the steady-state level of quantum yield was detected (Table 1). The effect of light was considerably greater than the effect of temperature.

No significant effect of temperature and light intensity on the regeneration potential of NPQ and PS II quantum yield was detected by two-factor ANOVA (Table 2). The dark relaxation time seemed to be sufficient to regenerate F_v/F_m at any condition applied, which is indicated by the lack of statistically significant differences (Tukey–Kramer post hoc test) between the initial F_v/F_m and the quantum yield values after the light treatment followed by 20 min of dark relaxation (Fig. 3).

The photosynthetic characteristics of the isolated photobionts (Fig. 4; Tables 3, 4) were more effected by the conditions applied as described above, compared to the entire lichen thalli, indicated by lower steady-state levels of dF/F'_m and higher NPQ levels during illumination. In particular, at moderate PPFD combined with 4/12 °C (Fig. 4b, d), and at high PPFD (Fig. 4e, h), NPQ exceeded the values obtained from the lichen thalli and was not saturated after 12 min of illumination. NPQ was not fully regenerated within 20 min at any applied condition except the combination of 20 °C and low light intensity.

By two-factor ANOVA, significant effects of temperature and light intensity variation could be detected, including a pronounced stronger effect of light intensity (Table 1). Additionally, the interactive effect of the two factors was significant.

The quantum yield of PS II reached the initial unstressed value after illumination at up to 200 µmol photons $m^{-2}\ s^{-1}$ at 12 °C, while at lower temperatures, quantum yield of PS II was significantly reduced after illumination at 200 and 1000 µmol photons $m^{-2}\ s^{-1}$ (Table 1; Fig. 4). After treatment at 20 °C and 200 µmol photons $m^{-2}\ s^{-1}$ and a subsequent recovery, quantum yield was only slightly but significantly reduced from 0.57 ± 0.05 to 0.47 ± 0.19. Two-factor ANOVA (Table 2) indicated that only light

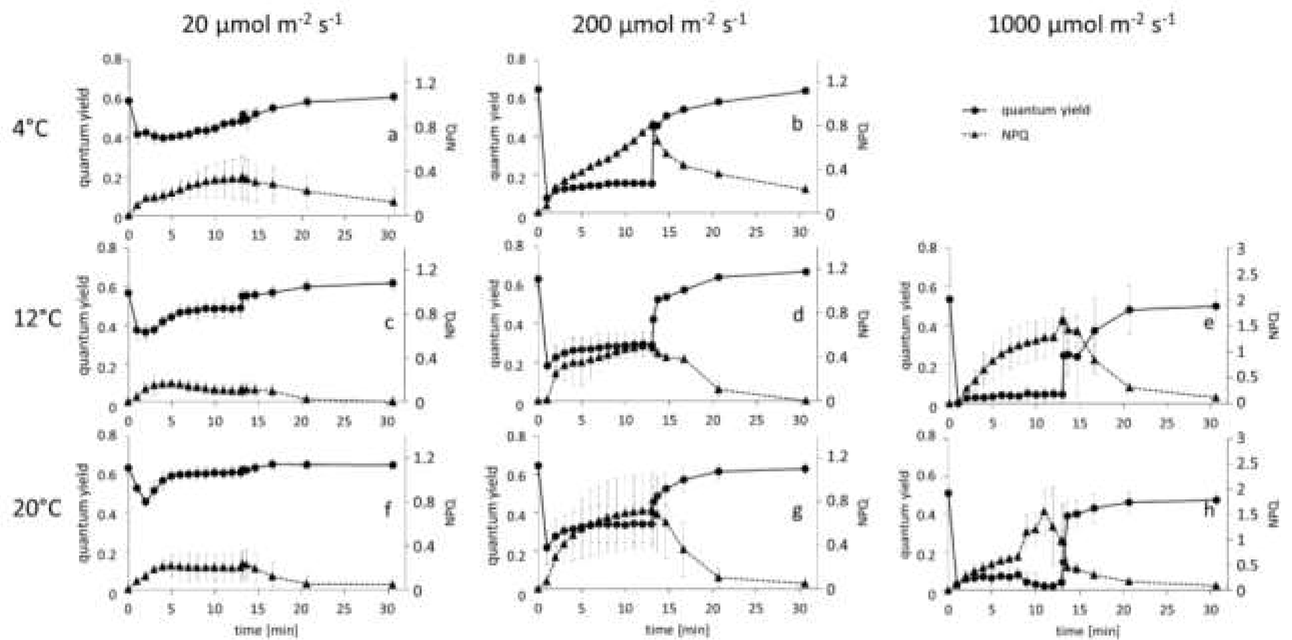

Fig. 3 Quantum yield of photosystem II and non-photochemical quenching (NPQ) derived from slow chlorophyll fluorescence induction in reactivated, dark-acclimatized entire *Umbilicaria decussata* thalli. Actinic light was switched on in minute 2 and switched off in minute 13. Applied temperatures: 4 °C (**a**, **b**); 12 °C (**c**–**e**); 20 °C (**f**–**h**). Applied light intensities (photosynthetically active photon flux density, PPFD): 20 µmol photons m^{-2} s^{-1} (**a**, **c**, **f**); 200 µmol photons m^{-2} s^{-1} (**b**, **d**, **g**); 1000 µmol photons m^{-2} s^{-1} (**e**, **h**). Values are presented as mean ± SD. Please note the higher NPQ scale in **e** and **h**

intensity, not the temperature, had a significant effect on dark recovered quantum yield values. By contrast, both factors as well as their interaction significantly affected the variation of NPQ recovery. Again, the light intensity contributed more to the parameter variation than temperature did.

Pigment analysis

NPQ of the photobionts was correlated with activation of the violaxanthin cycle as indicated by increased de-epoxidation state of the xanthophyll pool (DEPS) under moderate illumination (Fig. 5; Table 6). DEPS did not reach the relaxed initial state after 20 min of dark relaxation. By two-factor ANOVA, it could be shown that the illumination treatment and the photobiont origin (species level) both had a significant effect on the DEPS values. However, the contribution of light intensity was greater than that of photobiont species (Table 5). Furthermore, Tukey–Kramers post hoc test revealed that the photobiont of *Pleopsidium chlorophanum* showed significantly higher DEPS than the unstressed control after 5 min of irradiation. DEPS of the *Ubmilicaria decussata* photobiont was significantly elevated after 10 min (Fig. 5; Table 6).

Brownish thallus extracts of *Umbilicaria decussata* revealed absorption maxima of chlorophylls in the blue and red light, but also absorption due to other substances in the blue light and UV spectrum (Fig. 6). In the DMSO extract, the spectrum is characterized by increasing absorption in the blue and UV region toward a maximum at c. 270 nm (Fig. 6a). Extracted compounds of the isolated mycobiont showed a similar spectrum in DMSO and a brown coloration. It could be shown by chemical testing (Table 7) that the absorbance increasing with lower wavelengths is due to melanin present in both the thallus and the cultivated mycobiont. A blue light-absorbing compound of the thallus and the isolated mycobiont could be detected in the acetone extract. Acetone pre-rinsed mycobiont material extracted in DMSO showed reduced absorbance in the blue and near UV spectrum. Acetone and methanol extracts (Fig. 6b, c) of the lichen and mycobiont showed absorption peaks at c. 320 nm, but no brown coloration.

Discussion

Pigmentation

Light-screening pigments are a diverse class of secondary lichen substances which are often located in the cortex (Dietz et al. 2000). Some are able to protect against excess visible (photosynthetically active radiation, PAR) light, but most are UVR screening substances (Solhaug et al. 2003). A known UVR screening compound in *Umbilicaria*

Table 1 Two-factor ANOVA of the effects of temperature and light intensity on the steady-state quantum yield and maximum NPQ values obtained in the light-acclimatized state of *Umbilicaria decussata* photobionts and entire lichen thalli

Factor	Sum of squares	Degrees of freedom	Mean square	F	p value	H_0 rejected?
Steady-state photobiont quantum yield						
Temperature	0.03	2	0.02	39.55	2.26E−11	Yes
Light	0.83	2	0.41	991.69	7.16E−44	Yes
Interaction	0.05	4	0.01	27.90	1.13E−12	Yes
Within	0.02	55	0.00			
Total	1.98	63	0.03			
Maximum photobiont NPQ						
Temperature	0.65	2	0.33	8.15	7.94E−04	Yes
Light	13.91	2	6.96	173.14	1.84E−24	Yes
Interaction	1.37	4	0.34	8.54	1.96E−05	Yes
Within	2.21	55	0.04			
Total	37.09	63	0.59			
Steady-state lichen quantum yield						
Temperature	0.05	2	0.02	7.94	2.39E−03	Yes
Light	0.55	2	0.28	92.57	9.97E−12	Yes
Interaction	0.03	4	0.01	2.51	6.94E−02	No
Within	0.07	23	0.00			
Total	1.43	31	0.05			
Maximum lichen NPQ						
Temperature	0.07	2	0.04	0.17	8.46E−01	No
Light	0.96	2	0.48	2.22	1.31E−01	No
Interaction	0.17	4	0.04	0.20	9.36E−01	No
Within	4.95	23	0.22			
Total	9.58	31	0.31			

Significance level $\alpha = 0.05$. Null hypothesis H_0: no significant effect of the factor

decussata thalli is gyrophoric acid (Øvstedal and Lewis Smith 2001). Fungal melanins absorb PAR and especially UVR. Melanic compounds are also widespread among lichens (Beckett et al. 2012). Melanin-pigmented lichen cortices can serve as effective barriers against different harmful effects of excess insolation (Nybakken et al. 2004; Meeßen et al. 2013). This study presents the first proof of melanin in *Umbilicaria decussata*. The pigment may contribute substantially to the protection of the photobiont when the metabolically active *U. decussata* thallus is exposed to sunlight. The Antarctic endemic lichen *Buellia frigida* may also profit from its protective melanin-pigmented cortex. The *B. frigida* mycobiont, as that of *U. decussata*, also produces melanin in the isolated and cultured state, and it has been hypothesized that this pigmentation may protect the development of new-formed fungal tissue in *B. frigida* from UV radiation (Meeßen et al. 2013).

Microclimate

Continuous variations in air, substrate as well as thallus temperature measured at microsites of *U. decussata* for 24 h for 4 weeks from below 0 °C until about 20 °C occurred (Fig. 1). The relatively high temperatures are accompanied by increasing light intensities at noon (Fig. 2) when the thallus in general is dry (Ott in prep.). Because of low air humidity (Fig. 2), no dew fall occurred. Schlensog et al. (2013) analyzed a variety of plants and cryptogams of Léonie Island, Antarctic Peninsula and southern maritime Antarctic. In its more xeric microhabitat on Léonie Island, Marguerite Bay, *U. decussata* was moistened by precipitation of rain and snow during the measuring period exclusively, resulting in an erratic activity pattern and the shortest total activity time of that study.

As a cosmopolitan lichen (Øvstedal and Lewis Smith 2001), a broad ecological amplitude may be beneficial for *U. decussata* at habitats in North Victoria Land. Romeike et al. (2002) compared the hydration-dependent physiological activities of endemic and cosmopolitan lichen species across the maritime Antarctic until Alexander Island south of the maritime Antarctic, Antarctic Peninsula. In general, cosmopolitan species displayed broader ecological amplitudes. In particular, the bipolar lichen *Stereocaulon alpinum* showed adaptation toward high light intensity combined with high thallus humidity. As *U.*

Table 2 Two-factor ANOVA of the effects of temperature and light intensity on the recovered quantum yield and NPQ values obtained after light-acclimation and subsequent dark relaxation for 20 min of *Umbilicaria decussata* photobionts and entire lichen thalli

Factor	Sum of squares	Degrees of freedom	Mean square	F	p value	H_0 rejected?
Recovered photobiont quantum yield						
Temperature	0.00	2	0.00	0.01	9.88E−01	No
Light	0.12	2	0.06	12.13	4.32E−05	Yes
Interaction	0.05	4	0.01	2.27	7.38E−02	No
Within	0.28	55	0.01			
Total	1.56	63	0.02			
Recovered photobiont NPQ						
Temperature	0.23	2	0.12	29.66	1.83E−09	Yes
Light	0.64	2	0.32	81.36	3.69E−17	Yes
Interaction	0.93	4	0.23	59.11	2.83E−19	Yes
Within	0.22	55	0.00			
Total	8.94	63	0.14			
Recovered lichen quantum yield						
Temperature	0.00	2	0.00	0.23	7.98E−01	No
Light	0.00	2	0.00	0.10	9.02E−01	No
Interaction	0.01	4	0.00	0.56	6.93E−01	No
Within	0.06	23	0.00			
Total	0.18	31	0.01			
Recovered lichen NPQ						
Temperature	0.01	2	0.00	0.81	4.59E−01	No
Light	0.00	2	0.00	0.03	9.71E−01	No
Interaction	0.00	4	0.00	0.14	9.67E−01	No
Within	0.09	23	0.00			
Total	0.28	31	0.01			

Significance level α = 0.05. Null hypothesis H_0: no significant effect of the factor

decussata, *S. alpinum* grows at cold, alpine sites in the northern hemisphere (Øvstedal and Lewis Smith 2001) and was found in meltwater-fed Antarctic sites. The similar distribution patterns of these two lichen species may cause similar adaption mechanisms on physiology.

Light energy conversion

Excess light energy can lead to the production of cytotoxic reactive oxygen species (ROS) in photosynthetic cells. Lichens can avoid excess light by anabiosis or by shading of the photobionts by a well-developed optically dense cortex. On the other hand, many lichens are highly susceptible to photoinhibition in the dry state. Susceptibility to photoinhibition is especially relevant during the drying process (Gauslaa et al. 2012). In the dry state, dissipation of excess energy can be induced by conformational changes of the photosynthetic apparatus (Heber 2008). As long dry periods with strong insolation may occur at Antarctic habitats, light energy quenching in the dry state may be crucial for lichen survival. While this mechanism can be very effective and is independent of pigment conversions, active photobiont cells can also tolerate excess light by xanthophyll-based NPQ reactions. Additionally, evidence for pigment-independent chlorophyll fluorescence quenching has been found in desiccated Antarctic lichen photobionts (Sadowsky and Ott 2012). In the illumination experiments concerning isolated and symbiotic photobionts (Figs. 3, 4), NPQ was more rapidly induced at higher temperatures (12 and 20 °C). In particular, in the isolated photobionts, NPQ reached significantly higher values when the temperature was higher at any given light intensity.

Reduction in photosynthetic capacity (photoinhibition) by light stress occurs especially at low temperatures. Active NPQ prevents the photosystem from oxidative damage and avoids long-term (chronic) photoinhibition (Schlensog et al. 2003). The isolated photobionts of *Umbilicaria decussata* (Fig. 4) displayed chronic photoinhibition (detected as incomplete recovery of F_v/F_m) at low temperature (4 °C), even if combined with low or moderate light intensity (20 and 200 µmol photons m^{-2} s^{-1}, respectively). At 12 and 20 °C, chronic photoinhibition could only be observed under high light conditions (1000 µmol photons m^{-2} s^{-1}), despite a minor reduction in quantum yield at 20 °C and 200 µmol photons m^{-2} s^{-1} (Fig. 4). Consequently, it can be hypothesized that the NPQ-based excess

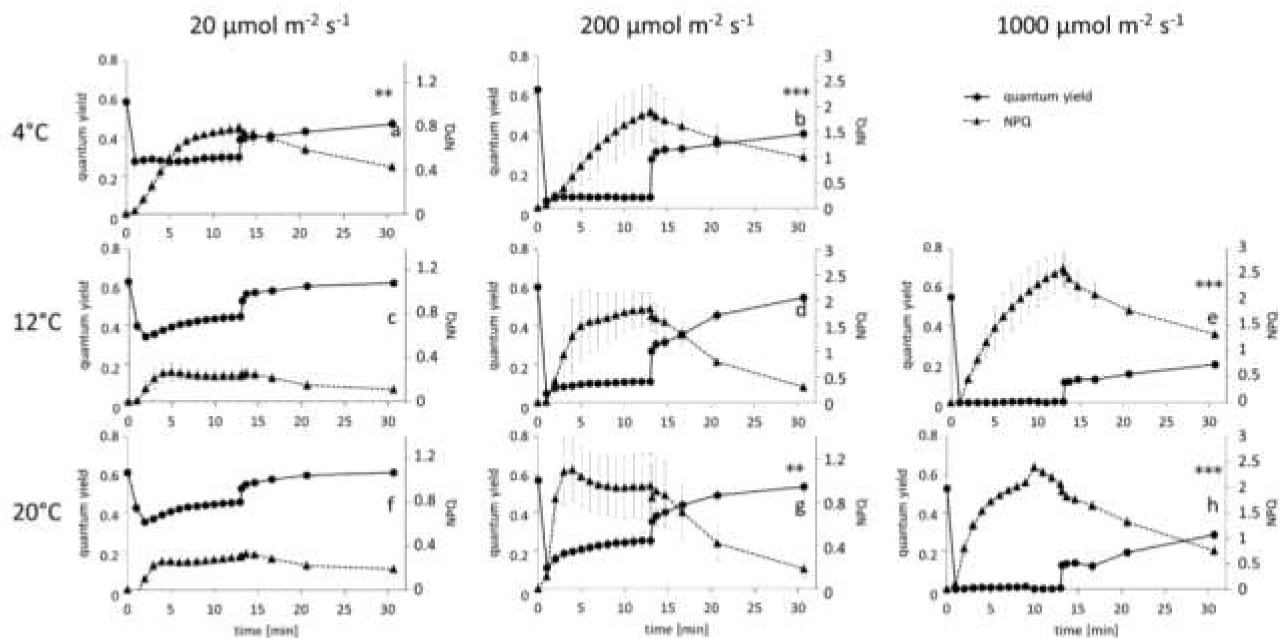

Fig. 4 Quantum yield of photosystem II and non-photochemical quenching (NPQ) derived from slow chlorophyll fluorescence induction in dark-acclimatized isolated, cultured *Umbilicaria decussata* photobionts. Actinic light was switched on in minute 2 and switched off in minute 13. Applied temperatures: 4 °C (**a**, **b**); 12 °C (**c**–**e**); 20 °C (**f**–**h**). Applied light intensities (photosynthetically active photon flux density, PPFD): 20 µmol photons m^{-2} s^{-1} (**a**, **c**, **f**); 200 µmol photons m^{-2} s^{-1} (**b**, **d**, **g**); 1000 µmol photons m^{-2} s^{-1} (**e**, **h**). Values are presented as mean ± SD. Please note the higher NPQ scale in **b**, **d**, **e** and **h**. *Asterisks* indicate significant differences (Tukey–Kramer multiple comparisons test) between recovered and unstressed quantum yield values: **$p < 0.01$; ***$p < 0.001$

Table 3 Non-photochemical quenching and photosystem II quantum yield of *Umbilicaria decussata* photobionts and entire lichen thalli under three different temperature conditions

	Dark	20 µmol m^{-2} s^{-1}	200 µmol m^{-2} s^{-1}	1000 µmol m^{-2} s^{-1}
Steady-state photobiont quantum yield				
4 °C	0.61 ± 0.03	0.30 ± 0.04	0.05 ± 0.01	
12 °C	0.59 ± 0.07	0.45 ± 0.02	0.11 ± 0.01	0.00 ± 0.00
20 °C	0.57 ± 0.05	0.46 ± 0.01	0.25 ± 0.03	0.01 ± 0.00
Maximum photobiont NPQ				
4 °C		0.81 ± 0.05	2.00 ± 0.38	
12 °C		0.24 ± 0.05	1.64 ± 0.21	2.14 ± 0.17
20 °C		0.30 ± 0.02	0.95 ± 0.30	2.07 ± 0.05
Steady-state lichen quantum yield				
4 °C	0.64 ± 0.02	0.49 ± 0.02	0.15 ± 0.03	
12 °C	0.55 ± 0.07	0.49 ± 0.05	0.20 ± 0.06	0.05 ± 0.05
20 °C	0.60 ± 0.07	0.63 ± 0.04	0.34 ± 0.10	0.04 ± 0.03
Maximum lichen NPQ				
4 °C		0.44 ± 0.01	0.81 ± 0.35	
12 °C		0.10 ± 0.05	0.41 ± 0.55	1.21 ± 0.82
20 °C		0.21 ± 0.10	0.72 ± 0.34	1.11 ± 0.65

Maximum quantum yield (F_v/F_m) was measured before light treatment in dark-acclimatized thalli, while steady-state quantum yield and maximum non-photochemical quenching (NPQ) were determined after acclimation to the indicated light/temperature conditions. Values are presented as mean ± SD

Table 4 Non-photochemical quenching and photosystem II quantum yield of *Umbilicaria decussata* photobionts as well as entire lichen thalli under three different temperature conditions

	20 μmol m^{-2} s^{-1}	200 μmol m^{-2} s^{-1}	1000 μmol m^{-2} s^{-1}
Recovered photobiont quantum yield			
4 °C	0.47 ± 0.02	0.39 ± 0.03	
12 °C	0.62 ± 0.01	0.55 ± 0.02	0.20 ± 0.02
20 °C	0.61 ± 0.00	0.47 ± 0.19	0.28 ± 0.02
Recovered photobiont NPQ			
4 °C	0.44 ± 0.03	0.97 ± 0.12	
12 °C	0.11 ± 0.05	0.28 ± 0.02	1.12 ± 0.00
20 °C	0.18 ± 0.02	0.16 ± 0.09	0.75 ± 0.07
Recovered lichen quantum yield			
4 °C	0.62 ± 0.01	0.64 ± 0.04	
12 °C	0.62 ± 0.03	0.60 ± 0.09	0.50 ± 0.09
20 °C	0.65 ± 0.01	0.63 ± 0.03	0.48 ± 0.04
Recovered lichen NPQ			
4 °C	0.20 ± 0.01	0.22 ± 0.12	
12 °C	0.00 ± 0.00	0.00 ± 0.00	0.09 ± 0.08
20 °C	0.04 ± 0.06	0.05 ± 0.06	0.09 ± 0.01

Measurements were taken after dark relaxation following the indicated illumination. Values are presented as mean ± SD

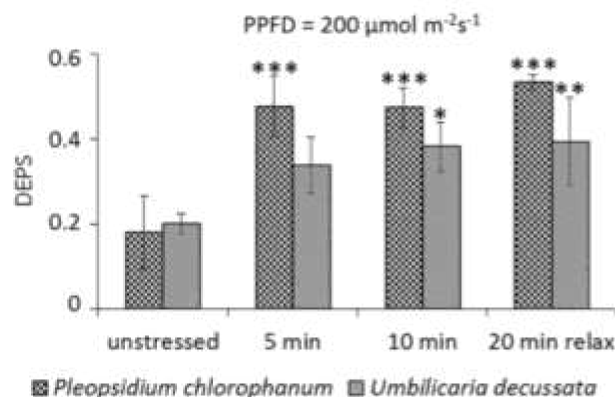

Fig. 5 De-epoxidation state of the xanthophyll pool (DEPS) of isolated photobionts of *Pleopsidium chlorophanum* and *Umbilicaria decussata* at 12 °C. The *first pair of bars* represents the unstressed DEPS of dark-acclimatized photobionts. The *second and third pair of bars* represent the DEPS values of photobionts illuminated at 200 μmol photons m^{-2} s^{-1} for 5 and 10 min, respectively. The last pair of values shows the DEPS after 10 min of illumination, followed by 20 min of dark acclimation. Values are presented as mean ± standard deviation. *Asterisks* indicate significant differences (Tukey–Kramer multiple comparisons test) from values obtained from the respective unstressed photobionts: $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$

light protection of the isolated *U. decussata* photobiont can effectively prevent photodamage at temperatures of 12 or 20 °C combined with a light intensity which exceeds the cultivation PPFD (at 20 μmol photons m^{-2} s^{-1}) tenfold. A PPFD of 200 μmol photons m^{-2} s^{-1} is in the range of maximum PPFD detected under snow cover in the measuring period. NPQ can be correlated with the de-epoxidation of the xanthophyll violaxanthin to zeaxanthin which quenches excess excitation energy from photosystem II (Havaux and Niyogi 1999).

The *P. chlorophanum* entire lichen thallus, unlike the *U. decussata* thallus (Meeßen et al. 2013, Fig. 6; Table 6), lacks melanin as a photoprotective metabolite. *P. chlorophanum* contains the UVR protective SLC rhizocarpic acid. Rhizocarpic acid shows little absorption of photosynthetically relevant wavelengths and can thus not be regarded as a protectant against excess PPFD (Hidalgo et al. 2002; Meeßen et al. 2013). Based on its pigmentation, the light-absorbing capacity of the *P. chlorophanum* thallus can be regarded as minor compared to *U. decussata*. The photobionts of *U. decussata* and of *Pleopsidium chlorophanum* belong to clade S *Trebouxia*. Despite the close phylogenetic position and despite the identical culture and experimental conditions of both isolated photobionts, the *P. chlorophanum* photobiont displayed faster activation of xanthophyll de-epoxidation. The photobiont originating from a less shady intrathalline environment may rely on more effective own photoprotection. The xanthophyll-based NPQ can be such a mechanism. Based on the NPQ-relevant xanthophyll composition of the isolated photobionts after illumination (Fig. 5), it can be concluded that the strategy of photoprotection was retained in the isolated, cultured state of the photobionts.

The entire lichen thalli of the study displayed no chronic photoinhibition at any light/temperature condition applied (Fig. 3). Barták et al. (2008) have demonstrated that in the umbilicate lichen *Lasallia pustulata* from a more temperate

Table 5 Two-factor ANOVA of the effect of light/dark treatment and photobiont origin (species) on the DEPS values

Factor	Sum of squares	Degrees of freedom	Mean square	F	p value	H_0 rejected?
Treatment	0.37	3	0.12	28.72	4.12E−08	Yes
Species	0.06	1	0.06	14.48	8.60E−04	Yes
Interaction	0.03	3	0.01	2.68	6.93E−02	No
Within	0.10	24	0.00			
Total	0.56	31	0.02			

Photobionts originate from the lichens *Umbilicaria decussata* and *Pleopsidium chlorophanum*. Significance level $\alpha = 0.05$. Null hypothesis H_0: no significant effect of the factor

Table 6 DEPS values of isolated photobionts after light treatment (200 μmol m^{-2} s^{-1})

	U. decussata	*P. chlorophanum*
Unstressed	0.20 ± 0.02	0.18 ± 0.02
5 min light	0.34 ± 0.07	0.48 ± 0.07
10 min light	0.38 ± 0.05	0.47 ± 0.06
20 min recovery	0.39 ± 0.02	0.53 ± 0.10

Photobionts originate from the lichens *Umbilicaria decussata* and *Pleopsidium chlorophanum*. Values are presented as mean ± SD

European site, photosynthetic processes were less influenced by light intensity than by the duration of irradiation. Laboratory experiments of this study indicated efficient use of light energy by moist, active *Umbilicaria decussata* thalli even at high PPFD and low temperatures, compared to the isolated photobiont. This is consistent with findings concerning lichens from across polar regions which show considerable adaptation to low temperatures (Kappen and Lange 1970, 1972). In the isolated state, the *U. decussata* photobiont, among other analyzed *Trebouxia* photobionts from various lichen species, even tolerates long-term freezing without taking serious damage to its photosynthetic capacity (Sadowsky and Ott 2012). NPQ was induced to a lesser extent in the entire *U. decussata* thalli, compared to the isolated photobionts. This may be due to reduced light intensity in the intrathalline algal layer of the lichen, especially caused by melanin pigmentation of the cortex. As the presented experiments show, the examined isolated photobionts are more likely stressed by excess light than by variations in temperature. Therefore, effective sun screening by the mycobiont can be a key feature of successful lichen colonization at continental Antarctic sites.

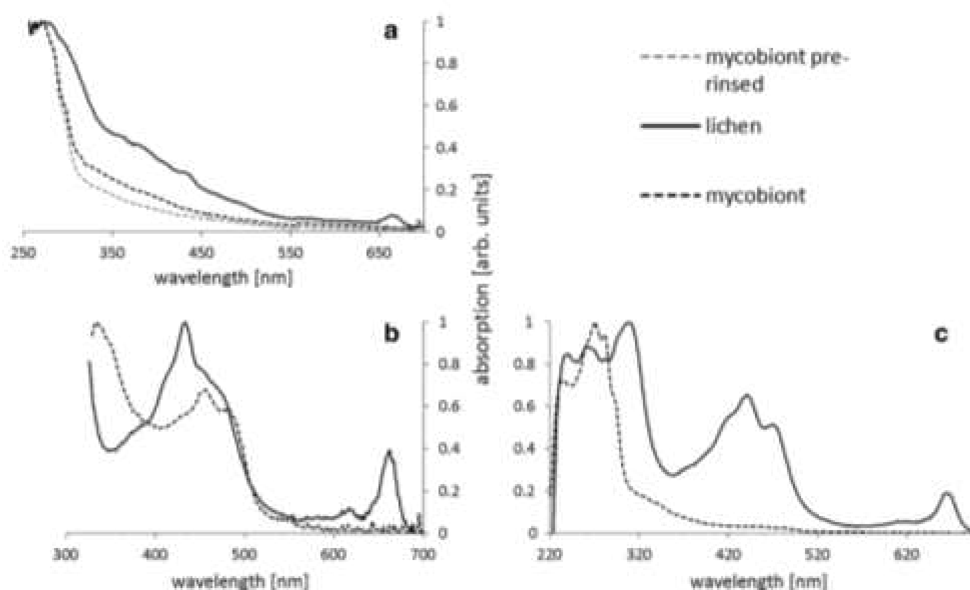

Fig. 6 Absorption spectra of *Umbilicaria decussata* lichen thallus and mycobiont extracts in different solvents. Pre-rinsed mycobiont material was rinsed with acetone prior to the DMSO extraction. Solvents are: **a** DMSO; **b** methanol; **c** acetone. Values are presented as arbitrary units, giving the highest absorption the value 1

Table 7 Chemical characteristics of synthetic melanin compared to acetone pre-rinsed (except test for solubility in acetone) mycobiont and thallus of the lichen *Umbilicaria decussata*

Chemical test	Synthetic	*Umbilicaria decussata*	
	Melanin >97 %	Thallus	Mycobiont
Solubility in			
Water	–	–	–
Methanol	–	–	–
Ethanol	–	–	–
Acetone	–	–	–
Hexane	–	–	–
Ethyl acetate	–	–	–
Toluene	–	–	–
Chloroform	–	–	–
DMSO	+	+	+
0.2 N NaOH	+	+	+
0.2 N NH_4OH	+	+	+
Precipitation by			
Alkaline $FeCl_3$	+	+	+
Bleaching in			
H_2O_2	+	+	+
NaOCl	+	+	+

Positive/negative results are indicated by +/–

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5.3 Extremotolerance of the symbiotic alga *Trebouxia* in the context of energy gain and sexual reproduction of the Antarctic endemic fungus *Buellia frigida*

Andres Sadowsky und Sieglinde Ott

Erklärung

Der vorliegende Artikel wurde vollständig durch den Erstautor verfasst.

Extremotolerance of the symbiotic alga *Trebouxia* in the context of energy gain and sexual reproduction of the Antarctic endemic fungus *Buellia frigida*

Key words: continental Antarctica, photobiology, *Trebouxia,* apothecia

Abstract

The crustose lichen *Buellia frigida* produces high numbers of functional generative structures under the harsh conditions of continental Antarctica. As sexual reproduction depends on the formation of energy consuming structures and a disruption of the lichen symbiosis, it is a cost-intensive strategy. Physiological investment in fruiting bodies and spores has to be complemented by the photobiont's carbon acquisition. The green algal symbiont tolerates extreme freezing treatments in the aposymbiotic state, while its limited photoprotection can be compensated in the symbiosis. *B. frigida* shows adaptations on the level of the lichen thallus as well as on the level of both symbionts which can assure reproductive success.

Introduction

Lichenising fungi are a polyphyletic group of ascomycetes and few basidiomycetes living a symbiotic lifestyle. The fungi, mycobionts, acquire photosynthetic partners, photobionts, whose photosynthetic activity nourishes the symbiotic association. The subsequently formed lichen thallus is a persistent poikilohydric life form which thrives especially under most extreme environmental conditions such as the Antarctic cold deserts (Kappen 2000).

Some lichen species colonising these harsh environments have lost their ability to reproduce sexually (Seymour *et al.* 2005, Ott *et al.* in prep.). This is especially the case for macro lichens (foliose and fruticose forms), while the crustose growth form in general shows sexual reproduction more often (Bowler and Rundel 1975). Sexual reproduction is an energy consuming process which depends on the formation of specialised morphological structures and spores (Lawrey 1980, Seymour *et al.* 2005). In the symbiotic association, sexual propagules are produced only by the mycobiont (Hill 2009),

which implies that sexual structures of lichen thalli comprise of large, compact photosynthetically inactive and therefore energy-consuming biomass, the metabolically active hymenium. Furthermore, the mycobiont, in general, can only form sexual structures in the symbiotic state (Stocker-Wörgötter 2001). Asexual reproduction of the lichen association can be achieved by specialised structures which carry both symbionts, as isidia or soredia, but also by thallus fragments (Bowler and Rundel 1975).

As germinating hyphae of fungal lichen spores need to find a suitable algal symbiont to complete the life cycle, low specificity concerning the algal partner may be advantageous for these mycobionts. Low photobiont specifity has been demonstrated in many of these mycobionts, compared to asexually reproducing lichens which incorporate a relatively narrow range of photobionts (Seymour *et al.* 2005).

Dyer and Mutargh (2001) found low genetic variation (based on ITS sequences) in the crustose Antarctic endemic lichen *Buellia frigida* Darb.. The low genetic variation of themycobiont is surprising, as the lichen produces numerous apothecia. As sexual reproduction forces genetic recombination, high genetic diversity would be expected in sexually reproducing mycobionts, compared to those which have lost this ability (Seymour *et al.* 2005, Singh *et al.* 2015).

The photobiont (genus *Trebouxia*) was also found to be identical in different locations at North Victoria Land (de los Rios *et al.* 2005, Sadowsky and Ott 2012). Therefore, photobiont selectivity can be described as very high in *B. frigida*. As previously explained, a different scenario characterised by low photobiont selectivity and high mycobiont variation would have been expected for a sexually reproducing lichen species (Seymour *et al.* 2005, Singh *et al.* 2015). The successful colonisation of Antarctic terrestrial habitats by *B. frigida* indicates particular features to ensure reproductive success at severe environmental conditions. This may include the protection of sexual structures against UV-induced DNA damage in the lichen symbiosis (de Vera *et al.* Adaptations on the biont level can be crucial during the establishment and differentiation of the thallus upon relichenisation. The photobiont physiology is of special importance as the photosynthetic partner has to provide carbon nutrition for the whole association. Physiological adaptations of the photobiont can be due to extreme abiotic factors of Antarctic terrestrial habitats (Sadowsky and Ott 2012, 2015). The study presented focuses on the freezing and excess light tolerance of the *B. frigida* photobiont.

Material and methods

Small rocks and rock fragments colonised by *Buellia frigida* thalli were collected at Terra Nova Bay, North Victoria Land, continental Antarctica. The material was collected air-dry and frozen until further analysis.

For microscopic analyses, thallus fragments and apothecia were carefully detached from the substrate and cut to thin slices (20 µm) using a cryotome (Reichert-Jung, Germany). Cytoplasm was stained with cotton blue solution. Micrographs of stained samples were taken by a Microscope (Axio Imager A1, Zeiss, Germany).

The green algal photobionts (genus *Trebouxia* Puymaly, ITS sequence identical to NCBI AY667580.1) were isolated from thallus fragments and cultivated as described in Sadowsky and Ott (2012). Photosynthetic performance and energy quenching was assessed via chlorophyll *a* fluorescence by a pulse-amplitude modulated fluorimeter (Mini-PAM, Walz, Germany) according to Maxwell and Johnson (2000). Prior to physiological experiments, photobionts were cultivated on nitrocellulose filter discs placed on solid *Trebouxia* organic medium (Ahmadjian 1967) for four weeks to achieve the necessary amount for the experiments.

To assess the shock freezing tolerance, dark-acclimatised filter cultures were immersed in liquid nitrogen for 15 minutes and subsequently thawed at 20°C. Maximum quantum yield of photosystem II (Fv/Fm) was determined during the thawing process in the dark.

Light energy quenching of dark-acclimatised photobiont samples was determined during a series of eight light intensities in an increasing range (photosynthetic photon flux densities, PPFD, up to 1107 µmol photons $m^{-2}s^{-1}$) and subsequent relaxation in the dark (rapid light curves, according to White and Critchley, 1999). Acclimation to three different light intensities was investigated by slow chlorophyll fluorescence induction (Roháček 2002) followed by twenty minutes of relaxation in the dark. Quantum yield of photosystem II is referred to as Y(II). The relative electron transport rate of photosystem II was calculated as

ETR(II) rel. = Y(II) * PPFD.

Data analysis was performed in MS Excel and GraphPad Prism. For nonlinear regression of light saturation data and Fv/Fm regeneration during the thawing process the following equation was used:

$Y=Y_0 + (Y_{max}-Y_0) * X/(t_{half} + X)$

Parameter definitions: Y = NPQ, Y(II) or ETR(II) rel.; Y_0 = initial Y; Y_{max} = maximal Y; X = time or light intensity; t_{half} = X at $Y_{max}/2$

Results and discussion

Concerning the vegetative thallus, especially the photobiont-rich young areolae are covered with a strongly melanic upper cortex (Meeßen *et al.* 2013). In addition to UV, the photosynthetic partner of the symbiosis may experience stress from excess high photosynthetically active radiation, which is a common factor at lichen sites in the Antarctic summer (Sadowsky and Ott 2015, Schlensog *et al.* 2003). Investigations of the photobiology of the isolated photobiont revealed high susceptibility to long-term high light intensity (Fig. 3). But the photobiont is capable of autonomous quenching of excess light energy by xanthophyll conversion. Only minor damage to the functionality of photosystem II appears after short durations (< 2 min) of high light intensity treatment in the laboratory (1120 µmol photons / (m^2s), Fig. 2A & B). Short exposures to excess light can therefore be tolerated even by the aposymbiotic photobiont. Exposure of the isolated photobiont to high light intensities for more than 2 minutes causes chronic photoinhibition, defined by incomplete regeneration of Y(II) and NPQ in the dark (Fig. 3). To avoid photoinhibition, effective sun-screening of the photobiont can be advantageous. Also during the initial developmental steps of reestablishment of the symbiosis, the formation of melanic fungal hyphae enveloping photobiont cells may provide an efficient sun-screening effect in the first steps of thallus differentiation.

Freezing, another common event in Antarctic terrestrial ecosystems, cannot be seen as a major stressor to the *Buellia frigida* photobiont. In studies previously carried out, the alga could retain its physiological activity for extended periods under subzero temperatures (Sadowsky and Ott 2012). Even extreme shock-freezing events are tolerated (Fig. 2C). The freezing tolerance of the *B. frigida* photobiont exceeds that of many others in the

genus *Trebouxia* (Sadowsky and Ott 2012, Hájek *et al.* 2012) and can be interpreted as a prerequisite to nourish the mycobiont for successful colonisation of harsh Antarctic habitats.

It is still a matter of dispute whether or to which extent *Trebouxia* algae occur free-living (Ahmadjian 1988, Mukhtar *et al.* 1994, Beck *et al.* 2014). Currently, only one strain of photobionts is known from *Buellia frigida* thalli (de los Rios *et al.* 2005, Sadowsky and Ott 2012). The potentially suitable photobionts for the relichenisation process of the sexually reproducing *Buellia frigida* fungus must be available in the environment. It can be postulated that the potential photobionts occur as non-lichenised algae, or as parts of degenerating or fragmented lichen thalli. The photobionts' susceptibility to long-term high insolation implies that free potential *B. frigida* photobionts are not to be expected on open sun-exposed rock surfaces. Shaded miniature fissures may provide appropriate micro habitats. The photobionts' outstanding freezing tolerance may besides other parameters distinguish these organisms from other terrestrial microalgae. Considering this stress tolerance, combined with the advantage of light-screening by a mycobiont, it can be speculated that these are crucial traits of the examined *Trebouxia* strain which are the basis of its selection as *B. frigida* photobiont. In the symbiotic state, it is crucially responsible for carbon nutrition of the highly demanding formation of apothecia and pycnidia, the generative features of the lichen species.

Apothecia including spores of continental Antarctic lichen *Buellia frigida* were formed in high numbers on the thallus surface (Fig. 1A-C). In culture, the ascospores were able to germinate and produce well-developed mycelia (Meeßen *et al.* 2013). Pycnidia have also been formed (Fig. 1D). As already known these structures produce pycnospores which may be responsible for fertilisation of the trichogynes, as it has been suggested for the life cycle of lichens (Henssen and Jahns 1974, Honegger 1984, Jahns and Ott 1994). The formation of vegetative diaspores has not been recognised in *Buellia frigida.*

Protection of these specialised generative structures may be crucial for the success of sexual reproduction. As *Buellia frigida* is frequently found on sun-exposed rock surfaces, shielding from potentially DNA damaging UV radiation as well as high irradiation can be regarded as a fundamental advantage. In this context, the intense occurrence of the dark pigment melanin in different developmental stages of *Buellia frigida* is especially relevant. The isolated mycobiont cultivated from spores forms melanin which is also

responsible for the black colour of the lichenised thallus (Meeßen *et al.* 2013). The deep black appearance of *B. frigida* apothecia is caused by accumulation of melanin in the hymenial layer including the individual spores (Fig. 1B & C). The secondary metabolite is essential to absorb UV and visible light. This substance may therefore be highly important as a sun-screening pigment in lichens (Meeßen *et al.* 2013, Sadowsky and Ott 2015).

It can be concluded that the combined adaptations of the two partners of the *Buellia frigida* symbiosis assure and protect the functionality of a complete sexual life cycle. The photobiont's outstanding freezing tolerance as well as the mycobiont's photoprotective capabilities can facilitate photosynthetic activity at continental Antarctic sites. Therefore, required carbon for the production of fungal spores can be assimilated. Furthermore, the single bionts' extremotolerance can enhance relichenisation success in a harsh environment.

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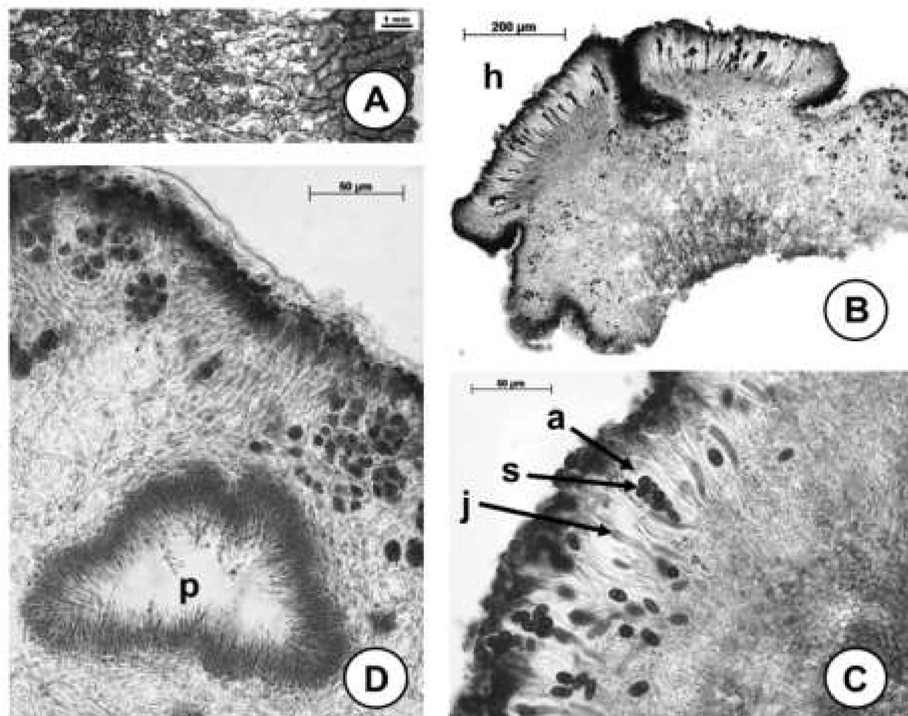

Fig. 1. Thallus structures of *Buellia frigida*. A: macroscopic image of the thallus surface. Younger marginal parts are on the right, dark spots in the older thallus area are apothecia. B: Cross-section through two apothecia, h: hymenium. C: magnified hymenium with asci (a), mature (s) and juvenile (j) spores. D: Cross-section through a pycnidium (p).

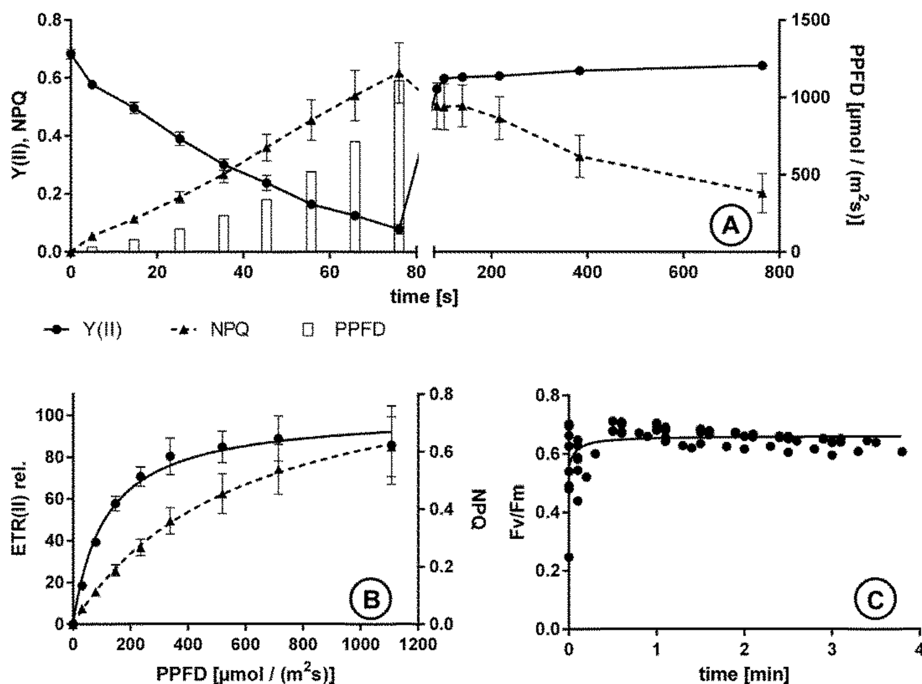

Fig. 2. High light and shock freezing treatment of isolated *Buellia frigida* photobionts. A: time course of quantum yield of photosystem II (YII) and non-photochemical energy quenching (NPQ) during exposure to increasing light intensities followed by a dark relaxation phase. B: light saturation of NPQ and relative electron transport rate of photosystem II (ETR(II) rel.). C: regeneration kinetics of maximum quantum yield of photosystem II (Fv/Fm) during thawing of shock-frozen photobionts. Single values are presented in C, while values are presented as means ± standard deviation (n = 5) in A and B. The curves in B and C are obtained by nonlinear regression.

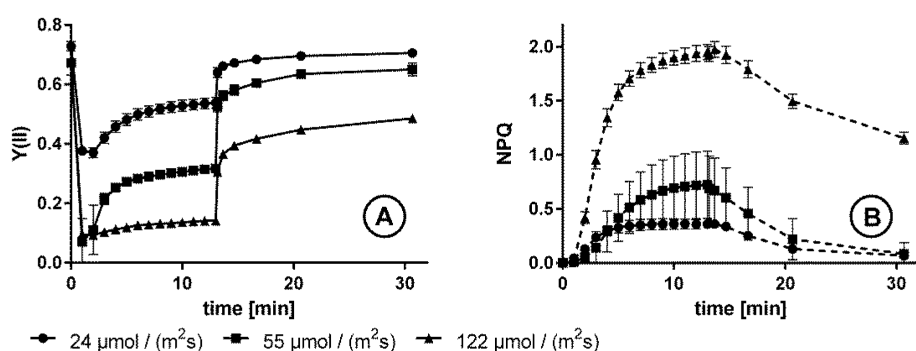

Fig. 3. Slow chlorophyll fluorescence induction of isolated *Buellia frigida* photobionts. First measurement of a time-course was taken in the dark-acclimatised state, followed by 12 measurements at the indicated light intensity (24, 55 and 122 µmol photons / (m^2s)) and a dark relaxation phase. Values are presented as means ± standard deviation, n = 5. A: photosynthetic quantum yield of photosystem II (YII); B: non-photochemical quenching (NPQ).

5.4 Response of the metabolite profile in the context of desiccation tolerance of Antarctic lichens from southernmost habitats

Andres Sadowsky, Tabea Mettler-Altmann und Sieglinde Ott

Erklärung

Der vorliegende Artikel wurde vollständig durch den Erstautor verfasst.

Response of the metabolite profile in the context of desiccation tolerance of Antarctic lichens from southernmost habitats

Keywords: *Trebouxia*, *Usnea lambii*, *Umbilicaria decussata, polyols, sugars*

Abstract

Desiccation tolerance is a feature of most lichens. These symbiotic associations of a fungal partner (mycobiont) and a photosynthetic partner (photobiont) experience severe desiccation on a regular basis. Many lichen species colonise extreme habitats such as the cold deserts of Antarctica. Although the stress physiology of lichens has been studied extensively, little is known about the photobionts' physiological potential. To assess the contribution of photobionts to the lichens' success in Antarctic terrestrial environments, the photosynthetic partners of *Umbilicaria decussata* (Umbilicariaceae, lichenised ascomycetes) and *Usnea lambii* (Parmeliaceae, lichenised ascomycetes) from extreme habitats in southern Antarctica have been isolated. Significant differences regarding stress tolerance of the two green algae had been shown previously, despite their close phylogenetic relationship. A deeper understanding and explanation of these differences are obtained by examination of the metabolite profiles and dynamics. Especially the role of sugars, polyols and amino acids is discussed. High concentrations of ribitol and sucrose among other metabolites characterise the *Usnea lambii* photobiont's mainly constitutive strategy of desiccation tolerance. A high degree of specialisation to dry environments can be assumed for this photobiont.

Introduction

Lichens are exosymbiotic associations of a fungus and a unicellular photosynthetic organism. The symbionts, called myco- and photobiont, form a macroscopic thallus structure (Ahmadjian 1965). In the thallus, most of the structural elements consist of mycobiont hyphae, while the photobiont is responsible for carbon nutrition of the whole association. Lichen symbiotic organisms are characterised by complex morphological and anatomical features but their water regulation is passive (Larson 1979). Depending on the habitat, desiccation can occur within hours or minutes (Lange *et al.* 1998). Upon desiccation the

lichens' poikilohydric terrestrial lifestyle causes a state of latent life, called anabiosis or anhydrobiosis (Kranner *et al.* 2008). In anabiosis, the lichen symbiosis tolerates extreme abiotic conditions such as strong irradiation, temperature extremes or exposure to space vacuum (Kappen 2000, de Vera *et al.* 2002, Sancho *et al.* 2007). Anabiotic lichens can be reactivated by moisturising within minutes (Lange *et al.* 1998). Even after years of dry and cold storage, full photosynthetic activity can be restored (Sadowsky & Ott 2015). This exceeds the reactivation capacity of spermatophyte resurrection plants (e. g. *Craterostigma plantagineum* Hochstetter, Bernacchia *et al.* 1996) by far, as those plants regain their full activity within hours to days. Cycles of dehydration and rehydration can be repeated innumerable times during the lichen life cycle. Dehydration/rehydration cycles may occur on a diurnal basis without disrupting and affecting the symbiosis (Ahmadjian 1965, Kappen 1988). It is characteristic for the lichen symbiosis that the latent life can be retained for prolonged periods up to years without disabling revitalisation (Honegger 2003, Sadowsky & Ott 2015).

Lichens and bryophytes, both described by poikilohydry, dominate the macroscopic flora of continental Antarctica (Øvstedal & Smith 2001). Antarctica is characterised by its isolated, cold and dry climate especially in the cold deserts of continental areas (Convey 1996a, b). At terrestrial continental habitats, precipitation occurs exclusively as snow. Substantial rainfall sufficient to activate lichens occurs in the maritime Antarctic (Barták *et al.* 2005). While two seed plants and few invasive species colonise sites in the northern maritime regions of the Antarctic Peninsula, they become completely replaced by cryptogams at lower latitudes and under more extreme conditions (Øvstedal & Smith 2001). Terrestrial life on the Antarctic continent and on the southern Peninsula does not follow a strict latitudinal or macroclimatic gradient but depends on suitable micro niches for colonisation (Pointing *et al.* 2015). The main limiting factor for lichens and any terrestrial life in these habitats is not temperature, but water availability (Kennedy 1993, Block 1996). Nevertheless, substantial temperature extremes occur at lichen sites even in the diurnal course (Sadowsky & Ott 2015). It is well known that especially lichens colonising sites at severe polar habitats can be photosynthetically active at very low, even subzero temperatures (Kappen 2000). Even aposymbiotic photobionts display considerable low temperature and freezing tolerance (Sadowsky & Ott 2012, Balarinová *et al.* 2013). An indirect effect of low temperatures is water restriction, which strictly limits plant life (Kennedy 1993, Block 1996). The combination of low precipitation and low availability of liquid

water due to subzero temperatures makes Antarctica the world's driest continent (Huiskes *et al.* 2006). Poikilohydric and highly desiccation-tolerant organisms replace plants and mosses in more water-restricted micro niches (Pointing *et al.* 2015). Continental Antarctic lichens fully depend on meltwater (Schlensog *et al.* 2003), leading to erratic patterns of physiological activity during the summer (Schroeter *et al.* 2011). Therefore, lichens need to be able to tolerate frequent desiccation. Also the isolated photobionts display desiccation tolerance and the potential to rehydrate (Sadowsky and Ott 2012), although it is enhanced in the symbiotic state (Kranner & Birtic 2005, Kosugi *et al.* 2009).

Water loss, although common in lichens, can cause severe stress in cells. Possible depletive effects of dehydration are mechanical stress, oxidative stress, protein denaturation and loss of membrane function. Water loss also changes intracellular pH and overall ion concentrations, influencing enzyme activity (Kranner *et al.* 2008). Poikilohydric organisms must possess mechanisms to avoid this kind of stress or be able to repair dehydration-induced impairments. In resurrection plants, repair mechanisms are of minor importance compared to stress-induced protective molecules (Martinelli *et al.* 2007, Gechev *et al.* 2012). Protective molecules can be proteins as, among others, late embryogenesis abundant (LEA) proteins, early-responsive to desiccation (ERD) factors, and early light-inducible proteins (ELIP). Free amino acids as gamma-amino butyric acid (GABA) may play a role as osmolytes, but also as signalling substances (Žárský 2015). Another class of protective substances are sugars and sugar alcohols (polyols). These molecules also may have a multiple function (Kranner *et al.* 2008). The hydrophilic sugars increase the osmotic potential in the cytoplasm and may replace hydration envelopes (Crowe *et al.* 2005). Due to their ability to form hydrogen bonds, highly concentrated sugar molecules such as trehalose can induce the glass-phase formation or vitrification of cytoplasm (Holzinger & Karsten 2013). In this dense state, protein, nucleic acid and membrane structures are preserved while physiological activity is stopped. Furthermore, some polyols have antioxidant properties (Kranner *et al.* 2008).

Although only few higher plants are considered desiccation tolerant, their seeds and pollen grains are desiccation tolerant in most cases (Black & Pritchard 2002). Seeds, for example, accumulate high amounts of sugars, especially sucrose (Pammenter & Berjak 1999).

Lichens may share some of the general mechanisms of desiccation tolerance which can be found in higher plants (Kranner *et al.* 2008). Green algal lichens display especially high polyol contents (Roser *et al.* 1992). Although it must be taken into account that polyols (ribitol and mannitol) are the transport form of assimilates in the lichen symbiosis, high polyol levels can be correlated with desiccation tolerance in lichens (Cowan *et al.* 1979, Nash III, 1996). Isolated green algal photobionts produce ribitol (Komiya and Shibata 1971), although general carbon metabolism undergoes substantial changes in culture, compared to the symbiotic state (Green & Smith 1974).

Polyol synthesis and high sugar content is common in free-living terrestrial algae, but not in aquatic such as *Chlamydomonas* Lindley species (Roser *et al.* 1992). As terrestrial green algae originated from aquatic ancestors, they had to evolve physiological features to cope with land environmental conditions. Compared to an aquatic lifestyle, especially lichen photobionts are exposed to higher levels of possible stressors as high light intensity and desiccation events (Kranner & Lutzoni 1999). Therefore, mechanisms of desiccation tolerance are presumably evolutionary old traits in lichen photobionts (Kranner *et al.* 2008).

Investigations on ecophysiology of lichens have been carried out across Antarctica, including a variety of different species (Kappen 2000). Although the main focus had been on maritime regions (Kappen & Redon 1987, Schroeter *et al.* 1995, Sancho *et al.* 2004, Schlensog *et al.* 2013, Bokhorst *et al.* 2015), some studies addressed continental habitats (Kappen 1989 Kappen & Breuer 1991, Pannewitz *et al.* 2003, Schroeter *et al.* 2011). Stress physiology and especially photosynthesis have been a major concern of this research. In order to clarify the contribution of the symbiotic partners to the adaptive strategies of lichens, few studies have also addressed photobiont stress physiology of Antarctic lichens (Sadowsky & Ott 2012, 2015).

This study focuses on features and dynamics of the metabolic profiles of two Antarctic lichen photobionts of the genus *Trebouxia* Puymaly. They originate from the cosmopolitic lichen *Umbilicaria decussata* Villars & Zahlbruckner (Øvstedal & Smith 2001), sampled in continental Antarctica, and from the antarctic endemic lichen *Usnea lambii* (Imshaug) Wirtz & Lumbsch (Wirtz *et al.* 2008), sampled on Coal Nunatak, southern maritime Antarctica. According to internal transcribed spacer (ITS) sequence based phylogeny, these photobionts belong to the same group (*Trebouxia* "*jamesii*" (Hildreth & Ahmad-

jian) Gärtner, clade S, Brandt 2011). In contrast to their close phylogenetic relation, they displayed substantial differences in stress physiology (Sadowsky & Ott 2012). Desiccation tolerance of the *U. lambii* photobiont was generally higher compared to the *U. decussata* photobiont.

It can be hypothesized that differences in stress tolerance are also reflected in the metabolite profiles of the two photobionts. The focus lies on hydrophilic compounds such as sugars, polyols, free amino acids and other carboxylic acids. These substances can directly influence the water potential of cells and especially polyols are considered important factors in the desiccation tolerance of terrestrial algae and lichens (Holzinger & Karsten 2013, Karsten & Holzinger 2014).

Material and methods

Lichen and photobiont

Umbilicaria decussata. thalli were collected at Terra Nova Bay, North Victoria Land, continental Antarctica. Thalli of *Usnea lambii*, synonym *Usnea sphacelata*, originate from Coal Nunatak, Alexander Island, southern maritime Antarctica. The green algal photobionts (genus *Trebouxia*) were isolated from thallus fragments after Bubrick and Galun (1988) and Yoshimura *et al.* (2002) and subsequently cultivated in *Trebouxia* organic medium (TOM) with 1 % (w/v) glucose (Ahmadjian 1967). Cultures were kept at low light intensity (20 µmol photons m^{-2} s^{-1}; diurnal cycle with 10 h of darkness) and 12 °C in a growth chamber (Rubarth Apparate GmbH, Germany).

DNA extraction and PCR

1 ml of cultures was harvested in triplicate and cells were separated from the majority of medium by centrifugation. Extraction of total DNA was performed with the DNeasy Plant Mini Kit (Qiagen, Germany). Cell pellets were ground in sterile quartz sand and lysis buffer including RNase with a sterile metal pistil. RNase digestion was elongated to 20 minutes. DNA quality was checked by spectrophotometry (NanoDrop ND1000, Thermo Scientific, USA). Sequences of actin, ribulose bisphosphate carboxylase/oxygenase – large subunit (rbcL) and internal transcribed spacer of ribosomal DNA (ITS) were amplified (thermocycler TGradient, Biometra, Germany). Primers and PCR programme: see

supplemental material. The size of amplified fragments was checked via gel electrophoresis.

Sequence alignment and distance

PCR fragments were sent to GATC, Germany for Sanger sequencing. Consensus sequences of three replicates were generated in the JEMBOSS software suite. The MEGA6 suite was used to align and blast the sequences as well as to create a distance tree and matrix for individual and combined markers.

Metabolome analysis

For the extraction of polar metabolites, four weeks old cultures were dark acclimatized for 30 minutes at 20°C. Cell density was determined with a Neubauer improved counting chamber and used to determine total cell count (n_{cells}) of the samples. The median single cell volume ($V_{single\ cell}$) was determined by microscopic measurement of cell radii (Axio Imager A1, Zeiss, Germany). 1 ml of culture volume was harvested per replicate ($n = 3$) and transferred to PVDF filters by vacuum filtration to remove the medium. Samples were either directly frozen in liquid nitrogen or previously subjected to dehydration in dry air (rel. humidity < 5%). 600 ml of precooled extraction buffer (methanol:chloroform 7:3, -20°C) was added to the frozen samples. Extraction was performed at 4°C in reaction tubes shook at 90 min^{-1} for 70 minutes using a ball mill (Retsch, Germany). The clean filter was removed and 300 ml ice-cold ultrapure water was added. After vigorous mixing and two freeze-thaw cycles in liquid nitrogen, the polar phase of the extract was separated by centrifugation and subsequently vacuum-dried.

The extracts were analysed by gas chromatography-mass spectrometry (GC-MS) according to Fiehn (2007) using a 7200 GC-QTOF (Agilent Technologies, USA). For peak integration to determine the signal strength per metabolite, the Mass Hunter Software (Agilent Technologies, USA) was used. For relative quantification, all metabolite peak areas were normalized to the according cell number used for extraction. Due to very strong ribitol and sucrose signals exceeding the detection range, samples were diluted 50-fold for determination of these two metabolites.

Principal component analysis (PCA) and plotting of individual metabolite levels was performed in R statistics software (R version 3.1.2 provided by the CRAN project, http://www.R-project.org). For statistical testing, two-way ANOVA and unpaired two-sided t-tests were performed in Microsoft Excel and GraphPad Prism. Clustering and cre-

ation of a heat map based on metabolite content was performed in Multiple Experiment Viewer (Saeed *et al.* 2003), using Euclidean distance. For PCA and hierarchical clustering, values were z-score normalised.

Results

Regarding the markers used (actin, ITS and rbCl), the two photobionts could not be separated in different groups, as there was no significant difference between the combined sequences (Fig. 1). They showed highest similarity with *Trebouxia jamesii*, confirming the results by Brandt 2011.

A total of 36 polar metabolites could be detected and quantified by GC-MS analysis (Fig. 2, Tables 1, 2). Ribitol and sucrose were very abundant in both photobionts as their extracts had to be diluted 50-fold to correctly determine these substances (Tables 1, 2).

Hierarchical clustering and principal component analysis

Principal component analysis (PCA, Fig. 3, Table 3) of the z-score normalised metabolite levels showed that unstressed and completely dehydrated sample sets form distinguished clusters, respectively. Samples taken during the desiccation process showed comparably higher variability and clustered together, including both steps (15 and 30 minutes of desiccation) as well as both photobionts. Regarding the samples taken during the desiccation process, there was a tendency of separating the photobionts along principal component (PC) 1 (Fig. 3 A). PC1 pronouncedly separated the control samples of the *U. lambii* photobiont from other sample sets of the study (Fig. 3A).

Hierarchical clustering (HCL) showed similar behaviour of the sugars maltose, fructose, lactose and xylose. They cluster with mannose, sorbitol and succinic acid (Fig. 4). Regarding the *Usnea lambii* photobiont, sugars, especially lactose, maltose and xylose, also form a major proportion of PC 2 according to PCA (Table 3). In the *U. lambii* photobiont, PC 2 separates the completely dehydrated samples clearly from all other samples taken. Regarding PC 1, treated samples are separated from the controls, while there is a tendency of approaching the controls during the proceeding treatment (Fig. 3E). The loadings of PC 2 regarding an analysis including both photobionts were mainly amino acids such as cysteine, isoleucine, valine, leucine, phenylalanine, proline, and tyrosine (Table 3). These

amino acids form a cluster in HCL (Fig. 4) as well. Other carboxylic acids as well as amino acids were the main components of PC 1, which explained c. 44.0 % of the data variation (Fig. 3A, Table 3). Analysing each of the photobiont species separately, it could be demonstrated that sugars clearly dominated PC3. This principal component however only explains 6.4% and 6.1% of the data variation, respectively (Fig. 3D, F).

Metabolite concentrations

Unstressed photobionts differed significantly concerning the contents of 22 metabolites (Fig. 5). Most of these were more abundant in the *Usnea lambii* photobiont, especially sucrose (c. 72-fold), ribitol (c. 43-fold) and malic acid (c. 17-fold). Isoleucine, leucine, phenylalanine, proline, valine and maltose (c. 4-fold and 3-fold, respectively) were present in higher concentrations in the *Umbilicaria decussata* photobiont.

Two-way ANOVA (Benjamini-Hochberg corrected, Table 4) indicated that the desiccation treatment had a significant effect on the photobionts concerning 91.2% of the detected metabolites. Interaction between treatment and photobiont origin (*U. lambii* and *U. decussata*, respectively) had a significant effect regarding 50.0% of the compounds. The interactive effect implies that the photobionts responded differently to the stimulus.

The desiccation treatment had a significant effect (Benjamini-Hochberg corrected two-way ANOVA, $\alpha = 0.05$) on all observed polyols (glycerol, myoinositol, ribitol and sorbitol) as well as on the sugars except maltose and mannose (Table 4). In the *Usnea lambii* photobiont, polyol and sugar levels were reduced during dehydration, and subsequently increased at the end of the treatment (Fig. 2). Considering glucose, sorbitol, ribitol and myoinositol, control values are regenerated, and, with xylose, exceeded (Table 2). Reduced polyol concentrations persisted in the desiccated *Umbilicaria decussata* photobionts. The initial levels of the sugars fructose, glucose, maltose and mannose were re-established (Table 1). Carboxylic acids (alpha-hydroxic and dicarboxylic acids) were strongly influenced by the desiccation treatment of the *U. lambii* photobiont in contrast to the *U. decussata* photobiont (Fig. 2).

GABA was found in both organisms and its content increased during desiccation, especially in the *U. lambii* photobiont (Fig. 2). Lower concentrations, as observed under control conditions, were regenerated at the end of desiccation (Table 2).

Discussion

The photobionts of the Antarctic macrolichens *Umbilicaria decussata* and *Usnea lambii* both belong to the same clade of the *Trebouxia jamesii* complex, as confirmed by sequence similarity of ITS, rbcL and actin-1 (Fig. 1). These markers are well suited to distinguish species and higher taxonomic levels, but do not provide any information on intra species diversity. *T. jamesii* is a widespread photobiont with a world-wide occurrence and can be found in a number of macro lichens across Antarctica (Friedl 1989, Beck 2002, Helms *et al.* 2001, Romeike *et al.* 2002, Helms 2003, Engelen 2008, Brandt 2011, Sadowsky & Ott 2012). The minor taxonomic distance of the *Umbilicaria decussata* and *Usnea lambii* photobionts is contrasted by the differences in physiological responses to cold and drought (Sadowsky & Ott 2012).

Sadowsky and Ott (2012) demonstrated superior resistance to freezing and desiccation treatments in the *U. lambii* photobiont compared to the *U. decussata* photobiont. This was pronouncedly represented by longer retention of photosynthetic activity under dehydrating conditions and subzero temperature, as well as higher regeneration capacity after the treatment. The photobionts can both be reactivated within seconds after immediate freezing as well as after slow dehydration. This ability can be described as a strong advantageous characteristic of poikilohydric organisms and by far exceeds the reactivation capacity of resurrection plants. To restore full activity, the spermatophytes need at least few hours up to days (Bernacchia *et al.* 1996). Lichens in extreme ecosystems such as cold and hot deserts inhabit preferably micro habitats which provide favourable combinations of environmental factors such as water availability, temperature and light intensity. The micro climate of these habitats can vary considerably during the annual and diurnal course, leading to short active periods of lichens (Convey 1996a, b, Huiskes *et al.* 2006, Schroeter *et al.* 1992, Romeike 2002). Water can be obtained and lost quickly from the thallus, and unlike resurrection plants, the lichen water relation is passive. Therefore, lichen water supply depends on the interplay of external factors and thallus structure, which influence the gradient of water potential between thallus and the surrounding air (Green & Lange 1995). The fruticose morphology of *U. lambii* implies a high surface to volume ratio compared to the foliose lichen *U. decussata*. Therefore, water may evaporate from and be absorbed more quickly by the fruticose lichen. Additionally, *Umbilicaria decussata* is frequently found close to meltwater channels and may therefore profit from

enhanced long-term water supply in the Antarctic summer (Schlensog *et al.* 2003). The erect growth form of *Usnea* thalli provides less opportunity to get hydrated by meltwater (Romeike 2002).

Sugars and sugar alcohols

Accumulation of soluble sugars and polyols is widespread in green plants (Viridiplantae). Besides their role as storage compounds, these metabolites serve as osmolytes and prevent ice formation. Seed plant embryos, for example, contain high amounts of sucrose which protect the integrity of membranes and proteins. Small non-reducing sugars such as sucrose and especially trehalose can stabilise protein structures in active plant tissues as well as in algae (Holzinger & Karsten. 2013). High cytoplasmic sucrose concentrations can, however, inhibit the metabolism, and trehalose was not detected in the studied lichen photobionts. Similarly, the desiccation-tolerant terrestrial green alga *Klebsormidium* contains only minor amounts of trehalose (Kaplan *et al.* 2012). Metabolites other than sucrose and trehalose can therefore be expected to play a more relevant role in direct desiccation tolerance of the *Usnea lambii* as well as *Umbilicaria decussata* photobiont. Sucrose can be a rapidly available energy source to fuel other mechanisms of a stress response. In the *U. lambii* photobiont, c. ¾ of the sucrose concentration was lost within the first 15 minutes of the desiccation treatment, which could not be recognised in the *U. decussata* photobiont. Possibly, degradation of sucrose for energy gain occurred in the *Usnea lambii* photobiont. The xylose pools of both photobionts studied were reduced as well.

Polyols (sugar alcohols) do not display the inhibitory effect of sucrose, but can also mediate desiccation tolerance (Yancey 2005). They can also be used as storage and transport compounds for chemical energy, even under conditions of stress and recovery (Karsten 2007). In addition, high polyol concentrations can reduce the water potential of the cytoplasm without inhibiting the metabolism. Especially ribitol was present in the photobionts examined, but also considerable levels of glycerol, myoinositol and sorbitol were detected. During the desiccation process, the concentrations of all these polyols decreased in both photobionts investigated. In the *U. lambii* photobiont, initial polyol concentrations were consistently higher and polyol concentrations increased again, up to full regeneration of the initial concentrations of, at the end of the desiccation phase. It can be concluded that, compared to the *U. decussata* photobiont, the *U. lambii* photobiont possesses more effective polyol-based protection mechanisms, and may also use this substance as a

source of chemical energy. These mechanisms may be based on higher constitutive polyol concentrations and re-establishment of the pool even before the next hydration cycle starts. Resynthesized polyols may derive from long-term storage compounds. Starch is however unlikely to be used at least in the earl stage of desiccation. A transcriptomic study addressing the drought response of the *U. decussata* and the *U. lambii* photobiont (Sadowsky *et al.* pers. comm.) revealed, that starch metabolism is downregulated, while the mitochondrial respiration is upregulated. Several protein-degrading enzymes are up-regulated at the same time. It can be suggested that protein degradation may additionally fuel the stressed metabolism of these algae.

Aquatic green algae such as the model organism *Chalmydomonas reinhardtii* P.A.Dangeard do not contain any ribitol. This substance is present in both photobionts examined, and especially in the *U. lambii* photobiont . Polyols are also found in terrestrial green algae as well as in land plants (Holzinger & Karsten 2013). The sugar alcohol glycerol is accumulated in stressed marine algae (Rathinasabapathi 2000) and was also detected in the photobionts examined. Only the *U. lambii* photobiont showed an increase of glycerol concentrations at the end of the desiccation phase, while glycerol levels of the *U. decussata* photobiont remained stable.

The occurrence of mechanisms of sugar and polyol mediated desiccation tolerance throughout the plant kingdom can be interpreted as a sign of an evolutionary old trait (Oliver *et al.* 2002).

Amino acids and other carboxylic acids

Free amino acids were involved in the desiccation response, some of which are known to increase tolerance against osmotic stress in plants and algae. Accumulation of free proline occurred in the *Umbilicaria decussata* photobiont during the dehydration phase. Proline is a well-described osmoprotectant in higher plants (Mattioli *et al.* 2008) as well as in green algae (Delauney & Verma 2002). The *Usnea lambii* photobiont did not show any response of the proline pool, therefore proline accumulation may be a mechanism of drought tolerance only present in the *U. decussata* photobiont. A similar reaction was observed regarding increasing valine levels in the *U. decussata*, contrasting to the *U. lambii* photobiont. Valine accumulation has also been shown under dehydration stress of higher plants (Sharma *et al.* 2014). Cysteine may have a similar protective function

(Anjum *et al.* 2014), but showed little dynamics in both examined organisms and is therefore considered of minor importance.

The free amino acid GABA displayed a pattern of accumulation during the desiccation phase of both photobionts. GABA accumulation has been reported from various members of the Viridiplantae, and is generally supposed to be involved in stress response. Particularly in the drought response, it can act as an osmolyte (Sharma *et al.* 2014). GABA may also be a stress signal, activating downstream stress responses. In animals, the role of GABA as a stress-related neuro transmitter is well-established, but also in higher plants, a GABA receptor was recently identified (Žárský 2015). The role of GABA in green algae as a stress signal is still in discussion, but acting in desiccation tolerance (as an osmolyte and membrane/protein protectant) is quite likely (Sharma *et al.* 2014).

Conclusions

Dehydration caused substantial changes in the metabolite pools of both photobionts examined. Despite their close relationship, both the photobionts of *Umbilicaria decussata* and *Usnea lambii* displayed significantly different dehydration responses and significantly different metabolite levels in the unstressed state. The *U. lambii* photobiont displayed several features which may contribute to superior dehydration tolerance (Sadowsky & Ott 2012). Constitutive levels of many protective compounds are much higher compared to the *U. decussata* photobiont. This is especially pronounced regarding sugars and polyols, which are able to protect cellular structures directly and serve as energy sources. The *U. lambii* photobiont may therefore be more efficiently prepared for rapidly occurring drought stress. The *U. decussata* photobiont's metabolome in the completely dehydrated state was completely different to the unstressed situation. Despite major changes during the desiccation phase, many metabolite levels were re-established in the dehydrated *U. lambii* photobiont metabolome, recreating the superior constitutive desiccation tolerance.

During the response to desiccation, some mechanisms distinguished the two photobionts studied from each other. While the *U. lambii* photobiont apparently relied on the use of polyols and sugars, the *U. decussata* photobiont accumulated some protective amino acids.

Although closely related taxonomically, the different physiology of the two photobionts under identical experimental conditions indicates genetically fixed differences. The respective lichen associations differ fundamentally regarding morphology/anatomy and

geographic distribution. The superior desiccation tolerance of the *U. lambii* photobiont might be correlated to the respective lichen's fruticose anatomy and to its distribution only in the dry terrestrial environments of Antarctica (Øvstedal & Smith 2001, Wirtz 2008). *Umbilicaria decussata*, by contrast, has a cosmopolitic distribution, inhabiting cold and alpine regions on both hemispheres (Øvstedal & Smith 2001). In the extremely dry and cold habitats of *U. lambii,* maintaining high levels of osmotically active non-reducing sugars and polyols may be especially advantageous. The costs of solute synthesis may be high (Yeo 1983), but compensated by the ability to maintain photosynthetic activity at low water availability. This can be interpreted as a high degree of specialisation to extreme Antarctic habitats. Unlike *Usnea lambii*, which is only found in extremely dry habitats (Øvstedal & Smith 2001), *U. decussata* can be found alongside *U. lambii* as well as in less extreme habitats. Its cosmopolitic distribution may correlate with less specialisation (Muggia *et al.* 2014), reflected in the photobiont physiology (Sadowsky & Ott 2012).

Based on these results, the *U. decussata* photobiont is quite likely to show higher ecological plasticity. Therefore, warming of Antarctic lichen habitats, as it has been proved on the Antarctic Peninsula (Davies *et al.* 2014), resulting in enhanced water availability, may be tolerated more easily by *Umbilicaria decussata*.

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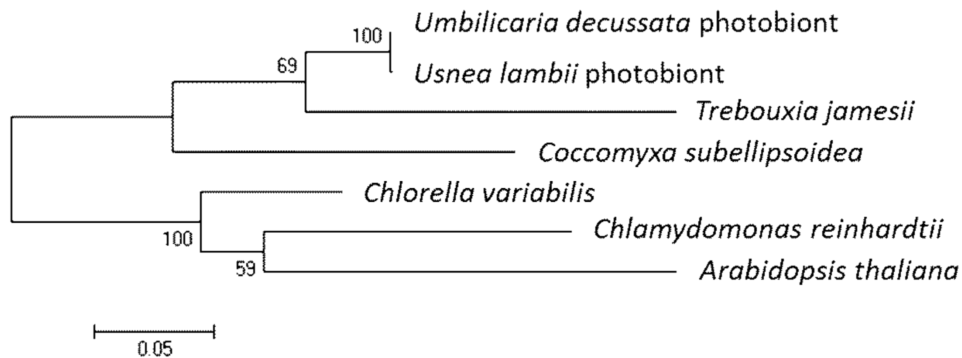


Fig. 1 Hierarchical tree of *Umbilicaria decussata* (D) and *Usnea lambii* (L) photobiont sequences. Numbers at branchings indicate the bootstrap-value (10,000 iterations), branch length indicates the phylogenetic distance.

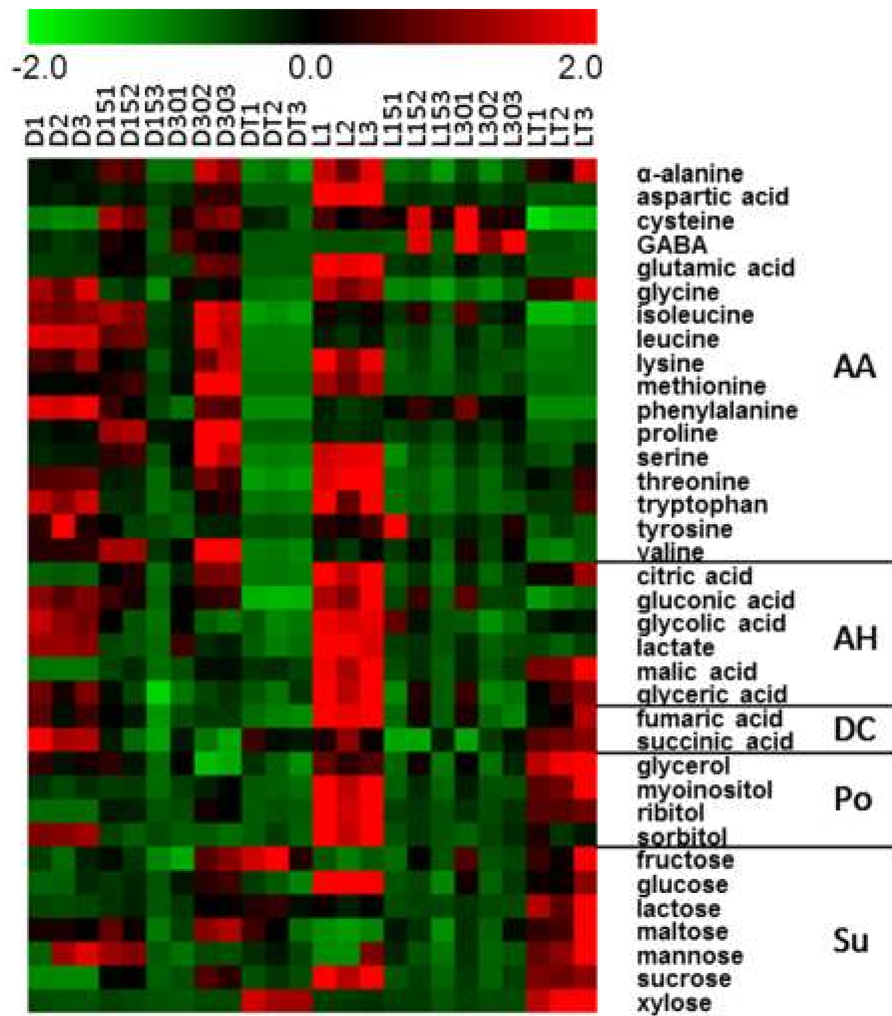


Fig. 2 Heat map of metabolite level z-scores. D = *Umbilicaria decussata* photobiont, L = *Usnea lambii* photobiont. Indices show the duration of desiccation (30 minutes, 15 minutes, and T = end of process, and the replicate numbers 1-3). Metabolites are classified as amino acids (AA), alpha-hydroxic acids (AH), dicarboxylic acids (DC), polyols (Po), and sugars (Su)

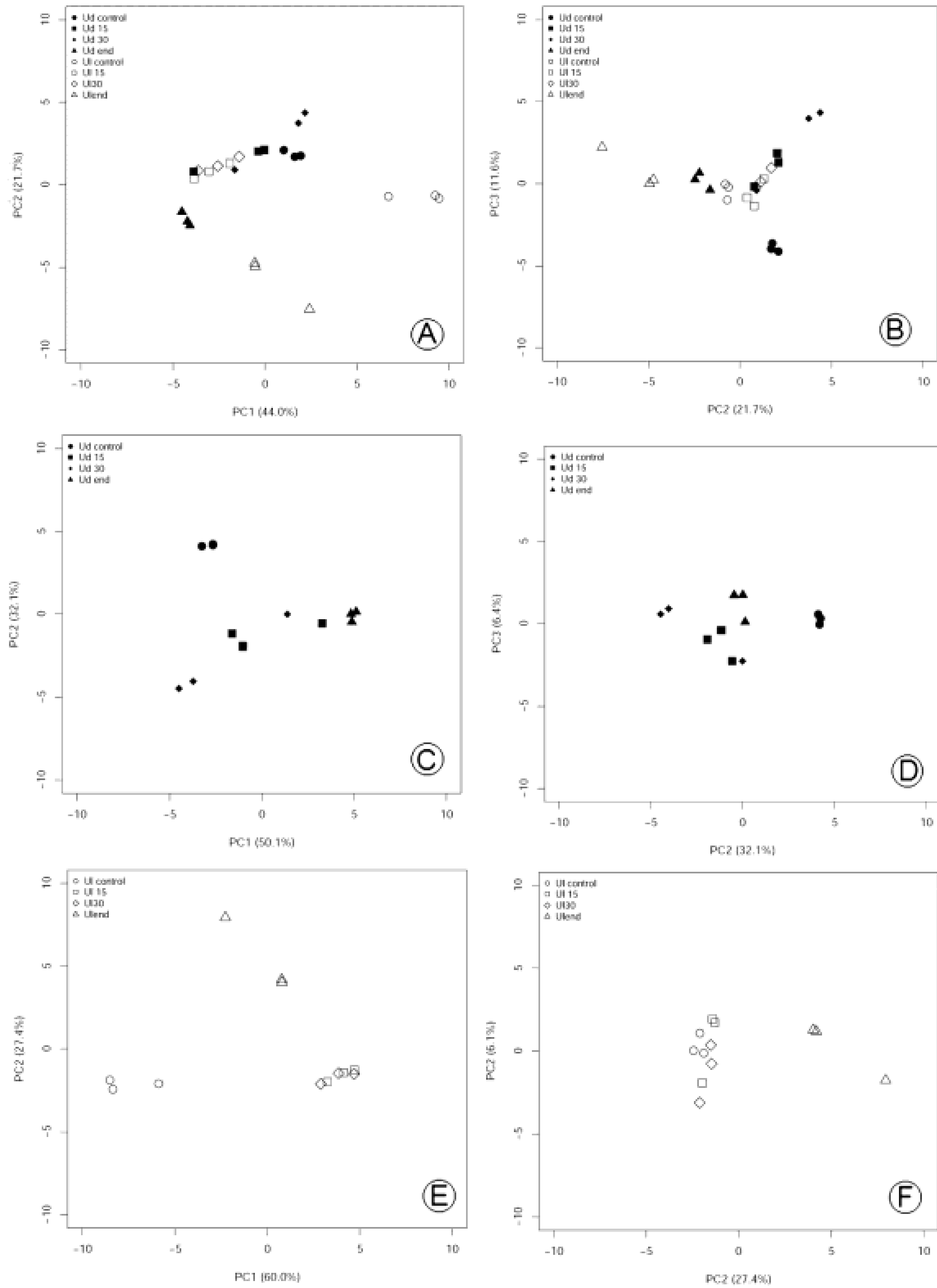


Fig.3 Principal component analysis of metabolite level z-scores. Principal components (PC) 1, 2 and 3 form the dimensions of coordinate systems.

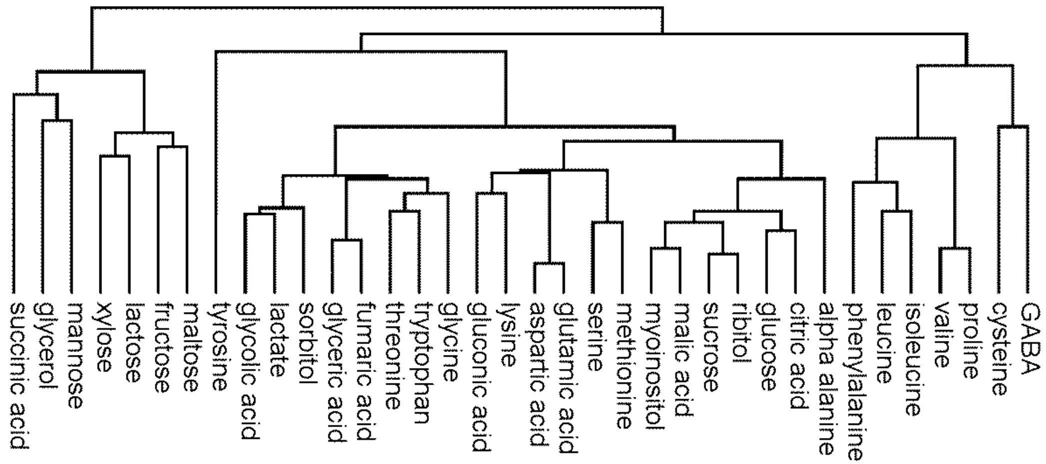


Fig. 4 Hierarchical clustering of z-score normalised metabolite levels (Euclidean distance, average linkage).

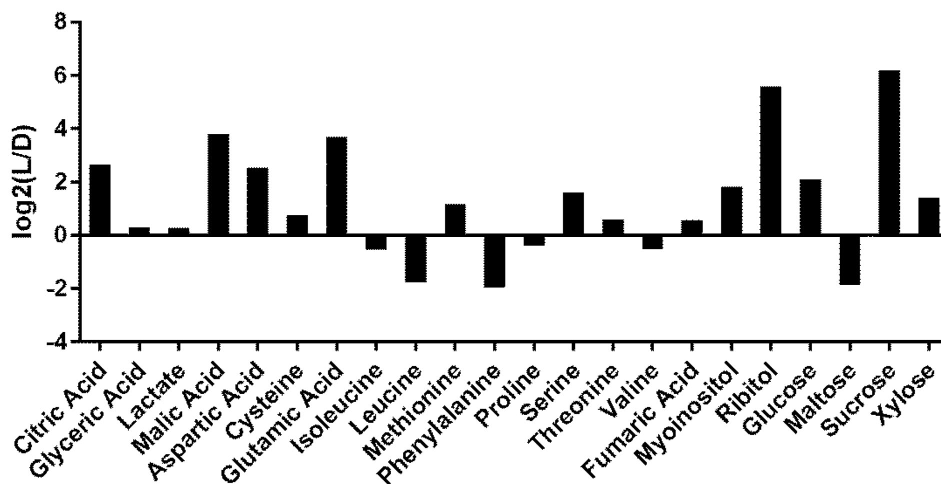


Fig. 5 Significant differences (two-sided unpaired t-test, $\alpha = 0.05$, Benjamini-Hochberg corrected) between metabolite levels of the two investigated photobionts. Values are presented as log2 of the ratio. Positive values indicate a higher concentration in the *Usnea lambii* photobiont.

Table 1 Relative concentrations of metabolites in the *Umbilicaria decussata* photobiont under different experimental conditions. Values are presented as means ± standard error of the mean.

	Umbilicaria decussata **photobiont**			
compound	**control**	**15 min**	**30 min**	**end**
alpha hydroxy acids				
Glyceric Acid	1.6E+3 ± 9.8E+1	9.6E+2 ± 1.9E+2	1.0E+3 ± 6.7E+1	**1.0E+3** ± 5.6E+1
Citric Acid	1.4E+4 ± 7.4E+2	2.9E+4 ± 1.0E+4	5.1E+4 ± 1.2E+4	**7.1E+3** ± 7.2E+2
Gluconic Acid	1.6E+3 ± 6.2E+1	1.1E+3 ± 1.9E+2	1.3E+3 ± 6.8E+1	**4.4E+2** ± 1.0E+1
Glycolic Acid	3.5E+2 ± 2.1E+1	1.7E+2 ± 3.0E+1	1.6E+2 ± 2.9E+1	**1.3E+2** ± 1.2E+1
Lactate	2.3E+4 ± 3.2E+2	8.2E+3 ± 6.5E+2	1.3E+4 ± 2.0E+3	**6.6E+3** ± 6.3E+2
Malic Acid	7.1E+2 ± 2.7E+1	2.0E+3 ± 5.9E+2	3.0E+3 ± 6.1E+2	**2.6E+3** ± 1.9E+2
amino acids				
alpha Alanine	2.8E+4 ± 8.5E+2	3.3E+4 ± 9.8E+3	4.2E+4 ± 1.4E+4	**1.0E+4** ± 2.2E+3
Aspartic Acid	1.4E+4 ± 1.2E+3	1.1E+4 ± 3.0E+3	2.4E+4 ± 6.7E+3	**2.1E+2** ± 2.3E+1
Cysteine	2.0E+2 ± 4.5E+0	3.8E+2 ± 7.0E+1	4.1E+2 ± 3.2E+1	2.7E+2 ± 2.2E+1
Gaba	1.9E+3 ± 2.9E+2	3.2E+3 ± 1.5E+3	5.2E+3 ± 1.0E+3	**2.4E+2** ± 1.5E+1
Glutamic Acid	3.0E+4 ± 1.4E+3	7.7E+4 ± 2.6E+4	1.4E+5 ± 5.6E+4	**1.0E+2** ± 1.4E+1
Glycine	6.7E+3 ± 6.3E+2	2.0E+3 ± 5.3E+2	3.7E+3 ± 2.5E+2	**1.5E+3** ± 6.4E+1
Isoleucine	2.9E+3 ± 8.1E+1	2.5E+3 ± 6.3E+2	3.0E+3 ± 7.4E+2	**3.9E+2** ± 5.3E+1
Leucine	5.1E+3 ± 4.9E+1	2.7E+3 ± 7.6E+2	3.8E+3 ± 1.1E+3	**7.6E+1** ± 1.2E+1
Lysine	2.8E+3 ± 3.7E+2	1.2E+3 ± 3.5E+2	2.9E+3 ± 8.7E+2	**2.5E+1** ± 7.0E+0
Methionine	1.8E+3 ± 1.1E+2	2.0E+3 ± 6.1E+2	5.2E+3 ± 1.8E+3	**1.8E+1** ± 1.3E+0
Phenylalanine	8.2E+3 ± 7.1E+2	2.8E+3 ± 7.9E+2	3.4E+3 ± 1.4E+3	**2.7E+0** ± 2.0E-01
Proline	2.7E+3 ± 1.8E+2	6.7E+3 ± 2.0E+3	1.1E+4 ± 4.0E+3	**2.0E+2** ± 3.4E+1
Serine	2.9E+3 ± 1.1E+2	4.0E+3 ± 1.2E+3	7.4E+3 ± 2.0E+3	**3.7E+2** ± 5.4E+1
Threonine	3.1E+3 ± 6.6E+1	1.4E+3 ± 3.0E+2	2.6E+3 ± 4.8E+2	**2.5E+2** ± 6.0E+1
Tryptophan	3.5E+3 ± 2.7E+2	6.6E+2 ± 2.5E+2	1.3E+3 ± 4.5E+2	**7.1E+1** ± 3.1E+1
Valine	8.1E+3 ± 9.4E+1	1.0E+4 ± 2.9E+3	1.5E+4 ± 4.3E+3	**1.6E+3** ± 1.6E+2
dicarboxylic acids				
Fumaric Acid	1.9E+3 ± 1.6E+2	1.4E+3 ± 2.4E+2	1.3E+3 ± 2.9E+1	**1.0E+3** ± 4.8E+1
Succinic Acid	3.0E+2 ± 3.3E+1	5.5E+1 ± 3.1E+1	5.0E+1 ± 3.3E+1	**1.4E+2** ± 2.1E+1
polyols				
Glycerol	1.5E+4 ± 7.5E+2	1.3E+4 ± 1.6E+3	1.0E+4 ± 2.2E+3	**1.1E+4** ± 1.0E+3
Myoinositol	2.2E+4 ± 1.6E+3	1.9E+4 ± 3.3E+3	2.7E+4 ± 3.4E+3	**1.4E+4** ± 1.2E+3
Ribitol	1.5E+5 ± 5.9E+3	1.3E+6 ± 3.6E+5	1.8E+6 ± 5.7E+5	**5.2E+5** ± 6.1E+4
Sorbitol	1.6E+3 ± 6.6E+1	2.0E+2 ± 5.1E+1	7.2E+1 ± 3.8E+1	**1.7E+2** ± 7.4E+1
sugars				
Fructose	5.1E+2 ± 5.6E+1	5.1E+2 ± 9.4E+1	7.4E+2 ± 2.6E+2	1.2E+3 ± 1.9E+2
Glucose	1.6E+2 ± 2.8E+1	1.8E+2 ± 2.0E+1	3.2E+2 ± 3.9E+1	1.3E+2 ± 3.2E+1
Lactose	6.2E+1 ± 1.0E+1	1.1E+2 ± 5.6E+1	2.4E+2 ± 6.3E+1	**3.9E+2** ± 6.6E+1
Maltose	1.5E+3 ± 6.0E+1	1.4E+3 ± 4.4E+2	2.0E+3 ± 5.2E+2	1.3E+3 ± 3.5E+2
Mannose	1.3E+2 ± 5.3E+1	1.1E+2 ± 4.1E+1	3.3E+1 ± 6.9E+0	5.6E+1 ± 6.4E+0
Sucrose	3.5E+5 ± 2.8E+4	7.4E+6 ± 2.1E+6	1.1E+7 ± 3.3E+6	**5.0E+6** ± 6.6E+5
Xylose	1.3E+2 ± 2.4E+1	2.0E+2 ± 5.3E+1	1.6E+2 ± 4.3E+1	**2.6E+3** ± 1.5E+2

Table 2 Relative concentrations of metabolites in the *Usnea lambii* photobiont under different experimental conditions. Values are presented as means ± standard error of the mean.

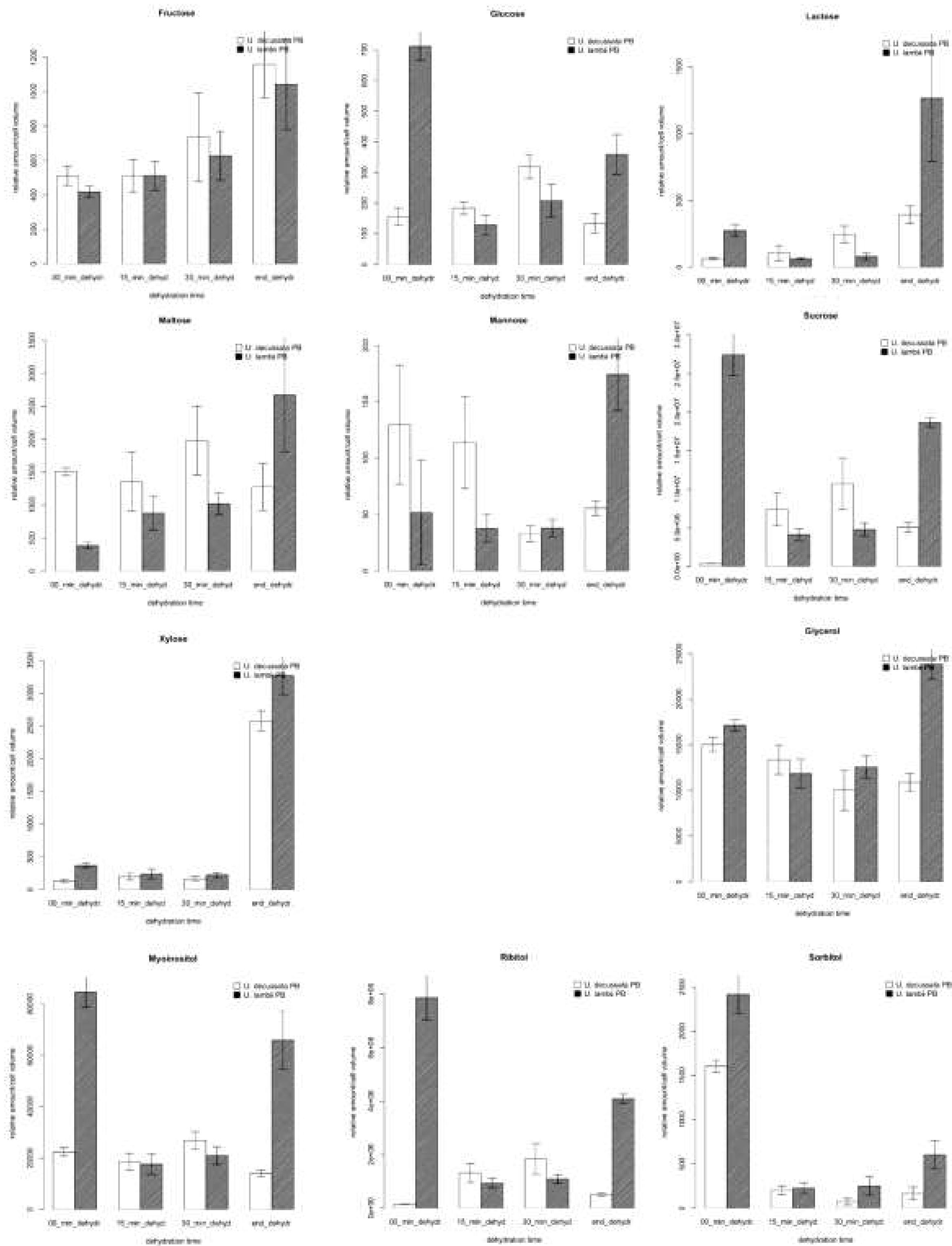
compound	*Usnea lambii* photobiont control			15 min			30 min			end		
alpha hydroxy acids												
Glyceric Acid	1.9E+3	±	2.4E+2	1.1E+3	±	1.6E+2	1.1E+3	±	1.9E+2	1.6E+3	±	8.6E+1
Citric Acid	8.0E+4	±	1.7E+4	1.5E+4	±	4.4E+3	2.0E+4	±	4.3E+3	**5.5E+4**	±	1.2E+4
Gluconic Acid	1.6E+3	±	4.3E+2	1.0E+3	±	1.8E+2	1.1E+3	±	1.9E+2	7.3E+2	±	5.2E+1
Glycolic Acid	3.6E+2	±	6.5E+1	2.2E+2	±	4.4E+1	1.3E+2	±	1.4E+1	**2.1E+2**	±	3.3E+0
Lactate	2.5E+4	±	5.9E+3	9.4E+3	±	6.4E+2	9.1E+3	±	1.1E+3	**8.6E+3**	±	9.2E+2
Malic Acid	1.0E+4	±	1.1E+3	2.0E+3	±	4.2E+2	2.4E+3	±	4.4E+2	1.0E+4	±	1.9E+3
amino acids												
alpha Alanine	5.3E+4	±	7.5E+3	1.2E+4	±	2.9E+3	1.6E+4	±	3.3E+3	4.8E+4	±	1.3E+4
Aspartic Acid	6.8E+4	±	2.7E+4	7.1E+3	±	1.1E+3	9.5E+3	±	1.8E+3	**1.4E+3**	±	3.3E+2
Cysteine	3.0E+2	±	7.4E+1	4.1E+2	±	6.4E+1	4.2E+2	±	7.2E+1	**1.4E+2**	±	2.2E+0
Gaba	6.8E+2	±	6.3E+1	4.6E+3	±	4.3E+3	1.4E+4	±	2.6E+3	3.2E+2	±	2.6E+2
Glutamic Acid	3.1E+5	±	1.2E+5	3.3E+4	±	1.0E+4	5.3E+4	±	1.3E+4	**1.1E+3**	±	3.9E+2
Glycine	6.0E+3	±	6.2E+2	1.0E+3	±	2.1E+2	1.6E+3	±	2.7E+2	6.3E+3	±	1.2E+3
Isoleucine	1.4E+3	±	5.0E+2	1.5E+3	±	3.3E+2	1.9E+3	±	3.4E+2	**2.6E+2**	±	9.7E+1
Leucine	1.1E+3	±	4.0E+2	7.7E+2	±	2.0E+2	1.1E+3	±	2.3E+2	**1.9E+2**	±	1.2E+2
Lysine	3.5E+3	±	1.4E+3	4.5E+2	±	1.4E+2	7.1E+2	±	1.4E+2	**1.5E+2**	±	4.1E+1
Methionine	3.2E+3	±	1.2E+3	3.5E+2	±	6.9E+1	6.5E+2	±	1.5E+2	**3.8E+1**	±	1.2E+1
Phenylalanine	1.5E+3	±	5.9E+2	3.1E+3	±	4.8E+2	3.6E+3	±	8.4E+2	**4.7E+0**	±	6.1E-01
Proline	1.5E+3	±	5.4E+2	1.3E+3	±	2.6E+2	2.3E+3	±	4.1E+2	**2.8E+2**	±	1.8E+2
Serine	7.6E+3	±	2.2E+3	9.2E+2	±	5.0E+2	2.2E+3	±	3.7E+2	**2.2E+3**	±	6.7E+2
Threonine	4.3E+3	±	9.2E+2	8.0E+2	±	1.0E+2	1.2E+3	±	1.8E+2	**2.2E+3**	±	4.9E+2
Tryptophan	3.1E+3	±	1.0E+3	1.9E+2	±	1.0E+2	3.7E+2	±	1.1E+2	1.6E+3	±	6.5E+2
Valine	4.4E+3	±	1.1E+3	4.3E+3	±	1.1E+3	5.9E+3	±	1.1E+3	**2.1E+3**	±	6.0E+2
dicarboxylic acids												
Fumaric Acid	2.7E+3	±	4.2E+2	1.4E+3	±	2.0E+2	1.3E+3	±	2.5E+2	2.2E+3	±	3.5E+2
Succinic Acid	1.9E+2	±	2.7E+1	3.6E+1	±	3.6E+1	6.9E+1	±	3.4E+1	2.3E+2	±	8.4E+0
polyols												
Glycerol	1.8E+4	±	1.3E+3	1.2E+4	±	1.6E+3	1.3E+4	±	1.2E+3	2.5E+4	±	1.5E+3
Myoinositol	7.6E+4	±	1.1E+4	1.8E+4	±	3.9E+3	2.1E+4	±	3.5E+3	7.3E+4	±	1.2E+4
Ribitol	7.9E+6	±	8.3E+5	9.4E+5	±	1.6E+5	1.1E+6	±	1.7E+5	4.1E+6	±	1.7E+5
Sorbitol	2.0E+3	±	4.7E+2	2.3E+2	±	6.1E+1	2.5E+2	±	1.1E+2	**4.6E+2**	±	8.1E+1
sugars												
Fructose	5.2E+2	±	1.3E+2	5.1E+2	±	8.5E+1	6.3E+2	±	1.4E+2	1.1E+3	±	3.4E+2
Glucose	6.1E+2	±	1.3E+2	1.3E+2	±	3.1E+1	2.1E+2	±	5.3E+1	**3.9E+2**	±	8.0E+1
Lactose	4.5E+2	±	2.0E+2	6.2E+1	±	7.7E+0	7.8E+1	±	2.7E+1	1.4E+3	±	6.4E+2
Maltose	7.3E+2	±	4.0E+2	8.8E+2	±	2.6E+2	1.0E+3	±	1.7E+2	3.1E+3	±	1.0E+3
Mannose	7.2E+1	±	4.4E+1	3.8E+1	±	1.2E+1	3.8E+1	±	7.4E+0	2.0E+2	±	3.3E+1
Sucrose	2.7E+7	±	2.7E+6	4.1E+6	±	7.5E+5	4.8E+6	±	8.5E+5	1.9E+7	±	5.7E+5
Xylose	9.5E+2	±	6.8E+2	2.4E+2	±	7.5E+1	2.2E+2	±	3.4E+1	**3.5E+3**	±	8.8E+1

Table 3 Loadings of principal components of z-score normalized metabolite levels. Values are given as percentages.

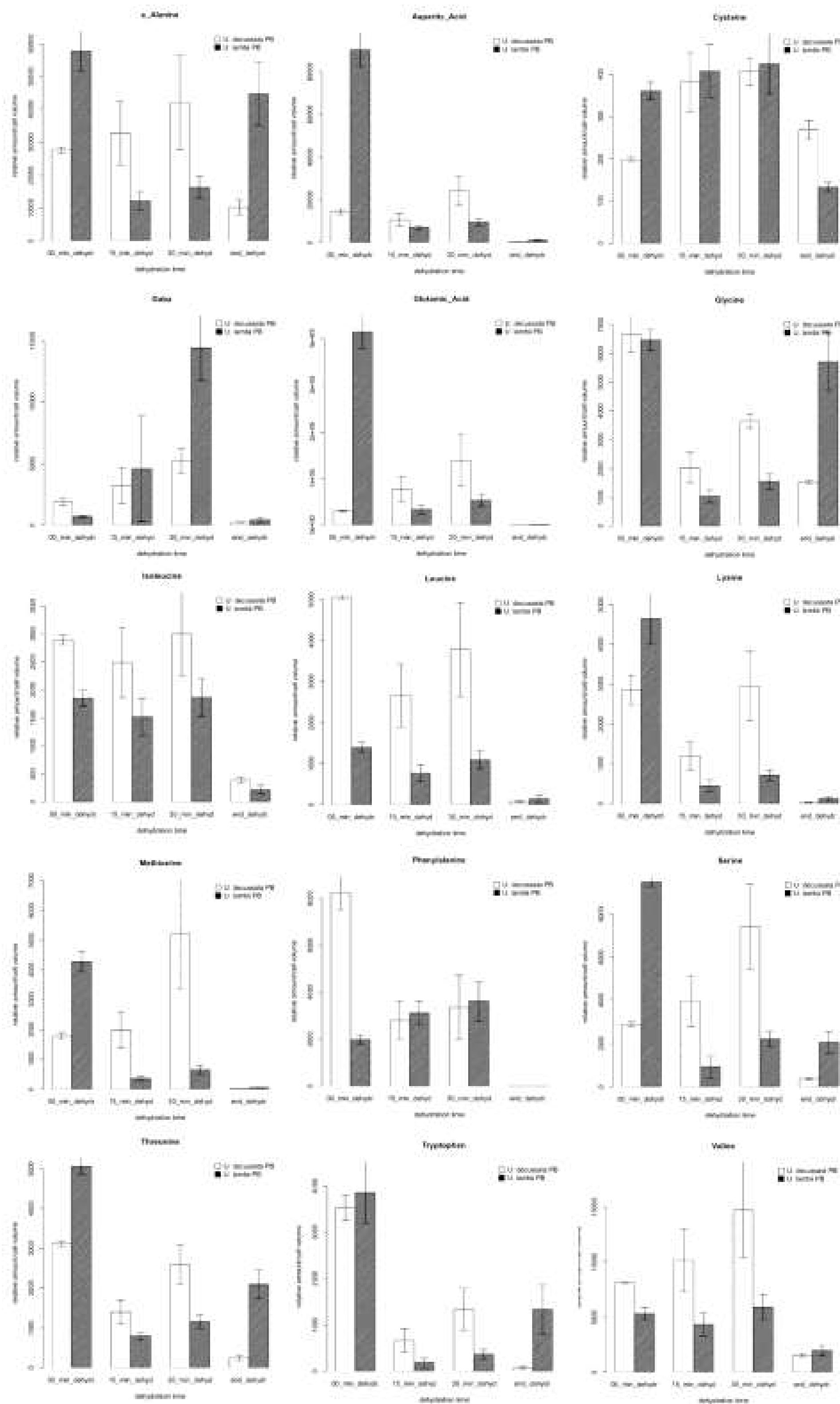
	total			*Umb. dec.* PB			*Usn. lam.* PB		
compound	**PC1**	**PC2**	**PC3**	**PC1**	**PC2**	**PC3**	**PC1**	**PC2**	**PC3**
alpha hydroxy acids									
Glyceric Acid	4.13	1.71	1.87	2.32	3.00	5.56	4.18	0.38	3.80
Citric Acid	4.25	0.94	3.73	2.49	3.43	0.44	4.52	0.53	1.30
Gluconic Acid	3.85	3.28	1.60	4.65	1.66	3.22	3.13	3.74	4.62
Glycolic Acid	3.72	0.03	5.16	2.47	4.90	1.06	3.72	1.62	3.65
Lactate	4.03	1.01	4.19	2.71	3.96	1.35	4.13	2.72	1.04
Malic Acid	3.41	4.27	2.64	0.03	1.76	2.39	4.13	3.02	0.83
amino acids									
a Alanine	4.07	0.56	3.77	4.03	2.88	2.07	4.05	2.28	1.91
Aspartic Acid	4.27	0.91	0.27	1.73	0.92	0.18	4.72	3.59	0.98
Cysteine	0.25	4.38	4.19	1.36	4.72	0.98	0.57	5.64	8.43
Gaba	1.03	2.82	1.70	1.25	1.07	3.97	2.55	3.07	12.96
Glutamic Acid	4.14	1.29	1.73	1.93	2.43	0.52	4.52	3.63	0.42
Glycine	3.80	1.77	2.52	3.38	3.83	1.33	3.88	3.05	0.57
Isoleucine	1.72	5.94	0.99	5.96	1.09	1.23	0.35	3.49	4.98
Leucine	1.58	4.93	0.94	6.04	1.11	0.63	0.48	1.33	1.86
Lysine	4.14	2.88	0.19	4.25	0.12	2.03	3.98	3.17	0.11
Methionine	3.22	3.72	3.83	4.60	4.44	2.03	2.91	2.38	0.31
Phenylalanine	0.97	4.84	3.60	5.61	3.94	2.95	0.79	2.83	4.26
Proline	0.93	4.64	5.55	4.79	5.89	0.08	0.10	0.99	1.57
Serine	4.02	2.25	3.41	3.97	3.75	0.85	3.99	1.93	0.63
Threonine	4.64	0.55	0.78	4.15	0.97	0.07	4.52	0.61	0.46
Tryptophan	4.25	0.40	2.77	3.92	3.51	3.43	4.20	0.24	1.15
Tyrosine	1.04	2.08	4.89	2.73	4.23	1.48	0.21	2.17	3.90
Valine	1.32	5.21	4.13	5.61	4.23	0.48	0.19	1.64	3.66
dicarboxylic acids									
Fumaric Acid	4.37	1.58	1.05	2.40	1.95	0.95	4.49	0.30	3.14
Succinic Acid	1.65	2.76	5.66	0.98	6.74	6.64	1.96	3.15	3.79
polyols									
Glycerol	1.97	4.99	1.03	0.50	2.89	2.97	2.11	5.57	3.00
Myoinositol	3.96	3.54	1.32	1.10	0.41	0.82	4.43	2.49	1.18
Ribitol	4.01	2.43	2.05	0.78	1.97	0.16	4.61	0.44	1.37
Sorbitol	3.98	0.58	4.49	1.84	4.59	2.23	4.32	1.60	2.03
sugars									
Fructose	0.77	3.14	4.73	1.61	2.67	19.73	0.15	4.69	7.40
Glucose	4.24	1.56	2.25	1.36	1.94	0.73	4.79	0.05	1.68
Lactose	0.78	5.52	3.26	0.70	1.13	5.21	1.34	7.47	3.60
Maltose	0.02	1.99	4.01	2.38	1.89	8.15	0.42	6.69	5.22
Mannose	0.85	2.65	0.42	1.81	2.28	1.36	0.81	5.60	2.08
Sucrose	3.73	2.71	3.63	0.78	3.40	1.59	4.39	1.51	1.26
Xylose	0.92	6.14	1.65	3.77	0.28	11.14	0.36	6.39	0.85

Table 4 Results of two factor ANOVA of metabolome data, presented as p-values. The Benjamini-Hochberg corrected q defines the threshold for p values considered significant (H_0: no significant effect of the factor), ns = not significant.

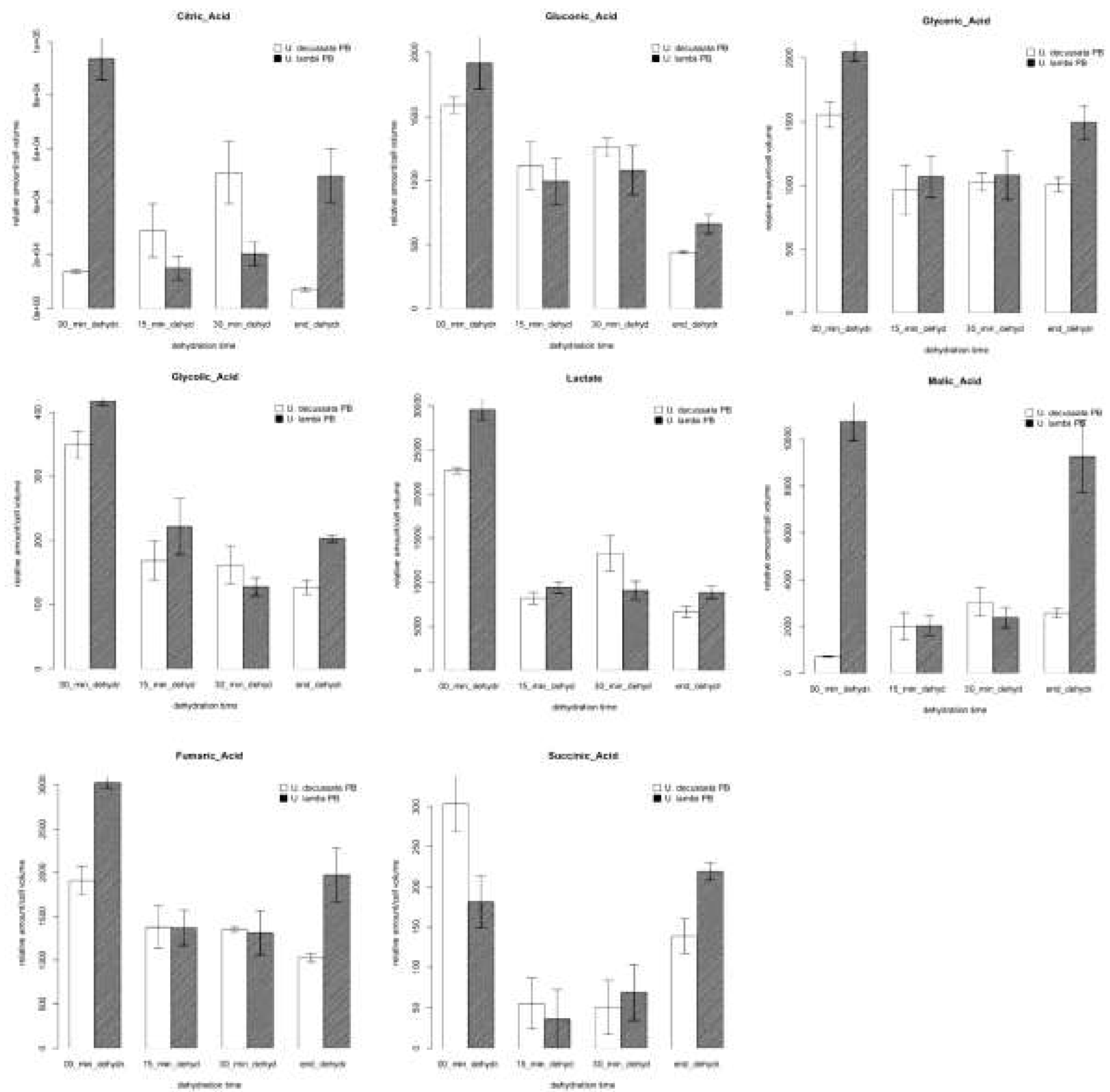
	p-values of factor effect		
compound	**interaction**	**photobiont**	**treatment**
alpha hydroxy acids			
citric acid	4.4E-06	2.0E-03	3.9E-03
gluconic acid	ns	ns	3.3E-06
glyceric acid	ns	7.9E-03	7.0E-05
glycolic acid	ns	ns	7.3E-08
lactate	5.7E-04	ns	9.5E-12
malic acid	2.3E-06	7.3E-07	5.4E-05
amino acids			
α alanine	9.5E-04	ns	ns
aspartic acid	1.1E-08	7.5E-05	2.7E-09
cysteine	ns	ns	5.7E-04
GABA	ns	ns	5.7E-04
glutamic acid	2.9E-07	3.0E-03	1.9E-06
glycine	5.9E-05	ns	1.7E-07
isoleucine	ns	8.1E-03	1.4E-04
leucine	ns	2.9E-05	9.5E-05
lysine	2.0E-03	ns	1.4E-06
methionine	9.7E-04	ns	1.2E-03
phenylalanine	4.7E-04	ns	2.1E-05
serine	1.9E-05	ns	1.1E-04
threonine	7.2E-06	ns	8.3E-09
tryptophan	ns	ns	3.3E-07
valine	ns	6.7E-03	3.8E-03
dicarboxylic acids			
fumaric acid	ns	1.9E-03	5.1E-05
succinic acid	ns	ns	1.2E-05
polyols			
glycerol	7.4E-04	1.1E-03	1.7E-03
myoinositol	5.2E-06	1.9E-06	1.7E-05
ribitol	2.0E-08	9.2E-08	7.2E-06
sorbitol		2.8E-04	2.4E-11
sugars			
maltose	ns	ns	ns
fructose	ns	ns	5.0E-03
glucose	1.8E-06	8.8E-05	5.7E-05
lactose	ns	ns	1.7E-03
mannose	ns	ns	ns
sucrose	1.6E-07	1.1E-05	1.1E-03
xylose	ns	9.0E-03	1.4E-13
q	0.034	0.024	0.046
% significant	50.00	47.06	91.18



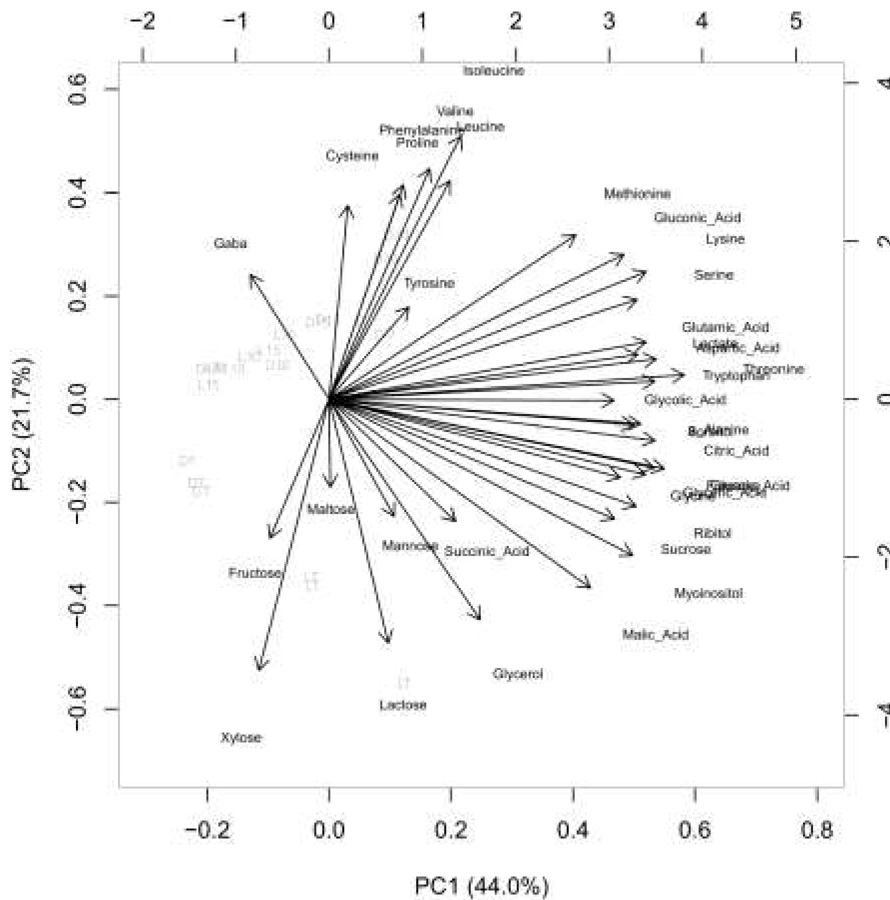
Supplemental figure 1 Relative metabolite levels of sugars and polyols. Values are presented as means ± standard error of the mean.



Supplemental figure 2 Relative metabolite levels of amino acids. Values are presented as means ± standard error of the mean.



Supplemental figure 3 relative metabolite levels of carboxylic acids. Values are presented as means ± standard error of the mean.



Supplemental figure 4 Biplot of principal component analysis. Contribution of variables (metabolites) to principal components (PC) 1 and 2. Black arrows and compound names indicate the vector of the variable's influence, reduced to two dimensions. Grey scripture indicates the position of samples in the two-dimensional space. Percentages associated with the PCs indicate the proportion of data variance explained by the respective PC.

Supplemental table 1: Primer sequences used for marker PCR.

locus	name	sequence (5'-3')	direction	literature	annealing
actin1	actin	AGCGCGGGTACAGCTTCAC CAGCACTTCAGGGCAGCGGAA	forward reverse	Skaloud and Peksa 2010	61°C
Internal transcr. spacer (rDNA)	ITS	GTWGTWCCAGTATTRGACAT AACCRAATCCANAYAAACAA	forward reverse	Werth and Sork 2008	54°C
rubisCO, large SU	rbcL	GAATCWTCWACWGGWACTTGGACWAC CCTTCTARTTTACCWACAAC	forward reverse	Nelsen *et al.* 2011	54°C

Supplemental table 2: PCR programme.

step	duration	temperature	comments
initial activation	5 min	95°C	
denaturation	1 min	95°C	35 cy-cles
annealing	1 min	See table 1	
extension	1 min	72°C	
final extension	7 min	72°C	

Supplemental table 3 Principal component analysis of metabolome data z-scores.

	Proportion of variance
PC1	0.4395
PC2	0.2173
PC3	0.1162
PC4	0.1036
PC5	0.0396
PC6	0.0241
PC7	0.0174
PC8	0.0124
PC9	0.0080
PC10	0.0067
PC11	0.0038
PC12	0.0033
PC13	0.0022
PC14	0.0017
PC15	0.0014
PC16	0.0011
PC17	0.0006
PC18	0.0005
PC19	0.0003
PC20	0.0001
PC21	0.0001
PC22	0.0001
PC23	0.0001
PC24	<0.0001

5.5 Revealing the molecular mechanisms of desiccation tolerance in lichen algae: transcriptome analysis of two Antarctic lichen photobionts

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Erklärung

Der vorliegende Artikel wurde vollständig durch den Erstautor verfasst.

Revealing the molecular mechanisms of desiccation tolerance in lichen algae: transcriptome analysis of two Antarctic lichen photobionts

Abstract

The lichen symbiosis is a poikilohydric association of a fungus (mycobiont) and a photosynthetic partner (photobiont). Frequent dehydration is common for most lichen species which are therefore desiccation tolerant. Upon dehydration of the lichen thallus, a state of latent life, called anabiosis, is established. Rapid recovery of metabolic activity is obtained by rehydration of the symbiosis. Especially in the harsh Antarctic terrestrial environment, desiccation tolerance can be considered a key prerequisite of the lichens' successful colonisation. Little is known about the desiccation tolerance of isolated lichen photobionts and hence about their contribution to the symbiosis' performance. Especially the molecular basis of desiccation tolerance of Antarctic lichen photobionts is unknown. Two photobionts, originating from an Antarctic endemic and a cosmopolitic lichen, were examined by transcriptome analysis. The transcriptome response as well as the unstressed transcriptomes of the two organisms examined differed significantly, reflecting their differences in stress physiology. Mechanisms of protein and membrane protection as well as a controlled metabolic shutdown are considered major factors of desiccation tolerance. Factors similar to land plants were identified, suggesting ancient adaptations to dry conditions.

Introduction

Lichens are exosymbiotic consortia which consist of at least a heterotrophic fungus, the mycobiont, and a photosynthetic organism, the photobiont. While the former provides relevant structural aspects and relevant secondary substances such as parietin and melanin (Meeßen *et al.* 2013, Sadowsky and Ott 2015), the latter is responsible for carbon nutrition of both partners (Henssen and Jahns 1974). The structure formed in the symbiotic state, the lichen thallus, possesses physiological and anatomical features that one of the aposymbiotic partners display. The lichen physiology is characterised by poikilohydry

that can be described as a key aspect of the lichen symbiosis. Lichens are not able to control actively their water relation and therefore, fully depend on the water regime in their micro environment of their habitat (Larson 1979). Desiccation leads to a state of latent life, called anabiosis (Kranner *et al.* 2008).

Anabiotic lichens are able to survive extremes of abiotic factors which would be lethal to other organisms and also to physiologically active lichens. The symbiosis can be revitalised after treatments such as freezing (Sadowsky and Ott 2015), UV irradiation (Sancho *et al.* 2007, Sánchez *et al.* 2014) or space vacuum exposure (Brandt *et al.* 2015).

Lichens tolerate desiccation to a much higher degree than known for higher plants (Kranner *et al.* 2008) quite likely based on their poikilohydre nature. These symbiotic organisms can resist artificial dehydration to less than 5 % relative humidity, as studied by silica gel dried atmospheres. Lichens are characterised by desiccation tolerance. Desiccation occurs frequently during their life cycle, especially in xeric habitats (Ahmadjian 1965, Kappen 1988). Gradual differences in the degree of desiccation tolerance between lichen species appear generally.

Isolated photobionts as well can survive dehydration, although rapid water loss impairs the reactivation of photosynthesis (Sadowsky and Ott 2012). Lichens and photobionts are able to reactivate their photosynthetic activity rapidly. Photosynthetic recovery mainly occurs within seconds to minutes upon rehydration, which exceeds the reactivation capacity of resurrection plants such as *Craterostigma plantagineum* by far (Bernacchia *et al.* 1996).

Dehydration of cells causes stress on numerous functions (Kranner *et al.* 2008, Leprince and Buitink 2015). Mechanical stress by cell lumen shrinkage can disrupt membranes. Lowered water potential has physiological effects on all intracellular processes. Membranes depend on an aqueous environment to retain their structure. Free and membrane-bound proteins fold according to a hydration envelope. Osmolyte concentration and pH influence protein folding and all metabolic functions.

Cellular mechanisms of desiccation tolerance cover a wide and complex range of different functions. Oxidative stress caused by water loss can be counteracted by enzymatic and non-enzymatic antioxidative systems (Kranner *et al.* 2002, 2008). To prevent water efflux by increasing the osmotic potential, sugars and sugar alcohols (polyols) can be accumu-

lated (Sadowsky *et al.* 2015). These solutes can also directly protect membrane and protein structures (Alpert 2006). Protein-based mechanisms of structural protection can be achieved by different classes of gene products, such as the late embryogenesis abundant (LEA) or early responsive to desiccation (ERD) proteins (Moore *et al.* 2009). Heat shock proteins (Hsp) ensure correct folding of proteins under stress conditions (Buitink *et al.* 2002).

Antarctic terrestrial habitats are characterised by harsh environmental conditions for colonistion. Especially in continental areas, the climate can be described by low temperatures and the water availability is modest (Convey 1996a, b). Low temperatures alone are not a major stressor for lichens in Antarctica, as many of them are cold tolerant and show considerable freezing resistance (Kappen 2000). As a consequence of cold, water becomes less available. The extremely low precipitation especially at higher latitudes makes Antarctica the world's driest continent (Huiskes *et al.* 2006). Water strictly limits plant life at these habitats (Kennedy 1993, Block 1996). Macro vegetation can mostly be found in micro niches which provide favourable conditions (Pointing *et al.* 2015). At extreme habitats, lichens, besides microbial crusts, form the dominant vegetation (Øvstedal and Smith 2001).

Across Antarctica, photobionts of the *Trebouxia* clade S (phylogeny after Helms 2003) dominate in the non-crustose lichens. It has been hypothesised that special adaptions to the extreme Antarctic habitats can be found in this group (Sadowsky and Ott 2012, Romeike *et al.* 2002) Both, the photobionts of *Usnea lambii* and *Umbilicaria decussata* belong to this widespread group. Their stress physiology however differed significantly, especially concerning the desiccation tolerance (Sadowsky and Ott 2012, Sadowsky *et al.* 2015). The *U. lambii* photobiont displayed higher potential stress tolerance, but the underlying molecular mechanisms remain unclear. It can be hypothesised that the transcriptomes of these two photobionts reflect their respective potential on desiccation tolerance.

The genomic information on algae of the genus *Trebouxia* is very limited to date. Sequencing the complete genome of the lichen photobiont *Trebouxia* 'TR-9' has started 2012, but is not yet completed (Bioproject PRNJA82781). The NCBI database, however, yields many fragments of genomic data on *Trebouxia* photobionts, mainly for phylogenetic analyses such as the internal transcribed spacer (ITS) regions. Phylogeny is still a major topic of molecular lichenology, and some lichen families such as the Parmeliaceae are

well described (Thell *et al.* 2012). The photobiont of the genus *Trebouxia* still provides challenges regarding molecular-based phylogeny (Sadowska-Deś *et al.* 2014). Recent ‘omics technologies foster in-depth analysis of processes in lichen thalli such as symbiont interaction (Wang *et al.* 2014) or lichen-associated bacteria (Grube *et al.* 2014). A proteomic study revealed small changes of the protein composition and high constitutive expression of protective proteins in the isolated lichen photobiont *Asterochloris erici* during dehydration (Gasulla *et al.* 2013). Two transcriptome studies with lichens are present to date. The Swedish lichen *Cladonia rangiferina* was subjected to a transcriptome analysis of dehydration (Junttila and Rudd 2012, Junttila *et al.* 2013) and the isolated photobiont *Trebouxia gelatinosa* was analysed (Candotto Carniel 2014).

This study presents the first transcriptome analysis of Antarctic lichen photobionts, which is also the first transcriptomic approach to any Antarctic lichen-related organism. Additionally, a direct comparison of the transcriptomes of two *Trebouxia* lineages was performed for the first time. The results provide novel insights in the functioning of these widespread symbiotic algae and will add to the knowledge on their success in severe environments of Antarctic terrestrial ecosystems.

Material and methods

Lichens and photobionts

Umbilicaria decussata (Vill.) Zahlbr. thalli were collected at Terra Nova Bay, North Victoria Land, continental Antarctica. Thalli of *Usnea lambii* (Imshaug) Wirtz & Lumbsch originate from Coal Nunatak, Alexander Island, Southern maritime Antarctica. The green algal photobionts (genus *Trebouxia* Puymaly) were isolated from thallus fragments after Yoshimura *et al.* (2002) and cultivated in *Trebouxia* organic medium (TOM) with 1 % (w/v) glucose (Ahmadjian 1967). Cultures were kept at low light intensity (20 µmol photons m^{-2} s^{-1}; diurnal cycle with 10 h of darkness) and 12 °C in a growth chamber (Rubarth Apparate GmbH, Germany). Four weeks old cultures were sampled by vacuum filtration on sterile, RNase free glass fiber filter discs.

Stress treatment and RNA extraction

Freshly harvested photobionts were immediately frozen in liquid nitrogen to stop metabolic processes for control samples. Desiccation treatment was performed by placing the freshly harvested photobionts in a desiccator at less than 5 % relative air humidity obtained by silica gel. In the dark, dehydration was performed for 15 minutes. Metabolism of the photobionts examined did not cease completely after this treatment (Figure 1 *Chlorophyll* **fluorescence parameters of the *Umbilicaria decussata* (A) and *Usnea lambii* photobiont (B) during the dehydration treatment. Sampling time (15 minutes) is indicated by grey circles.** Afterwards, the treated samples were immediately frozen in liquid nitrogen. From this point, the samples were handled with sterile, RNase-free instruments only. Frozen photobionts were transferred to plastic reaction tubes for total RNA isolation. The NucleoSpin® RNA Plant mini kit (Macherey-Nagel) was used. The subsequent DNase digestion was perfomed on the coloumns with RNase-free DNase I (New England Biolabs). The quality and purity of the obtained total RNA samples was checked by photometry (NanoDrop® ND-1000, Thermo Scientific) and miniature gel electrophoresis (Bioanalyzer®, Agilent Technologies).

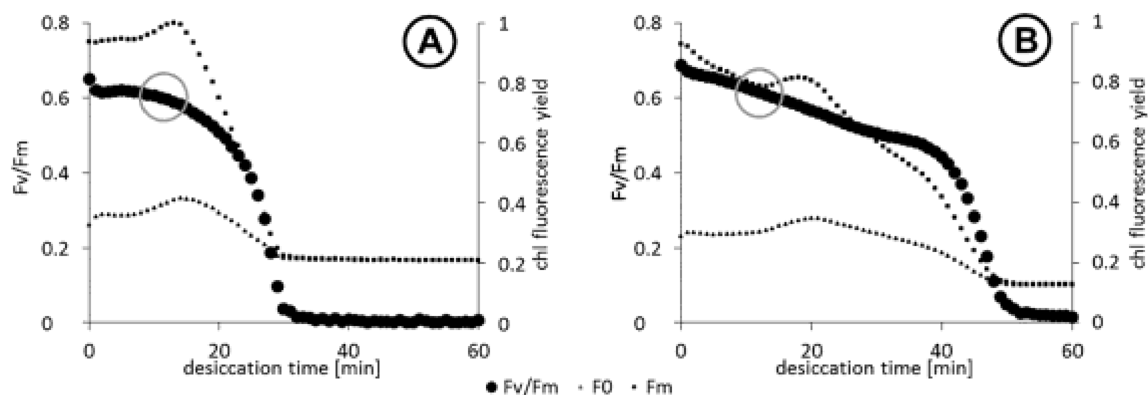

Figure 1 Chlorophyll fluorescence parameters of the *Umbilicaria decussata* (A) and *Usnea lambii* photobiont (B) during the dehydration treatment. Sampling time (15 minutes) is indicated by grey circles.

Construction and sequencing of cDNA libraries

Purification of mRNA, reverse transcription and purification of obtained cDNA libraries was performed with the TruSeq™ RNA Sample Prep Kit v2 (Illumina). The normalised libraries were sequenced on an Illumina HiSeq2000 platform. Paired-end reads of 100 bp length were obtained.

Assembly and mapping of the transcriptomes

The *Illumina* reads were cleaned from adapter sequences and assembled to contigs by the Trinity software suite (http://trinityrnaseq.sourceforge.net/). For BLAST search and functional annotation of the contigs, the Blast2GO suite (Conesa *et al.* 2005) was used. Heterologous mapping of reads for quantification of expression levels was performed based on the transcriptome of *Coccomyxa subellipsoidea* (Blanc *et al.* 2012). Transcript abundance was calculated as reads per million reads (RPM). Statistical analysis was performed in R statistics software (version 3.1.2, http://www.R-project.org) using the edgeR package after Robinson and Smyth 2007, 2008, Robinson *et al.* 2010. McCarthy *et al.* 2012, Zhou and Robinson 2014. Visualisation was performed in R and MS Excel.

Results and discussion

Characterisation of the transcriptome

cDNA libraries were successfully synthesised, sequenced and assembled for both photobionts. The transcriptome of the *Umbilicaria decussata* photobiont (PBD) had a total length of 43,581,831 bp with 50.65 % GC content, distributed over 55,990 contigs with an average length of 778 bp. 75% of the total sequence length was contained in 37.3% of the contigs. The total length of the *Usnea lambii* photobiont (PBL) transcriptome was 47,114,394 bp (GC content 50.44 %), distributed over 64,655 contigs with an average length of 665 bp (Figure 2 A). In 40.5% of these contigs, 75% of the total sequence length was represented. The transcriptome sizes are within the range of known genome sizes for the *Trebouxiophyceae*. The free-living algae *Chlorella variabilis* and *Coccomyxa subellipsoidea* possess genomes of the size of 46.2 and 49.0 Mbp, respectively (Blanc *et al.* 2010, 2012). A larger genome is represented in the *Asterochloris* photobiont of *Cladonia grayi* (106.7 Mbp estimated by QPCR, Armaleo and May 2009). A smaller genome was found in the free-living alga *Nannochloris* sp. (12.6Mbp, Yamamoto *et al.* 2001).

BLAST search of contig sequences revealed that in most cases, highest sequence similarities were obtained with the published sequenced genome of the Antarctic green alga *Coccomyxa subellipsoidea* C-169 (portion of BLAST top hits in PBD: 47.4%; PBL: 42.1%, Figure 2 A, B). This was followed by *Chlorella variabilis* (PBD: 12.8%, PBL: 11.3%). BLAST hits were also obtained with phylogenetically more distant organisms such as the

model bryophyte *Physcomitrella patens* (PBD: 0.6%, PBL: 0.9%) and *Arabidopsis thaliana* (PBD: 0.8%, PBL: 11.3%). *Trebouxia* sequences are much less abundant in the NCBI database, and therefore less than 1% of the top BLAST hits were obtained with *Trebouxia* sequences (*T. aggregata*). A considerable proportion of contigs could be aligned to *Volvox carteri* sequences, especially encoding for proteins of and associated with the flagellar complex. Algae of the genus *Trebouxia* are capable of releasing flagellate zoospores, which does not occur in the symbiotic state (Slocum *et al.* 1980).

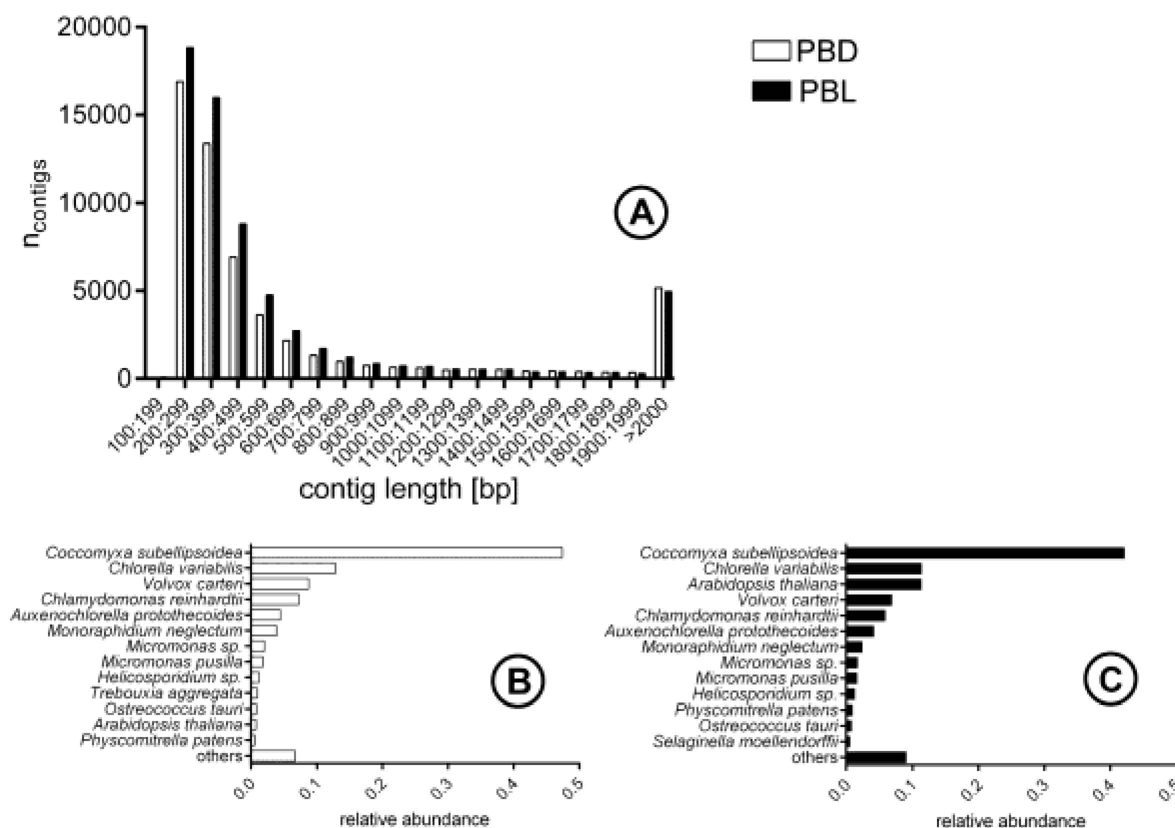

Figure 2 Properties of the assembled transcriptomes of the *Umbilicaria decussata* (PBD) and *Usnea lambii* (PBL) photobionts. A, B: species distribution of best BLAST hits for the PBD and PBL contigs, respectively. C: distribution of contig length classes

As reported by Beck *et al.* (2015), three candidate genes for ancient horizontal gene transfer (HGT) from fungi to algae are present in the lichen photobiont *Trebouxia decolorans*. One of these genes is an oxidoreductase. A contig (# 20547, 211 bp long) of the PBD transcriptome was identified with 88% sequence identity to the oxidoreductase of the ascomycete *Cladophialophora carrionii* (syn. *Cladosporium carrionii*) with an, the e-value of 0.68 due to low query cover. The identification as *Coccomyxa subellipsoidea* homo-

logue (e-value 4.18^{-6}) is more reliable. Therefore, no evidence for HGT as found in *T. decolorans* has been found in the examined transcriptomes of the *T. jamesii* photobionts.

Quantification of transcripts

Assignment and quantification of the reads was performed by heterologous mapping to the published transcriptome of *Coccomyxa subellipsoidea* C-169. Of the 5,915 genes mapped, 1,636 experienced differential expression upon desiccation in the PBD. In the PBL, 1.071 genes were differentially transcribed and 5,917 genes were mapped. Taking into account that c. 1,000 and c. 450 genes were exclusively differentially transcribed in one of the respective photobionts (Figure 3 B), a total of c. 2,000 transcriptionally regulated genes involved in the stress response has to be considered. Few transcripts were only found in one of the respective photobionts (110 in the PBD and 112 in the PBL, Figure 3 A), but only few reads were obtained from those. Therefore, these differences between the photobionts are considered negligible. Principal component analysis shows that the sample sets form distinct clusters (Figure 4).

Under control conditions, the transcript abundance differed significantly between the PBD and the PBL concerning 2196 genes (Figure 3 B). This comprises about 36.4 % of the 6028 mapped transcript sequences. Due to this, the transcriptomic investment of the two photobionts examined displayed different patterns (Figure 5). The differences were consistently significant in several functional categories, including effector and regulator genes. Most of these genes could be assigned to functions of RNA signalling, DNA synthesis, DNA repair and protein degradation. Significant effects of the treatment could also be observed in a variety of functional categories in primary as well as stress metabolism (Figure 5, Table 4).

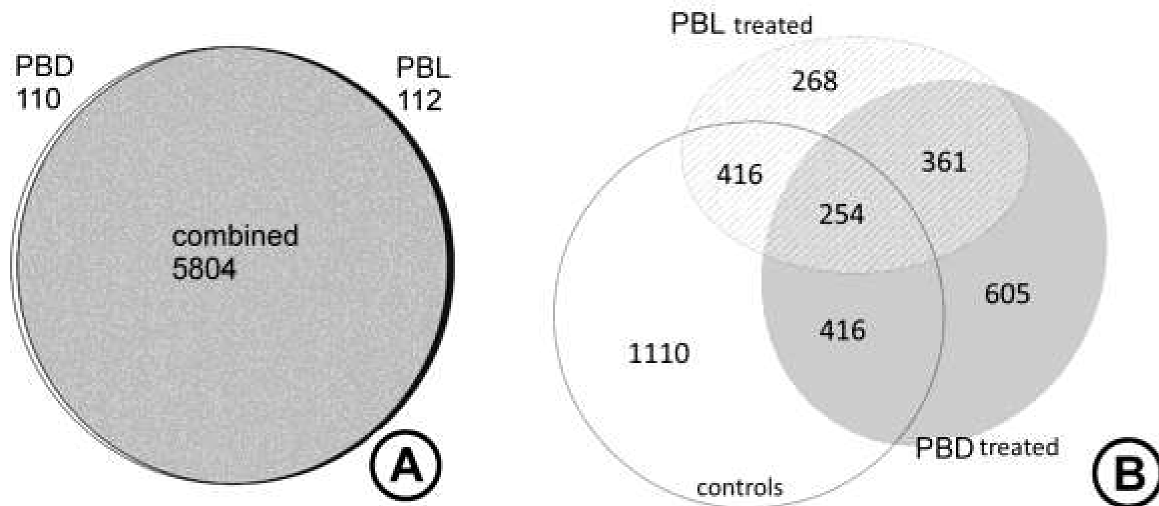

Figure 3 Venn diagrams of mapped transcript identities for the *Umbilicaria decussata* (PBD) and *Usnea lambii* (PBL) photobiont. A: total genes identified, B: significantly different expression levels.

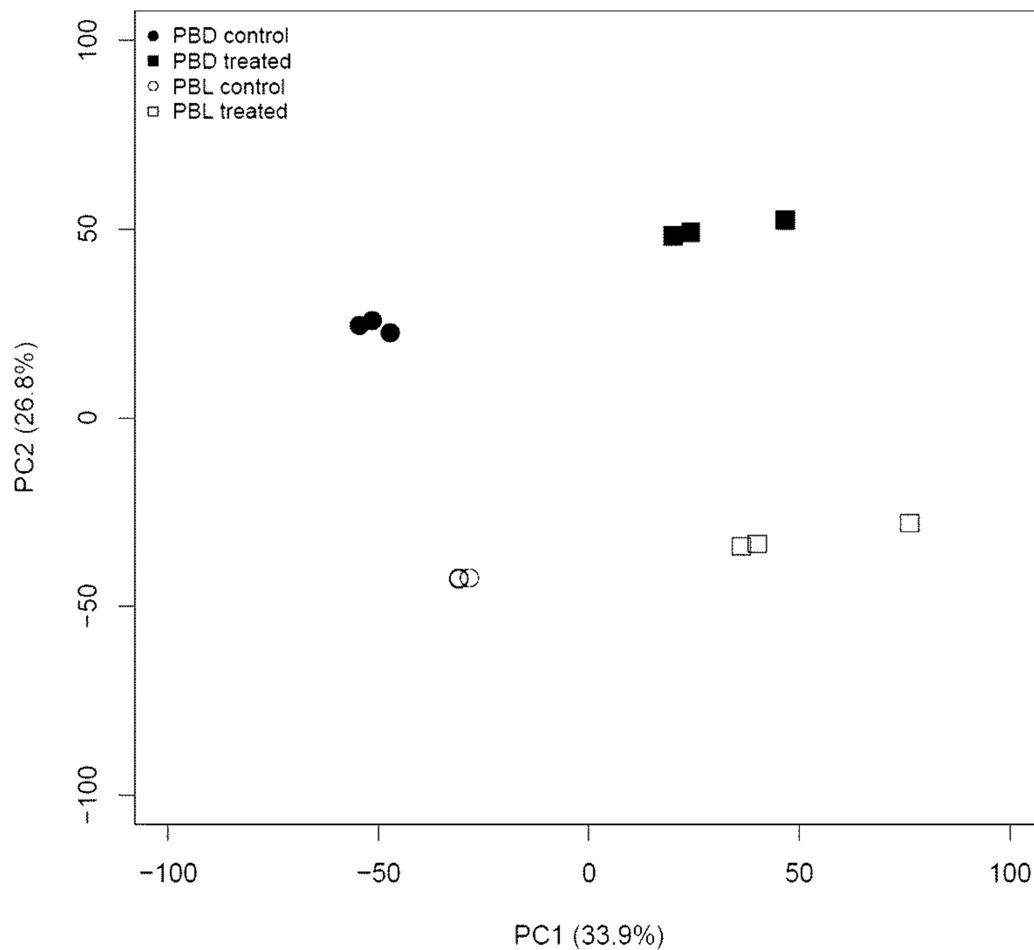

Figure 4 Two-dimensional visualisation of principal component analysis. Samples are represented as points. Principal components (PC) 1 and 2 are the three axes of the coordinate system.

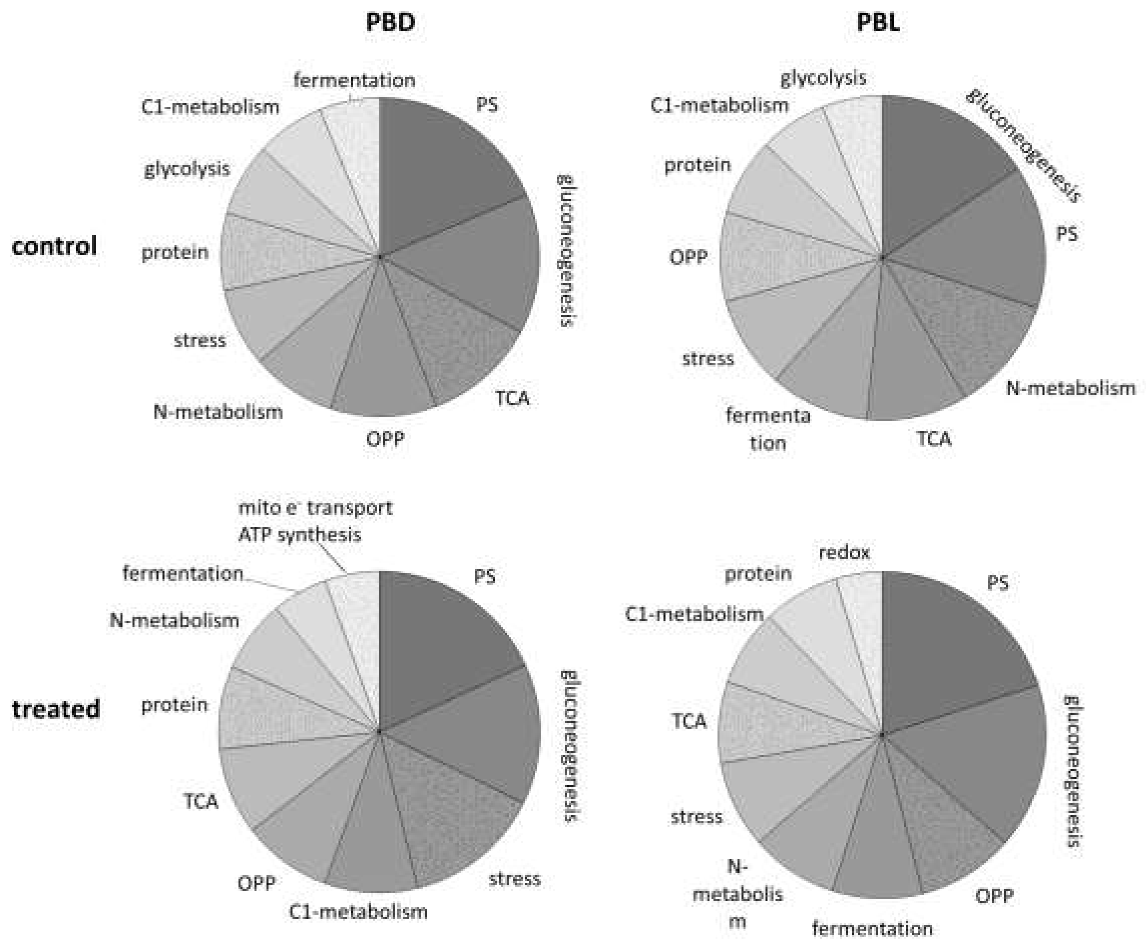

Figure 5 Transcriptomic investment in the control and treated state. The ten categories with highest RPM per gene are presented.

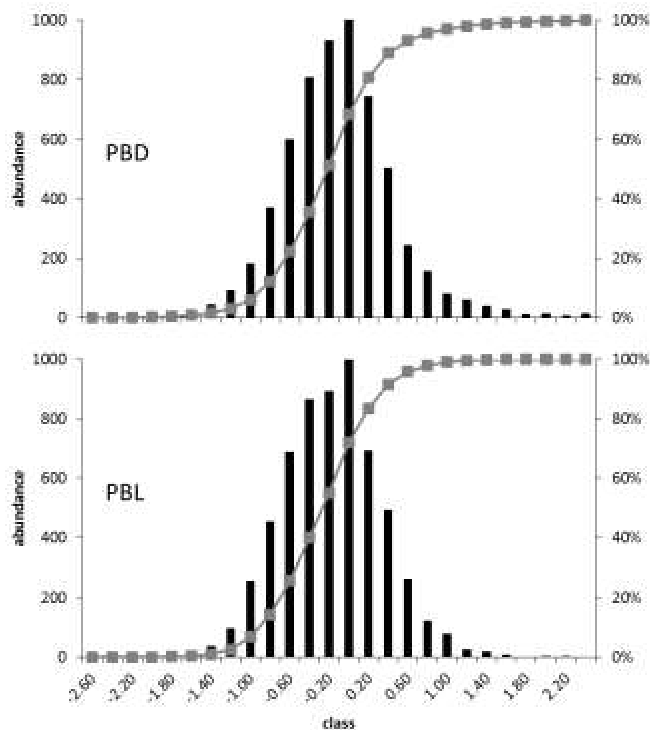

Figure 6 Distribution of log_2 fold-change values in the transcriptomes of the PBD and PBL. Cumulative abundance is presented in grey.

Table 4 p-values for significant effects of the treatment on transcript levels in functional categories (Wilcoxson rank-sum test, Benjamini-Hochberg corrected).

MAPMAN bin	n(genes)	p (PBD)	p (PBL)
photosynthesis	104	ns	0.00E+00
major CHO metabolism	47	9.15E-13	1.22E-09
minor CHO metabolism	53	1.36E-02	8.06E-03
glycolysis	44	4.25E-05	2.57E-02
OPP	13	3.29E-02	ns
TCA / org transformation	39	1.09E-07	3.89E-02
mito. electron transport / ATP synth.	54	2.58E-03	2.05E-08
cell wall	65	1.15E-03	4.26E-03
lipid metabolism	169	1.28E-03	1.70E-05
amino acid metabolism	151	2.45E-12	1.12E-07
secondary metabolism	129	1.21E-02	4.19E-02
tetrapyrrole synthesis	41	7.58E-06	1.13E-03
stress	109	ns	5.57E-03
redox	87	ns	4.79E-03
polyamine metabolism	11	4.13E-04	6.98E-04
nucleotide metabolism	83	6.50E-09	8.54E-07
C1-metabolism	21	3.34E-02	ns
misc	290	3.62E-02	ns
RNA	540	4.28E-16	9.07E-47
DNA	240	6.34E-07	3.05E-11
protein	1119	5.87E-10	1.11E-18
signalling	231	8.81E-03	3.42E-13
cell	256	ns	9.09E-06
development	160	1.09E-02	1.19E-06
transport	365	3.95E-02	1.52E-07
not assigned	5476	0.00E+00	0.00E+00

Abiotic stress response genes

Oxidative stress

Constitutive transcription of four ascorbate peroxidase (APX) genes (homologues of *Arabidopsis* plastid *SAPX*, as well as three cytosolic *APX*: *APX2*, *APX6*, and very low quantities of *APX5*) and two catalase genes (*catalase 1*, and *catalase 2* homologues) was found. *APX2* constitutive expression level was c. 1.5-fold higher in the PBL compared to the PBD. Upon desiccation, no differential expression of APX genes was detected despite very little increase of *APX2* transcription in both photobionts.

Catalase transcripts were slightly more abundant in the PBL. Two possible isoforms of catalase 1 were detected in both organisms. In the PBD, catalase 2 was upregulated in response to desiccation while in the PBL, catalase 2 was less, but also significantly upregulated and transcription of a superoxide dismutase (Fe superoxide dismutase 1) was slightly increased.

Early responsive to desiccation and late embryogenesis abundant protein transcripts

Two orthologs of early-responsive to desiccation (ERD) 4 genes were significantly higher expressed in the PBL (orthologs to the *Arabidopsis thaliana* genes *AT4G02900.1* and *AT1G30360.1*) under control conditions, although at very low expression levels (maximum 19 RPM in the unstressed PBD, Table 5). No significant effect of desiccation was observed in any of the ERD transcripts detected.

An ortholog of the late embryogenesis abundant (LEA) protein gene *AT4G13230.1* was highly expressed in both photobionts. Upon desiccation, its transcription was significantly upregulated c. two-fold in the PBL. Further four LEA orthologs were identified, but showed low expression levels (Table 5).

Table 5 Expression levels (reads per million, RPM) of late embryogenesis abundant (LEA) and early-responsive to desiccation (ERD) orthologs. Values are presented for control and treated (t) samples.

		RPM				log2 fold-change	
type	matching *Arabidopsis* gene	PBD	PBD t	PBL	PBL t	PBD	PBL
LEA	*AT4G13230.1*	713	872	538	1150	0.29	1.09
	AT3G50790.1	39	21	36	17	-0.90	-1.01
	AT3G62580.1	28	23	32	27	ns	ns
	AT1G34340.1	12	16	11	13	ns	ns
	AT5G49950.1	6	6	4	6	ns	ns
ERD	*AT1G30360.1*	9	7	19	11	ns	ns
	AT4G02900.1	7	5	10	4	ns	ns

Heat shock proteins

A general effect of desiccation, which may also impact photosynthetic mechanisms, is conformational change including loss of function in proteins. Heat shock proteins (Hsps), possessing chaperone function, protect and establish correct protein folding. A necessity for the correct functioning of proteins. During various abiotic stress treatments, organisms use Hsps as a part of the stress response (Wang *et al.* 2004). The transcription of Hsp genes was significantly different between the two photobionts. The PBL displayed significantly more transcription of chaperones, predominantly of the Hsp70 and Hsp40/DNAJ type. A total of 13 chaperones were identified in higher amounts in the PBL, of which 10 could be associated to membranes, six to the chloroplast and one to the thylakoids (Table 6).

The notable upregulation of heat shock proteins in the PBD indicates that the protection of protein conformation may be a key factor of this organism's desiccation tolerance. This interpretation is supported by the finding that the second most upregulated gene encodes for a plastidary chaperone. It is orthologue to a chloroplast protein import cpHsc70-1 family protein (Fes1A in *Arabidopsis thaliana*).

Table 6 Significant (t-test corrected for multiple comparison) differences between Hsp transcript levels under control conditions. The ratio of expression in PBL vs. PBD is given as decimal value. X marks a target or domain known for the corresponding sequence

ortholog/category	PBL/PBD exp. ratio	target				Hsp domains		
		cytosol	membrane	chloroplast	thylakoid	Hsp 70	Hsp 90	Hsp40/ DNAJ
Ftn2 (cyanobacterial)	2.59		X	X				X
GRP94	2.03						X	X
Chaperone DnaJ-domain superfamily	1.82							X
BiP3 & BiP2	1.74					X		X
heat shock protein 70 (Hsp 70) family	1.65		X	X		X		
GRV2	1.58			X				X
HSP81-1	1.53	X	X				X	X
DNAJ heat shock N-terminal domain-containing	1.40							X
Stromal 70 kDa heat shock-related, chloroplast precursor	1.30			X		X		
Heat shock 70 kDa protein, mitochondrial precursor	1.19		X	X		X		
HSP70	1.17	X	X			X		
HSP70	1.12	X	X			X		
DNAJ-1	1.06		X					X
Hsp101	0.76		X	X	X	X		
DNAJ domain protein localized in ER membrane	0.75		X					X
chloroplast-targeted 90-kDa Hsp, stroma	0.72		X	X			X	
DNAJ heat shock family protein	0.56							X

Photosynthetic response

Chlorophyll fluorescence and activity of the light reaction

Slight differences in the inactivation kinetics of photosynthesis were more pronounced at slower desiccation, especially concerning the slow phase of loss of photosynthetic activity followed by rapid decline of photosynthetic activity (Figure 1). Notably, the PBL showed relatively long retention of high quantum efficiency of photosystem II compared to other photobionts studied in these experiments even under conditions of faster desiccation (Sadowsky and Ott 2012), including the closely related PBD. Reactivation of photosynthetic activity during rehydration revealed an increase of minimum chlorophyll fluorescence (F_0) in the clade S *Trebouxia* photobionts (Sadowsky and Ott 2012).

Measurements with higher time resolution detected a very rapid increase of F_0 during rehydration in all photobionts of the study. Parallel measurements of PS II and PS I activity showed that both photosystems can be reactivated within seconds to minutes, but are also more susceptible to light stress after desiccation. A contrary effect has been found in the Swedish lung lichen *Lobaria pulmonaria* (Fernández-Marín *et al.* 2010). During desiccation in the dark, this intact lichen from a temperate habitat activated a photoprotective mechanism based on xanthophyll conversion. This strategy enhanced its excess light tolerance during rehydration. In the isolated Antarctic photobionts of *Usnea lambii* and *Umbilicaria decussata*, no activation of the xanthophyll cycle could be detected (Sadowsky and Ott, 2012). Transcriptome analysis revealed no transcriptional activation of the central enzyme violaxanthin de-epoxidase. This is consistent with the observation that pigments of the xanthophyll cycle were not converted during dehydration in the dark (Sadowsky and Ott, 2012). It is, however, possible that by dissipation or inactivation of PS II light harvesting complexes (LHC), which can be reflected by a decrease in F_0, the absorption capacity of PS II is downregulated. This would provide excess light protection during desiccation and during a few seconds of the rehydration process

Photosynthetic pigments

While there is evidence for degradation of Proteins of the photosynthetic light reaction during desiccation, both photobionts exhibited no change in chlorophyll contents (Sadowsky and Ott 2012). Chlorophyll synthesis or degradation functions are not upregulated. On the contrary, the tetrapyrrole synthesis pathways are consistently downregulated by desiccation. It can be concluded that the photobionts examined are homoiochlorophyllous, as many other lichen photobionts are in the symbiotic state (Kranner *et al.* 2003). Homoiochlorophyllic organisms do not degrade chlorophyll and preserve their photosynthetic apparatus during desiccation (Sherwin and Farrant 1996).

Proteins of the light reaction

The PBL showed upregulation especially of light-harvesting complex (LHC) II and LHC I components during desiccation. New LHC peptides formed from the mRNA may replace damaged parts of the photosynthetic apparatus. Turnover of PS proteins is a common stress response in the plant kingdom and can also be observed under light stress (Andersson and Aro 2001). Heat shock proteins can maintain protein folding, and therefore protect protein function, but are also necessary during *de novo* protein synthesis (Wang *et al.* 2004). Many heat shock proteins, such as the chaperones which are activated

during desiccation of the PBD, displayed higher constitutive expression in the PBL (table X). As in the PBD, most of these may function in the chloroplast and its membrane systems. Considering these possible strategies to protect the integrity of the photosystems during desiccation, the PBL can establish longer retention of high PS II activity during desiccation while both photobionts may profit from recruiting intact LHC II proteins during rehydration, which can be reflected by an increase of F_0 during rehydration.

The strategy of keeping the photosynthetic apparatus intact during dehydration is not common in poikilohydrous autotrophs. Rather, a controlled shutdown of the photosynthetic processes is reported (Collett *et al.* 2003, Liu *et al.* 2008). The study presented examines an early stage of dehydration. Photosynthetic processes have not completely ceased, and especially the PBL still showed relatively high quantum yield of PS II after the treatment. The PBD, by contrast, lost its photosynthetic activity quickly after the treatment. Therefore, the overexpression of PS elements is consistent with its longer retention of activity.

Shift from anabolism to catabolism

Proteins

Regarding protein synthesis, positive transcriptomic regulation can be considered. Both photobionts significantly increase the expression of ribosomal proteins, which are necessary for *de novo* synthesis of proteins. Positively regulated chaperones target the chloroplast membrane systems remarkably often (Table 7). As elements of the photosynthetic light reaction were upregulated especially in the PBL, it can be concluded that upregulated protein synthesis of this photobiont is largely due to the enhanced turnover of photosynthetic proteins during desiccation stress.

Notably high upregulation of highly expressed protein degradation factors could be observed, especially in the PBD (Table 7). As many ubiquitin-dependent enzymes were encoded by the upregulated transcripts, controlled degradation of damaged proteins may be a major reason. As neither genes involved in starch nor lipid degradation were upregulated, it is also possible that storage proteins were used as an energy reserve in the examined photobionts.

Table 7 Top ten significantly upregulated transcripts related to protein degradation.

matching *Arabidopsis* gene	RPM				log2 fold-change	
	PBD	PBD t	PBL	PBL t	PBD	PBL
AT5G35690.1	18	349	33	82	4.20	1.30
AT5G06420.1	120	672	136	379	2.47	1.48
AT2G26000.2	72	395	96	141	2.45	0.55
AT5G40200.1	195	863	203	260	2.14	0.35
AT4G36860.1	58	259	48	110	2.14	1.18
AT5G42390.1	138	474	148	111	1.78	ns
AT4G04180.1	105	325	134	193	1.62	0.52
AT1G51710.1	207	516	214	293	1.32	0.45
AT3G27925.1	281	644	237	302	1.20	0.34
AT4G05320.2	3053	6224	3177	5297	1.03	0.74

Growth and proliferation

Apart from protein metabolism, large portions of the basic metabolism were downregulated in both photobionts. Several elements connected to cell growth and proliferation displayed significant downregulation in both photobionts studied. Broad downregulation occurs in carbon assimilation, cell wall synthesis, DNA synthesis and chromatin modelling. Also starch metabolism and small carbohydrate metabolism is downregulated, concerning degradation as well as synthesis. Functions which are putatively associated with growth and proliferation processes are also affected by the desiccation treatment. Polyamine synthesis is downregulated in both photobionts, and comprises the most intensively downregulated functional category in the PBL. Polyamines are essential for cell division processes in a large number of organisms, including plants (Paschalidis *et al.* 2005) and algae (Theiss *et al.* 2002).

Sugars and polyols

A metabolomics analysis of the PBD and the PBL revealed high amounts and dynamics of polyols and sugars in these photobionts during the desiccation process (Sadowsky *et al.* 2015). After 15 minutes of desiccation, the levels of several sugars and polyols dropped. This was especially pronounced in the PBL. It could therefore be speculated that declining concentrations of sugars and polyols occurred due to katabolic use of these resources under stress conditions. The energy gained may foster processes such as the enhanced photosystem turnover observed in the PBL. Nevertheless, no significant upregulation of related transcripts could be observed. Nevertheless, post-transcriptional control of the corresponding genes is possible and should therefore be investigated in future research.

The mitochondrial respiration chains, by contrast, were significantly transcriptionally upregulated in both photobionts of the study. Besides sugars and polyols, it is also possible that storage proteins are used to maintain metabolism (see above).

Conclusion

The study presents the first transcriptomic study of an Antarctic lichen photobiont as well as the first approach comparing the transcriptomes of two *Trebouxia* lineages. Compared to photobionts from more temperate environments, the effects of dehydration were more pronounced. Although it must be taken into account that the treatment differed in the study regarding *Trebouxia gelatinosa* from Europe (Candotto Carniel 2014), the examined Antarctic photobionts showed different behaviour. Pathways affected covered a wide range of stress tolerance and primary metabolism. Some strategies can be considered evolutionary ancient, as they can be found in many *Viridiplantae*, including higher plants. These are especially the activation of heat shock and LEA proteins. The large proportion of regulated genes is however negatively affected. They encode for functions of anabolism, such as growth and cell division. This finding is consistent with a controlled metabolic shutdown of cell metabolism. Catabolic processes are upregulated and may provide energy for the protection and turnover of proteins to maintain activity without destruction. The retention of intact protein structures may enhance the reactivation capacity. Many protective functions were not regulated but displayed considerable significant differences between the photobionts under control conditions. Especially heat shock and antioxidative proteins were more present in the PBL. A dominance of constitutive protection mechanisms was also concluded from the results of proteomic and transcriptomic studies of lichen photobionts (Gasulla *et al.* 2013, Candotto Carniel 2014) and a lichen (Junttila *et al.* 2013). Constitutive mechanism of desiccation tolerance may therefore be characteristic for many lichen species and their photobionts. Energy invested in constitutive protection mechanisms may be a major reason for the slow growth of these organisms, but enable to survive in a rapidly changing environment (Kranner *et al.* 2008). As the fruticose, erect growth form of *Usnea lambii* thalli may lead to more rapid desiccation than in the foliose *Umbilicaria decussata* thalli, higher constitutive stress tolerance of the PBL can be regarded as an adaptation to its symbiotic lifestyle in the harsh Antarctic terrestrial environment (Sadowsky *et al.* 2015). Although the PBD and PBL are closely related, it can be concluded that different mechanisms of desiccation tolerance are preserved in the respective algae.

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6. Summary

In the present study, various levels of adaptations of lichen photobionts towards Antarctic extreme sites are proved. Photobionts of the genus *Trebouxia* were isolated from five different Antarctic lichens as well as from one European lichen. These photobionts' responses to drought, cold and high light intensities was examined. Pronounced differences of the physiological potentials of the organisms examined are demonstrated.

In Antarctic macro lichens, the dominant group of photobionts is *Trebouxia* sp., clade S. Within this clade, significant differences of physiological stress responses were shown. Regarding freezing and desiccation tolerance, photobionts from Antarctic endemites and bipolar lichens displayed higher potential. Photobionts of *Usnea lambii*, *Pleopsidium chlorophanum* and *Umbilicaria antarctica* surpassed the photobiont of the cosmopolitic lichen *Umbilicaria decussata* in stress tolerance. Therefore, the photobiont isolated from the Antarctic endemic crustose lichen *Buellia frigida* (clade A *Trebouxia* sp.) displayed similar stress tolerance as the clade S photobionts from bipolar and endemic lichens. Contrastingly, the *U. decussata* photobiont's stress tolerance resembled that of the photobiont of the Swedish lichen *Fulgensia bracteata* (clade I *Trebouxia* sp.). The growth form of the respective lichen thalli must also be taken into account. The *U. lambii* photobiont's high desiccation tolerance can be an advantage, as the lichens erect and branched growth form hampers long-term moistening by melt water.

Because of their significantly different response to desiccation, the clade S photobionts of *U. lambii* and *U. decussata* were chosen for in-detail examination of metabolites and gene expression. No Antarctic lichen photobionts have been examined in such detail before. The type of study is a novelty in Antarctic lichen research. Its results yield valuable new knowledge on the basal mechanisms of the examined photobionts' stress response.

During the desiccation process, metabolites as especially sugars, polyols and amino acids displayed strong dynamics. Degradation of energy- rich sugars and sugar alcohols as well as the possible role of several amino acids in stress tolerance can be interpreted as controlled acclimation of the metabolome towards rapid desiccation. The higher stress tolerance of the *U. lambii* photobiont compared to the *U. decussata* photobiont could be correlated to its metabolome. Especially constitutively high levels of ribitol and sucrose may mediate its superior desiccation tolerance.

Significant differences in gene expression of the *U. lambii* and the *U. decussata* photobiont were detected regarding various metabolic functions. Especially differences in constitutive transcription could be correlated to the *U. lambii* photobiont's higher desiccation tolerance. Regarding its longer retention of photosynthetic activity under desiccating conditions, transcriptomic regulation could be identified as a major factor. Overexpression of genes encoding for proteins of the photosynthetic light reaction as well as upregulation of protein synthesis and protein folding functions were detected. Especially chaperones ensuring the correct folding of plastid membrane proteins were upregulated. The combination of these regulated pathways can be interpreted as a strategy to ensure the functioning of photosynthesis during desiccation as well as to recruit functional proteins for the reactivation phase. Apart from that, both photobionts examined displayed a shift from anabolism to catabolism. It can be concluded that during desiccation of the photobionts, a transcriptionally controlled metabolic shutdown towards anabiosis was conducted by the organisms.

It has been hypothesised that the examined Antarctic clade S *Trebouxia* spp. may share similar adaptations to extreme environments. This hypothesis had to be rejected, as despite of the identical cultivation procedure of all photobionts, their stress responses and potentials differed significantly. These differences could be correlated with the according lichens' geographical distribution and growth forms. Therefore, it can be concluded that ecologically relevant hidden diversity is present in *Trebouxia* clade S.

The results show remarkable physiological potentials of the photobionts examined. Generalist and highly specialised adaptation strategies, as in the photobionts isolated from endemic lichens, could be identified. Based on these results, it can be postulated that warming of lichen habitats, which can be accomplished by enhanced water availability, may favour species with a broad ecological amplitude before specialised endemic species.

Based on the knowledge obtained by this study, the current state is characterised. This characterisation is relevant to predict future developments of Antarctic terrestrial ecosystems.

Erklärung

Ich versichere an Eides Statt, dass die Dissertation von mir selbständig und ohne unzulässige fremde Hilfe unter Beachtung der „Grundsätze zur Sicherung guter wissenschaftlicher Praxis an der Heinrich-Heine-Universität Düsseldorf“ erstellt worden ist.

Die Dissertation wurde in der vorgelegten oder in ähnlicher Form noch bei keiner anderen Institution eingereicht. Ich habe bisher keine erfolglosen Promotionsversuche unternommen.

Düsseldorf, d. 12.11.2015

(Andres Sadowsky)

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