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Effects of enhanced UV-B radiation in the field on the concentration of phenolics and chlorophyll fluorescence in two boreal and arctic–alpine lichens

Jarle W. Bjerke [a,*], Dylan Gwynn-Jones [b], Terry V. Callaghan [c,d]

[a] *Department of Biology, Faculty of Science, University of Tromsø, Tromsø N-9037, Norway*
[b] *Institute of Biological Sciences, University of Wales, Aberystwyth, Ceredigion, Wales SY23 3DA, UK*
[c] *Department of Animal and Plant Sciences, University of Sheffield, Western Bank, Sheffield S10 2TN, UK*
[d] *Abisko Scientific Research Station, Abisko 981 07, Sweden*

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Abstract

Lichens constitute a prominent part of the vegetation at high latitudes and altitudes, but the effects of UV-B radiation on these symbiotic organisms are not well known. In a northern boreal site (Abisko, northern Sweden), the usnic acid-producing lichens *Flavocetraria nivalis* and *Nephroma arcticum* were exposed to enhanced UV-B radiation, corresponding to 25% ozone depletion, for two and one growing seasons, respectively. They were compared with lichens grown under ambient UV-B and harvested fresh from the field. The treated thalli of *F. nivalis* had been transplanted from a site 24 km from the treatment site. From this source locality, untreated thalli were also harvested. Enhanced UV-B did not affect concentrations of usnic acid and the two depsides phenarctin and nephroarctin. A gradual decline of usnic acid, probably coupled to unusually long periods of dry, sunny weather, was observed both under enhanced and ambient UV-B and in untreated thalli. Photosystem II efficiency in both species was slightly reduced by enhanced UV-B. However, differences between seasons were larger than differences between treatments, which indicate that UV-B effects are minor in comparison to other climatic variables. Concentrations of UV-B-absorbing phenolics in lichens do not show a simple relationship to UV-B dose and therefore cannot be used as bioindicators of UV-B levels.

Keywords: Ultraviolet-B; Lichen; UV-absorbing substances; Usnic acid; Depsides; Photosystem II efficiency; Seasonal trends

1. Introduction

The discovery of the severe depletion of the stratospheric ozone layer in the mid 1980's led to increased attention to the potential effects of enhanced ultraviolet-B (UV-B; 280–315 nm) radiation on all living organisms. Most studies were conducted on vascular plants, in particular on economically important crop species. During the late 1990s more focus was given to terrestrial ecosystems, in particular at high latitudes, since the highest relative increases of enhanced UV-B are assumed to occur near the poles (McKenzie et al., 2003).

High-latitude ecosystems are characterised by low vegetation with a high proportion of poikilohydric cryptogam species, especially bryophytes and lichens.

* Corresponding author. Tel.: +47-77644439; fax: +47-77646333.
E-mail address: jarle.bjerke@ib.uit.no (J.W. Bjerke).

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The number of reports on the effects of enhanced UV-B radiation on bryophytes has increased considerably in recent years. Despite the high abundance and diversity of lichens in polar and alpine environments, experimental field studies that attempt to relate lichen responses to UV-B radiation are restricted to some UV exclusion experiments (Bachereau and Asta, 1997, 1998; Swanson and Fahselt, 1997; Huiskes et al., 2001; Lud et al., 2001; Solhaug et al., 2003) and a few experiments using supplementary UV-B radiation (Sonesson et al., 1995; Heide-Jørgensen and Johnsen, 1998; Lud et al., 2001; Buffoni Hall et al., 2002; Solheim et al., 2002; Bjerke et al., 2003).

As briefly summarised by Bjerke et al. (2002, 2003), lichens vary considerably in their response to UV-B, and the variations seem to depend on response variables and species in question, type of experiment, and duration. Most focus has been given to the effects on the biosynthesis of secondary lichen substances, most of them phenolics and unique to lichens.

The yellow–green dibenzofuran usnic acid is the best known and most studied lichen phenolic. It has a high antibiotic activity, which may give the lichens protection against microbial and microfaunal attacks (see reviews by Cocchietto et al., 2002; Ingólfsdóttir, 2002). However, it also absorbs efficiently in the UV-C and UV-B ranges of the spectrum (Rancan et al., 2002), which makes it an interesting substance in relation to UV photobiology, since it renders photoprotection to fungal hyphae and photobiont cells. The possible use of lichens and their UV-absorbing substances (especially usnic acid) as bioindicators or biomonitors of UV-B radiation levels has been postulated (Galloway, 1993; Quilhot et al., 1994; Rikkinen, 1995). BeGora and Fahselt (2001a) observed a decrease in usnic acid concentrations when visible light and UV-A radiation was screened out, whereas Buffoni Hall et al. (2002) observed an increase in the tips of a *Cladonia* species exposed to enhanced UV-B radiation.

The efficiency of excitation capture by open photosystem II (PS II) reaction centres, measured as F_V/F_M (Genty et al., 1989), is another response variable that has been studied in lichens. It is generally used as an indicator of stress, often caused by photoinhibition. Huiskes et al. (2001), Lud et al. (2001) and Solhaug et al. (2003) did not observe any effects of altered UV-B radiation on photosystem II efficiency in various lichens.

The primary objective of the study described here was to assess whether simulated ozone depletion (viz. enhanced UV-B radiation) can affect the secondary metabolism in lichens outdoors, as compared to indoor experiments. It was particularly focused on the concentration of usnic acid, because of the general interest (see above) in this very common dibenzofuran. Supportive data on photosystem II efficiency were also acquired. Even if these aspects of the lichen symbiosis have been studied previously (see above), more information is needed to understand how lichens respond to enhanced UV-B radiation in the field. This study contributes with novel results by examining the two variables simultaneously, and by doing this in the field over longer time spans than used in most of the cited studies. Two widespread boreal and arctic–alpine lichens were examined, one that was treated during two growing seasons, and one that was treated during one season.

2. Materials and methods

2.1. Lichen material and treatment

The fruticose lichen *Flavocetraria nivalis* (L.) Kärnefelt and Thell and the foliose lichen *Nephroma arcticum* (L.) Torss. were included in the study. Both species are widespread in the Northern Hemisphere, and by far most common in arctic and alpine environments, although they also grow in warmer regions. *F. nivalis* was collected 5 June 2001 from low-alpine, lichen-rich heaths at Vassijaure, northern Sweden (68°26′ N, 18°16′ E, 490 m altitude) and transported to Abisko Scientific Research Station (68°21′N, 18°49′ E, 360 m altitude). The aerial distance between these two locations is 24 km. The climatic data from Abisko and Katterjåkk, a meteorological station ca. 2 km from our collection site at Vassijaure, show considerable year-to-year fluctuations both in temperature, sun hours, shortwave radiation and precipitation (Table 1). The growing season of 2002 was warmer, sunnier and drier than 2001. Katterjåkk received more precipitation and had considerably fewer sun hours than Abisko. The long-term precipitation rates between the two localities also differ considerably,

Table 1
Climatic data from Abisko and Katterjåkk (ca. 2 km E of Vassijaure), northern Sweden

	Month							
	May		June		July		August	
	2001[a]	2002[a]	2001[a]	2002[a]	2001[a]	2002[a]	2001[a]	2002[a]
(a)								
Temperature (°C), Abisko	3.1	6.1	10.1	11.8	10.8	12.5	10.2	12.2
Temperature (°C), Katterjåkk	2.1	4.8	9.5	11.5	10.0	12.0	9.9	12.4
Precipitation (mm), Abisko	18	11	62	10	108	82	55	20
Precipitation (mm), Katterjåkk	76	37	42	39	121	97	104	16
Shortwave radiation (MED per day)	5.35	5.93	6.30	7.04	5.47	5.87	3.70	5.05
Sun hours, Abisko	189	255	243	294	–	168	–	174
Sun hours, Katterjåkk	144	220	196	246	109	172	48	108

(b)

	Period							
	12 June–11 July (30 days)		27 June–11 July (15 days)		22 July–20 August (30 days)		6–20 August (15 days)	
	2001[a]	2002[a]	2001[a]	2002[a]	2001[a]	2002[a]	2001[a]	2002[a]
Abisko: days without rainfall	19	18	10	5	9	18	5	10
(days <1 mm in brackets)	(20)	(24)	(11)	(10)	(11)	(22)	(6)	(13)
Katterjåkk: days without rainfall	13	15	5	6	6	18	2	10
(days <1 mm in brackets)	(19)	(22)	(8)	(13)	(9)	(21)	(4)	(13)
Precipitation (mm) Abisko	73	20	34	17	93	42	45	20
Precipitation (mm) Katterjåkk	57	53	33	16	149	52	64	14

(a) Precipitation, mean monthly temperature, monthly minimum erythemal dose (MED per day) and sun hours for the months May–August 2001 and 2002. Note that the August data include only the 21 first days of the month, as these are the relevant days for the experiment. Some days with sun hour data from Abisko are lacking, and therefore not presented for July and August 2001. MED values are only available from Abisko. (b) Days without rainfall and precipitation rates for the 30 and 15 days periods prior to sampling.
[a] Year.

Katterjåkk/Vassijaure receiving more than the double during a year than Abisko (844 mm versus 304 mm) (Alexandersson and Eggertsson Karlström, 2001). *F. nivalis* is also found near the research station, but due to the relatively high number of necessary replicates and due to collection restrictions within the National Park, it was more convenient to sample from Vassijaure. The same day as they were collected, the lichen thalli were planted in trays, each with six cells (4.0 cm diameter) containing local nutrient-poor soil, gravel and sand. To avoid wind dispersal of thalli, the trays were wrapped in a thin nylon net with 1.0 cm mesh size. In total, 128 trays with 768 thalli were used in the experiment.

N. arcticum was sampled from the open subalpine birch forest in the Abisko area in 1994 and placed in rectangular wooden compartments (75 cm × 25 cm) with bottoms of nylon net. Some of the samples were used in previous studies (see Björn et al., 1997), but the samples used here grew vigorously under open conditions without being treated in any way before they were used in the present study. In early June 2002, the thalli were separated and planted in 10 cm pots containing local soil and gravel. Each pot was covered by nylon net. *F. nivalis* was watered three times during the two first weeks of the experiment in order to improve the establishment of the thalli, whereas *N. arcticum* was not watered.

Eight metal frames (2.5 m × 1.3 m × 1.5 m high), each with six fluorescent lamps (Q-PANEL UVB-313, Cleveland, OH, USA), were used in the treatment, according to methods described by Johanson et al. (1995a, b). Four of the frames served as controls by using window glass to exclude all radiation at wavelengths below 320 nm from the lamps. In the UV-B treatment, pre-burnt cellulose diacetate was used to cut off UV-C radiation. The UV-B treatment

was set up to simulate a 25% ozone depletion under clear sky conditions, by using the computer models developed by Björn and Murphy (1985) and Björn and Teramura (1993). UVR levels were measured at Abisko (as minimum erythemal dose, MED per hour; UVB Solarlight Biometer, Solarlight Co., Philadelphia, PA, USA) according to Björn and Holmgren (1996). Monthly means per day (MED per day) are given in Table 1a. Radiation data are not available from Vassijaure or Katterjåkk.

2.2. Harvesting

The treated samples of *F. nivalis* were harvested at the same dates in 2001 and 2002: 12 July (ca. 36 days after start of the experiment for the season) and 21 August. During the experiment, trays receiving the same treatment were once a week randomly moved between and within frames, so that each tray was considered an independent replicate. On each harvesting day, 16 trays per treatment were sampled. Samples were also collected from the Vassijaure population on the same dates, and on 6 June 2002. Sixteen randomly distributed thalli within an area of ca. 0.4 km^2 were collected each time. These samples are henceforth referred to as 'untreated'.

N. arcticum was harvested twice; 12 July and 21 August 2002. Fourteen pots per treatment were harvested each harvesting day. These pots had also been moved randomly between and within experimental units. Since the thalli of *N. arcticum* originated from the same area as the experimental set-up, it was not so important to monitor any potential transplantation effects. Thus, untreated samples of *N. arcticum* were not harvested.

2.3. Chlorophyll fluorescence

Chlorophyll *a* fluorescence was measured with a portable plant stress meter (Biomonitor S.C.I. AB, Umeå, Sweden) according to Öquist and Wass (1988), using a photon flux density of 400 $\mu mol\ m^{-2}\ s^{-1}$ during 5 s, which is the highest intensity on this meter. Measurements were done on samples harvested in 2002. One thallus of *F. nivalis* from each tray, 12–16 untreated thalli from Vassijaure and one lobe from each pot of *N. arcticum* were preconditioned overnight in a dark chamber with low incandescent light to recover from recent exposure to solar radiation. The thalli were hydrated, and during the preconditioning period, the thalli slowly air-dried. In the morning, the air-dried thalli were sprayed with distilled water, and for *F. nivalis* chlorophyll fluorescence was measured at 5 and 30 min, and at 1, 2, 4, 8 and 24 h after the first wetting. The measurements of *N. arcticum* were done after 5, and 30 min, and after 1, 2, 4, 8, 18 and 30 h. During these periods, the thalli were kept hydrated by placing them on moist filter paper and by regularly spraying with water, and water was non-limiting during these periods. The thalli were placed in room temperature (20–22 °C) with low-energy growth light during the entire period, except for the 15 min period prior to each measurement, when the lichens were dark-adapted. The lightweight clamp cuvettes used for dark adaptation were attached to the thallus tips of sterile lobes. The thalli from Vassijaure collected in July 2002 were not analysed at 8 h.

2.4. Content of UV-absorbing phenolics

Acetone and methanol extraction and quantitative analyses of usnic acid in the thallus tips of *F. nivalis* by means of reversed-phase high performance liquid chromatography (RP-HPLC) were done according to the method used for the same species by Bjerke et al. (2002). Residues of usnic acid in thalli macerated after completed extraction were minute (<0.4% of total concentration), and extraction procedures were therefore considered sufficiently efficient. Three thalli from each tray were randomly chosen and analysed for usnic acid levels.

The levels of phenolics in *N. arcticum* were analysed by the same RP-HPLC method, but with a longer gradient run. Four phenolics were measured, viz. usnic and isousnic acids, and the depsides phenarctin and nephroarctin. The proportion of methanol was increased from 30 to 72% during 4 min, and then to 90% during 26 min. The other solvent, 1% orthophosphoric acid in ultra-pure water, was decreased accordingly. The flow rate was 1.15 $ml\ min^{-1}$. Two thallus zones of *N. arcticum* were analysed, viz. the five outermost millimetres of the lobe tips, and the basal zone (12–16 mm from the lobe tip). Pure samples of usnic acid, nephroarctin and phenarctin were used to obtain linear standard curves with point of intersection at 0 ($r^2 > 0.990$). Pure samples of isousnic acid were not

available, but since the absorbance properties of usnic and isousnic acids are almost identical, the standard curve of usnic acid was used to calculate the amounts of isousnic acid.

2.5. Statistical analyses

The concentration data for *F. nivalis* were first analysed by a three-level nested ANOVA with measurement (two per thallus), thallus (three per tray) and tray (16 per treatment event) nested within treatment event. Since there was no evidence for variance components among subordinate levels, a 3×4 (treatment $\times$ time) fixed effects factorial ANOVA was applied on the data, using mean values per tray as replicates. A two-factor multiple comparison design was applied to compare mean values within treatment levels and harvesting events according to Toothaker (1993), i.e. cross-comparisons (e.g. enhanced UV-B in July 2001 versus ambient UV-B in August 2002) were not made. By reducing the number of compared contrasts, the required critical values are also reduced. The June mean values were compared to the other mean values of the Vassijaure population in a one-way ANOVA. Since there are four response variables, the chemical data for *N. arcticum* were first analysed by MANOVA and subsequently by multiple univariate ANOVA. The repeated measurements of chlorophyll fluorescence were analysed as univariate data by using the mean values for each subject. Multiple comparisons of these data were done by Tukey multiple comparison tests. The data on the untreated samples were not included in multiple comparisons of PS II recovery measurements, since one data point was lacking (8 h in July), and since the number of replicates varied (from 12 to 16). All analyses were done in S-Plus 6.1 statistical package for Windows (Insightful Corp., Seattle, USA). Logarithmic transformation of data was performed in some cases in order to obtain normality and minimise heteroscedasticity. *P*-levels lower than 0.05 were considered significant.

3. Results

3.1. UV-absorbing phenolics

Usnic acid was detected in both species. The two didepsides nephroarctin and phenarctin were detected

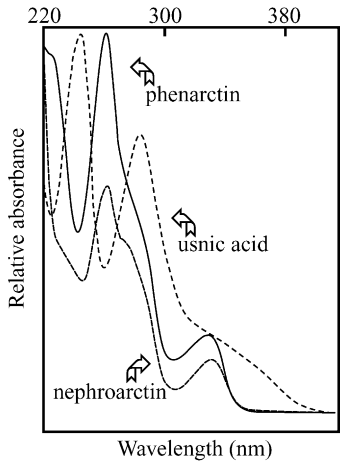

Fig. 1. UV spectra of nephroarctin (peaks at 253.2 and 342.0 nm), phenarctin (253.2 and 339.7 nm) and usnic acid (230.9 and 280.3 nm). The spectrum of the trace substance isousnic acid is almost identical to that of usnic acid, and is not shown. The spectra are obtained using an HPLC system equipped with a PDA detector. The substances pass the detector at different solvent ratios. The methanol to 1% orthophosphoric acid ratios lay between 65:35 and 75:25.

in *N. arcticum*. All these substances have their maximum absorption in the UV-C range of the spectrum (Fig. 1). Usnic acid has relatively high absorption in the UV-B range with a long tail through the UV-A range, whereas both nephroarctin and phenarctin absorb some UV-B and UV-A, with only small absorption peaks in the UV-A range. Some additional substances with low chromatogram peak sizes were sporadically detected in *N. arcticum*. Isousnic acid was among the trace substances. Since it was detected in nearly all specimens, it was included in the study, but the remaining unidentified trace substances with lower frequency were not analysed any further.

Mean concentrations of usnic acid in the two species did not differ between ambient UV-B and enhanced UV-B at any time (Fig. 2, Tables 2 and 3). For *F. nivalis*, the untreated Vassijaure samples had significantly ($P = 0.05$) and near-significantly ($0.05 < P \leq 0.10$) higher concentrations than the treated samples for the last three harvesting events.

Moreover, enhanced UV-B treatment did not have any significant effects on the concentrations of depsides and the total concentration of phenolics (Tables 2 and 3). A significant interaction between UV-B and thallus zone was observed for nephroarctin, which is

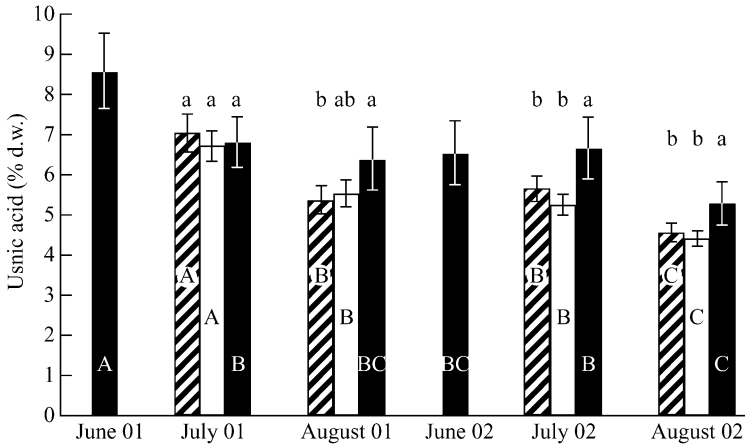

Fig. 2. Usnic acid concentrations (% of dry weight [d.w.] lichen) in the thallus tips of *Flavocetraria nivalis* in relation to time and treatment (hatched bars: enhanced UV-B; open bars: ambient UV-B; filled bars: untreated population at Vassijaure). Means are back-transformed. Error bars represent 95% confidence intervals. Lower-case letters above the bars symbolise differences among means from the same harvesting date. Capital letters placed on bars symbolise differences between means of UV-B treatment. Bars with different letters are significantly different.

explained by a trend towards higher concentrations in the basal thallus zone under enhanced UV-B than under ambient UV-B whilst mean concentrations in apices did not differ between UV-B conditions.

For all three treatment groups (ambient UV-B, enhanced UV-B and untreated), significant temporal declines in usnic acid concentration in *F. nivalis* were observed. However, the concentrations at the end of the growing season of 2001 did not differ from those in July 2002. Harvesting time had significant effects on the concentrations of usnic acid and phenarctin, and near-significant effects on the two other phenolics in *N. arcticum* ($0.05 \leq P < 0.10$). While mean usnic acid and isousnic acid concentrations decreased from July to August, the mean concentrations of the two didepsides increased. Since the dibenzofuranes and the didepsides balanced each other, harvesting time did not have any effect on the total amount of phenolics.

Strong differences were observed between the apical and basal thallus zones of *N. arcticum* for all phenolics, except for nephroarctin. The overall concentration of phenolics is 90% higher in thallus tips than in basal parts. There were few significant interactive effects. The concentration of usnic acid in basal thallus parts was more than halved from July to August, whereas in apical parts it remained constant. Therefore, a significant interactive effect of harvesting time and thallus zone was observed for usnic acid.

Table 2
MANOVA results for effects of UV-B treatment, harvesting time, and thallus zone on concentrations of usnic acid, isousnic acid, phenarctin and nephroarctin in *Nephroma arcticum*

Effect	Pillai's trace	*F*	*P*
UV-B (UV)	0.042	1.09	0.364
Time (*T*)	0.182	5.62	**<0.001**
Thallus zone (*Z*)	0.612	39.78	**<0.001**
UV × *T*	0.020	0.50	0.734
UV × *Z*	0.075	2.06	0.092
T × *Z*	0.143	4.22	**0.003**
UV × *T* × *Z*	0.051	1.35	0.258

Pillai's trace criterion for significance testing was used, and the associated approximated *F*-values are given. *P*-values lower than 0.05 are in bold.

3.2. Chlorophyll fluorescence

For both lichens, maximal PS II efficiency was significantly higher in August than in July, irrespective of UV-B treatment (Figs. 3 and 4). The PS II efficiency of *F. nivalis* increased rapidly during the two first hours, and remained more or less constant during the remaining measurement period (Fig. 3a), although in July, the thalli exposed to enhanced UV-B and the untreated thalli experienced a slight decrease in F_V/F_M values from 2 to 4 h after first wetting.

Table 3
Univariate tests for effects of UV-B treatment, harvesting time, and thallus zone on concentrations of usnic and isousnic acids, phenarctin, nephroarctin and total amount of phenolics in *Nephroma arcticum*

Time		12 July 2002				21 August 2002			
Thallus zone		Apex		Basis		Apex		Basis	
UV-B treatment		Enhanced	Ambient	Enhanced	Ambient	Enhanced	Ambient	Enhanced	Ambient
Usnic acid (% d.w.)	$\bar{u}$	3.87	3.86	0.98	1.42	3.44	3.97	0.54	0.47
	L_1	3.09	3.08	0.62	0.78	2.77	3.19	0.40	0.19
	L_2	4.85	4.85	1.55	2.58	4.27	4.95	0.74	1.13
Isousnic acid (% d.w.)	$\bar{u}$	0.052	0.055	0.026	0.037	0.037	0.048	0.027	0.022
	L_1	0.037	0.043	0.017	0.018	0.029	0.039	0.023	0.011
	L_2	0.071	0.071	0.041	0.075	0.048	0.060	0.032	0.042
Nephroarctin (% d.w.)	$\bar{u}$	1.38	1.51	1.32	1.14	1.54	1.57	1.80	1.17
	L_1	1.20	1.25	1.13	0.92	1.32	1.37	1.32	0.83
	L_2	1.59	1.82	1.55	1.42	1.81	1.80	2.45	1.66
Phenarctin (% d.w.)	$\bar{u}$	2.51	2.93	1.59	1.39	3.31	3.06	2.60	2.11
	L_1	2.03	2.34	1.21	1.10	2.61	2.51	1.44	1.53
	L_2	3.10	3.67	2.08	1.76	4.19	3.73	4.70	2.92
Total phenolics (% d.w.)	$\bar{u}$	8.11	8.70	4.20	4.57	8.62	8.90	5.21	4.12
	L_1	7.14	7.52	3.49	3.71	7.69	7.82	4.21	2.91
	L_2	9.21	10.06	5.06	5.63	9.67	10.13	6.43	5.84
	Univariate ANOVA								
		UV-B (UV)	Time (T)	Zone (Z)	UV × T	UV × Z	T × Z	UV × T × Z	
Usnic acid (% d.w.)	F	0.39	9.18	115.26	0.38	0.02	7.44	1.24	
	P	0.531	**0.003**	<**0.001**	0.537	0.880	**0.007**	0.269	
Isousnic acid (% d.w.)	F	0.59	3.01	15.74	0.45	0.15	<0.01	1.88	
	P	0.442	0.086	<**0.001**	0.503	0.702	0.945	0.174	
Nephroarctin (% d.w.)	F	2.70	2.78	2.62	1.53	5.59	0.40	054	
	P	0.103	0.098	0.109	0.219	**0.020**	0.528	0.466	
Phenarctin (% d.w.)	F	0.40	9.13	19.76	0.59	1.05	2.09	0.15	
	P	0.528	**0.003**	<**0.001**	0.445	0.308	0.152	0.700	
Total phenolics (% d.w.)	F	0.03	0.57	99.37	1.90	0.94	0.01	1.16	
	P	0.854	0.452	<**0.001**	0.171	0.333	0.918	0.283	

Back-transformed means ($\bar{u}$) are presented as percentage of dry weight lichen (% d.w.) and shown together with lower and upper 95% confidence limits (L_1 and L_2). P-values lower than 0.05 are in bold.

PS II efficiency reached considerably higher levels in *N. arcticum* than in *F. nivalis* (Fig. 3b). The F_V/F_M values of *N. arcticum* increased during the entire period, albeit the increase from 8 to 30 h was modest, in particular in August. Since the lichens had been preconditioned at low light levels before fluorescence measurements, differences between treatments at any time during the 24–30 h of wetting were considered as relevant treatment effects, and not only as transient, short-term effects of down-regulation of PS II.

Enhanced UV-B radiation had slight, negative effects on the recovery of PS II efficiency in both lichens. Although no significant differences were observed between the UV-B treatments within each month for *F. nivalis* (Fig. 4a), the overall mean F_V/F_M value was significantly lower for enhanced UV-B than for ambient UV-B (see figure text of Fig. 4). In July, *N. arcticum* had significantly lower mean F_V/F_M values under enhanced UV-B, but not in August (Fig. 4b). The July differences between treatments are most apparent in measurements between 30 min and 8 h (Fig. 3b). F_0 and F_M values were closely positively correlated with F_V/F_M values for all treatments, species and harvesting times (not

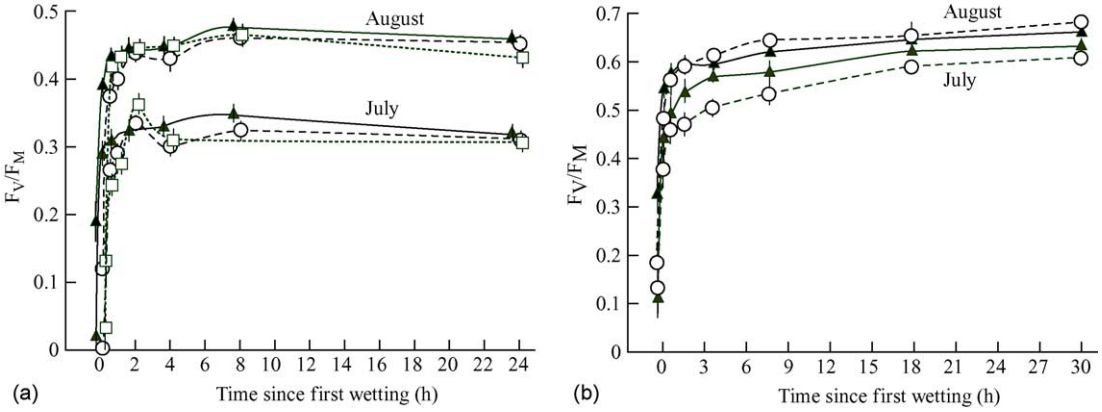

Fig. 3. Recovery of maximal photosystem II efficiency (F_V/F_M) of (a) *Flavocetraria nivalis* (wetted at time 0 and kept hydrated for 24 h) and (b) *Nephroma arcticum* (wetted at time 0 and kept hydrated for 30 h) in relation to radiation treatments and harvesting time. Open circles and stippled lines represent enhanced UV-B radiation, filled triangles and unbroken lines represent ambient UV-B radiation, and open squares with dotted lines represent the untreated population at Vassijaure. Symbols are slightly moved horizontally in order to visualise as many data points as possible. Error bars represent ±1 S.E., and are only shown when larger than symbols.

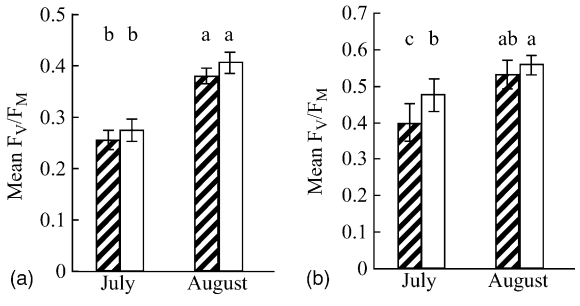

Fig. 4. Effects of UV-B treatment and harvesting time on overall mean F_V/F_M values of (a) *Flavocetraria nivalis* and (b) *Nephroma arcticum*. Open bars: ambient UV-B radiation; hatched bars: enhanced UV-B radiation. Error bars represent 95% confidence intervals. Bars with different letters are significantly different according to Tukey multiple comparison tests. *F*- and *P*-values for *F. nivalis* are 6.03; 0.017 (UV-B treatment), 196.93; <0.001 (harvesting time), and 0.05; 0.826 (UV-B × harvesting time), and for *N. arcticum* 7.31; 0.009 (UV-B treatment), 30.53; <0.001 (harvesting time), and 1.55; 0.218 (UV-B × harvesting time).

shown). Thus, the highest values for both parameters were observed in August after 24 or 30 h of recovery.

4. Discussion

Contrary to the results from some other UV studies (Buffoni Hall et al., 2002; Bjerke et al., 2002), usnic acid and depside concentrations in thallus tips did not increase with enhanced UV-B radiation. In fact, concentrations declined or were unaffected under all conditions (enhanced UV-B, ambient UV-B and untreated). The decline was most abrupt in samples of *F. nivalis* moved to Abisko, which indicates an effect of the transplantation.

Gauslaa and Solhaug (2001) showed that the production of UV-protective melanins in *Lobaria pulmonaria* only occurred during periods of frequent hydration. This dependency of humidity was further documented by Solhaug et al. (2003) and Solhaug and Gauslaa (2004), who showed that the synthesis of the UV-absorbing anthraquinone parietin in *Xanthoria parietina* and of melanins in *L. pulmonaria* only occurred in thalli being hydrated during exposure to UV-B radiation. It is likely that water availability played a similar role in regulating the phenolic content of the two lichens studied here. Since lichens are poikilohydric, they require sufficient wetting through rainfall or dew to become metabolically active. The periods for metabolic activity were fewer and shorter at Abisko than at Vassijaure, and also during the growing season in 2002 than in 2001 (Table 1). Thus, all physiological processes in the lichen thalli were probably adversely affected by the long, dry and sunny periods that were most pronounced at Abisko.

BeGora and Fahselt (2001a,b) suggested that usnic acid becomes degraded by UV-B radiation and resynthesised during periods with positive carbon balance.

Rancan et al. (2002) also observed some photodegradation of usnic acid, but amongst the many analysed commercial synthetic and other natural UV-B filters, usnic acid experienced the smallest degradation. Since concentrations in *F. nivalis* did not differ at any time between the enhanced UV-B and the ambient UV-B treatments, and since there probably was no or very low synthesis under the dry conditions, there is no evidence that the supplementary UV-B radiation in the present experimental set-up and corresponding to 25% ozone depletion, led to any significant photodegradation of usnic acid.

In the studied lichen samples usnic acid concentrations are not closely correlated with UV-B radiation levels. This does not imply that usnic acid is not an effective sunscreen in these lichens. A lack of correlation merely implies that measurements of UV-B-absorbing compounds do not necessarily provide a good indicator of tolerance to UV (Phoenix et al., 2002). The current results and other results (e.g. BeGora and Fahselt, 2001a; Bjerke et al., 2002, 2003, 2004; Nybakken et al., 2004) imply that the potential of using lichens and their UV-absorbing metabolites as bioindicators of near-ground UV-B radiation levels, is limited. The observed usnic acid concentrations in *N. arcticum* were unexpected, firstly because previous analyses have shown that usnic acid is generally present in minor amounts only, or even not detectable in this species (James and White, 1987; Bjerke and Dahl, 2002), and secondly because concentrations in the basal thallus zone were more affected by harvesting time than concentrations in the apical thallus zone. For being able to explain these results in more detail, additional experimentation is required.

The two didepsides phenarctin and nephroarctin showed a different temporal trend than the usnic acids, viz. they increased from July to August. Thus, the concentrations of phenarctin and nephroarctin were correlated with the F_V/F_M values, which also increased from July to August. This correlation is not regarded merely as a coincidence, because the synthesis of secondary metabolites is correlated with availability of photosynthates (BeGora and Fahselt, 2001a; Solhaug and Gauslaa, 2004), which at this locality are more readily available in early autumn (Sonesson et al., 1992; Sonesson, 2001). The differences in temporal responses and UV spectra between the two didepsides and the two dibenzofuranes point towards different ecological functions. To the best of our knowledge, the biological roles of phenarctin and nephroarctin have not been investigated previously, despite the frequent use of *N. arcticum* in ecophysiological studies (e.g. Sonesson et al., 1992; Palmqvist et al., 1994; Land and Lundström, 1998; Sundberg et al., 1999, 2001). Many depsides reduce the activity of viruses, bacteria, fungi and insect herbivores (Huneck, 1999; Müller, 2001; Bjerke et al., 2003), and it is reasonable to believe that phenarctin and nephroarctin play similar roles.

The statistical analyses of the chlorophyll fluorescence measurements show that both species experienced lower PS II efficiency in July than in August, and that enhanced UV-B radiation slightly further reduced the efficiency. These results contrast to those from the same locality by Sonesson et al. (1995), in which the PS II efficiency of three other fruticose lichens was found to be stimulated by enhanced UV-B radiation. However, that study differs from the current particularly in the exposure time and UV-B intensity, which was 45 h of high-energy, ecologically irrelevant, irradiation. Since the effects of enhanced UV-B radiation on PS II efficiency were minor in comparison to the effects of seasonal variation, it is reasonable to believe that other elements of global climatic change (warming, precipitation patterns, etc.) can have more pronounced impact on lichen activity than UV-B radiation. The differences in PS II efficiency between the two lichens may be due to different photobionts, *F. nivalis* possessing a *Trebouxia* photobiont and *N. arcticum* possessing a *Coccomyxa* photobiont, which have different carbon acquisition properties and thereby probably also different state transition properties (Palmqvist et al., 1994; Sundberg et al., 1997).

In conclusion, our data show that enhanced UV-B radiation can reduce slightly the PS II efficiency both of shade-adapted and light-tolerant lichens, but that the lichens during dry growing seasons do not respond to UV-B enhancement by accumulating more UV-absorbing phenolics. The natural seasonal fluctuations in solar radiation, temperature and precipitation may have more impact on the ecophysiology of boreal and arctic–alpine lichens than enhanced UV-B radiation. Nevertheless, it is noteworthy that modest negative short-term effects of UV-B radiation may lead to severe longer-term cumulative effects, as was the

case for the nitrogen fixation potential of the tripartite lichen *Peltigera aphthosa* from Abisko (Solheim et al., 2002). Given the slow growth and long life span of lichens in boreal, alpine and polar environments, studies covering several growing seasons and iterative sampling along climatic gradients in the field, with control measurements of spectral quality and other climatic variables, are required to improve our knowledge on how lichens respond to enhanced UV-B radiation and other elements of global climatic change.

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