

Effects of elevated UV-B radiation on UV-absorbing pigments and leaf anatomy of a sedge, *Eriophorum russeolum*

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Received 11 July 2011, final version received 5 Dec. 2012, accepted 8 June 2012

Mörsky, S. K., Haapala, J. K., Rinnan, R., Saarnio, S., Kyrö, E., Silvola, J., Martikainen, P. J. & Holopainen, T. 2013: Effects of elevated UV-B radiation on UV-absorbing pigments and leaf anatomy of a sedge, *Eriophorum russeolum*. *Boreal Env. Res.* 18: 414–424.

Long-term effects of elevated ultraviolet-B (UV-B) on the sedge *Eriophorum russeolum* were studied in a subarctic fen at Sodankylä in northern Finland for three consecutive growing seasons (2003–2005). Supplemental UV-B exposure (63%, 37% and 21% above ambient in 2003, 2004 and 2005, respectively) was conducted using fluorescent UV-lamps. The experimental plots were divided into three treatments ($n = 10$): ambient control, UV-A control and elevated UV-B. Elevated UV-B transiently increased the amount of cell-wall bound UV-absorbing pigments in *E. russeolum* leaves during the first exposure year, but the concentrations of soluble UV-absorbing compounds, total chlorophyll and carotenoids were not affected. Enhanced UV-B did not affect the leaf anatomy or senescence of *E. russeolum*. Additionally, there were no changes in carbon or nitrogen content and decomposition rate of *E. russeolum* leaves. These results show that *E. russeolum* responds to elevated UV-B radiation primarily by producing UV-absorbing compounds. Hence, projected UV-B radiation levels in the near future will not reduce vitality of *E. russeolum*.

Introduction

Peatlands form a significant part of the landscape especially at the northern latitudes. Pristine peatland ecosystems, such as minerotrophic fens, are either sinks (Aurela 2004) or sources (Carroll and Crill 1997) of atmospheric carbon dioxide and sources of methane, a significant greenhouse gas (e.g. Roulet *et al.* 1992, Rinne *et al.* 2007). In wetlands, *Eriophorum* species

play an important role in annual carbon balance and in peat accumulation. In open peatlands, these plants are freely exposed to harmful UV-B radiation (280–315 nm). Severe ozone depletion in the upper stratosphere has been reversed, but enhanced UV-B radiation levels may still occur (McKenzie *et al.* 2011).

Until now, there has been a limited number of published studies reporting possible adverse UV-B effects on peatland plant species. In their

meta-analysis, Newsham and Robinson (2009) highlighted the effects of UV-B radiation on bryophytes and angiosperms living in the polar regions. The meta-analysis showed that UV-B radiation enhanced concentrations of UV-B absorbing compounds, reduced aboveground biomass and plant height, and increased DNA damage. However, neither carotenoid or chlorophyll concentration, net photosynthesis, total biomass nor leaf area were affected by elevated UV-B. Another meta-analysis summarizing field studies on vascular plants at lower latitudes showed similar results (Searles *et al.* 2001). However, shoot biomass and, to some extent, leaf area can decrease under moderately elevated UV-B doses (> 20% ozone depletion).

UV-B tolerance of a sedge *Eriophorum vaginatum* was studied using peatland microcosms in open-field conditions in central Finland. When microcosms were exposed to enhanced UV-B (30% above ambient) for one growing season, the leaf cross-section area and the percentage of aerenchymatous tissue in *E. vaginatum* leaves were significantly reduced (Niemi *et al.* 2002a). In addition, enhanced UV-B increased the amount of UV-B-absorbing compounds in *Sphagnum papillosum* but decreased them in *S. angustifolium*. Furthermore, in another microcosm study with lower UV intensity, UV-B had no effects on morphology of *E. vaginatum* leaves. However, the concentration of chlorophyll and carotenoid pigments in *S. balticum* were increased by elevated UV-B (Niemi *et al.* 2002b). Grasses and sedges show similar responses to enhanced UV-B radiation. In their study, van de Staaij *et al.* (2002) showed that three grasses (*Deschampsia antarctica*, *Deschampsia borealis* and *Calamagrostis epigeios*) and the sedge *Carex arenaria* did not show drastic changes in the flavonoid concentrations when growing under elevated UV-B (12 weeks, 20% ozone depletion).

The effects of reduced UV-B radiation (10%, near-ambient; 80%, reduced) have been studied in southern Argentina using a UV-filtering approach (Searles *et al.* 2002, Robson *et al.* 2003). During a 3-year-long field study on the ombrotrophic bog, the morphology of vascular plants (*Empetrum rubrum*, *Nothofagus antarctica* and *Tetroncium magellanicum*) was not affected,

but there was a 10%–20% decrease in the amount of UV-B-absorbing compounds of *T. magellanicum* (Searles *et al.* 2002). However, during the fourth to sixth growing seasons, stem growth and branching frequency of *E. rubrum* and branching frequency of *N. antarctica* decreased under near-ambient UV-B (Robson *et al.* 2003).

UV-B radiation can affect the decomposition of plant litter by altering the chemical characteristics of the plant material (Cybulski *et al.* 2000, Pancotto *et al.* 2005). UV-B can also photodegrade plant material and change structure and activity of the decomposer community (Austin and Vivanco 2006, Brandt *et al.* 2007). Photodegradation may increase the decomposition rate, especially in arid ecosystems where microbial activity is limited by low availability of water (Brandt *et al.* 2007, Smith *et al.* 2010). In wetter conditions, the negative effect of UV-B on microbial activity becomes more pronounced and the decomposition rate can be decreased by supplemental UV-B (Moody *et al.* 2001, Pancotto *et al.* 2003, Smith *et al.* 2010). In addition, exposure to UV-B radiation increases the concentration of UV-B absorbing pigments in leaves, which can reduce the decomposability of the leaf litter (Gehrke *et al.* 1995, Brandt *et al.* 2007).

The present UV-B exposure study was conducted in a natural subarctic fen in Sodankylä (northern Finland) in 2003–2005, to assess both the ecosystem and plant responses to realistically elevated UV-B radiation levels. The earlier measurements had shown that, firstly net ecosystem CO₂ exchange (NEE) at light saturation was slightly higher under the UV-B treatment, but the rate of gross photosynthesis at the ecosystem level (Haapala *et al.* 2009) as well as, the leaf-specific CO₂ assimilation rate and fine structure of the dominant species *E. russeolum* and *Warnstorfia exannulata* were not affected by the UV-B exposure (Haapala *et al.* 2010). Secondly, elevated UV-B did not affect the net methane emission, but slightly increased the concentrations of acetate and propionate (substrates for methanogenesis) in peat pore water (Mörsky *et al.* 2012). Thirdly, during the third growing season (2005), the UV-B treatment significantly reduced the sucrose and the total soluble sugar concentrations in the *E. russeolum* leaves, but simultaneously increased the sucrose concentra-

tion in the storage organ, rhizome (Rinnan *et al.* 2008). Carbohydrate concentrations remained unaffected in the plant roots, but the total phenolics concentration increased as a result of elevated UV-B. There were no effects on microbial biomass in peat, but the decrease in the bacterial growth rate and the changes in the bacterial community composition in peat suggested UV-B-induced changes in below-ground carbon allocation (Rinnan *et al.* 2008). Finally, total emissions of biogenic non-methane volatile organic compounds (BVOC) were not affected by elevated UV-B (Faubert *et al.* 2010). In contrast, isoprene emissions, related to the *E. russeolum* leaf density, were significantly increased by the enhanced UV-B radiation during the second (2004) growing season (Tiiva *et al.* 2007).

Since leaf chemistry (e.g. pigments) and anatomy (e.g. plant height) are among the most sensitive parameters responding to elevated UV-radiation (Searles *et al.* 2001, Newsham and Robinson 2009) we performed a more detailed study of these topics using *E. russeolum* during the three exposure years (2003–2005).

Based on the present knowledge, we hypothesized that long-term elevated UV-B exposure would (1) increase UV-absorbing pigments in leaves, (2) alter the chlorophyll pigment concentration in leaves, (3) alter anatomy and senescence of leaves and (4) decrease the decomposition rate of leaf litter.

Material and methods

Experimental design

A natural subarctic fen ecosystem in Sodankylä (northern Finland, Halssiaapa, 67°22'N, 26°39'E, 179 m a.s.l) was exposed to elevated UV-B radiation for three growing seasons (2003–2005). The fen consists of hummock strings with wet flarks in between. The vegetation is dominated by two plant species: the sedge *Eriophorum russeolum* and the moss *Warnstorfia exanulata*. In the spring, the study site was flooded, and during the summer the water table remained a few centimetres below the peat surface.

The experimental setup consisted of 30 plots (120 × 120 cm) randomly divided into three

groups ($n = 10$): ambient controls, UV-A controls and elevated UV-B. The UV-A controls were included in the experiment because the UV lamps always emit UV-A radiation that can also affect plants (Newsham *et al.* 1996). Equal minor shading, as created by the lamp arrays, was achieved in the ambient-control plots by using similar frames as for the lamps. Irradiation treatments were conducted using four Philips TL 40W/12 RS fluorescent tubes (Philips Lighting, Eindhoven, The Netherlands) per study plot that formed a square 120 cm above the peat surface. In the UV-B treatment, the fluorescent tubes were covered with a thin (0.1 mm) cellulose-acetate filter (Expopak Oy, Jämskipohja, Finland) to cut off wavelengths below 290 nm. In the UV-A control, a thin (0.125 mm) polyester filter (Melinex/Polyfoil, KTA-yhtiöt Oy, Helsinki, Finland) was used to filter wavelengths below 315 nm.

The irradiation system was constructed to maintain UV-B irradiation 46% above the prevailing ambient level, thus simulating 20% ozone depletion. Irradiation levels in the plot centre and at the vegetation level were monitored by erythemally (CIE) weighted PMA1102 (UV-B) and PMA1111 (UV-A) sensors (Solar Light Co. Inc., Glenside, PA, USA). Air temperature at each plot was monitored using TinyTalk TK-0040 data loggers (Gemini Data Loggers Ltd., Chichester, West Sussex, UK). Water table was measured four times a week during the growing seasons from perforated pipes located within each study plot. A more detailed description of the experimental setup is reported in Haapala *et al.* (2009) and Mörsky *et al.* (2012).

For technical reasons (a regulation adjustment in the system in 2003, moisture related connection problems in UV lamps in 2004–2005), there was some variation in the UV-B enhancement between the growing seasons (Fig. 1). In the first growing season (2003), the cumulative UV-B dose (weeks 25–40) was 147 kJ m⁻² in the ambient and 239 kJ m⁻² (+63%) in the UV-B treatments. In the second growing season (2004), the UV-B doses (weeks 24–40) were 135 kJ m⁻² and 185 kJ m⁻² (+37%) in the ambient and UV-B treatments, respectively. In the third growing season (2005), the UV-B doses (weeks 23–39) were 165 kJ m⁻² in the ambient and 199 kJ m⁻² (+21%) in the UV-B treatments.

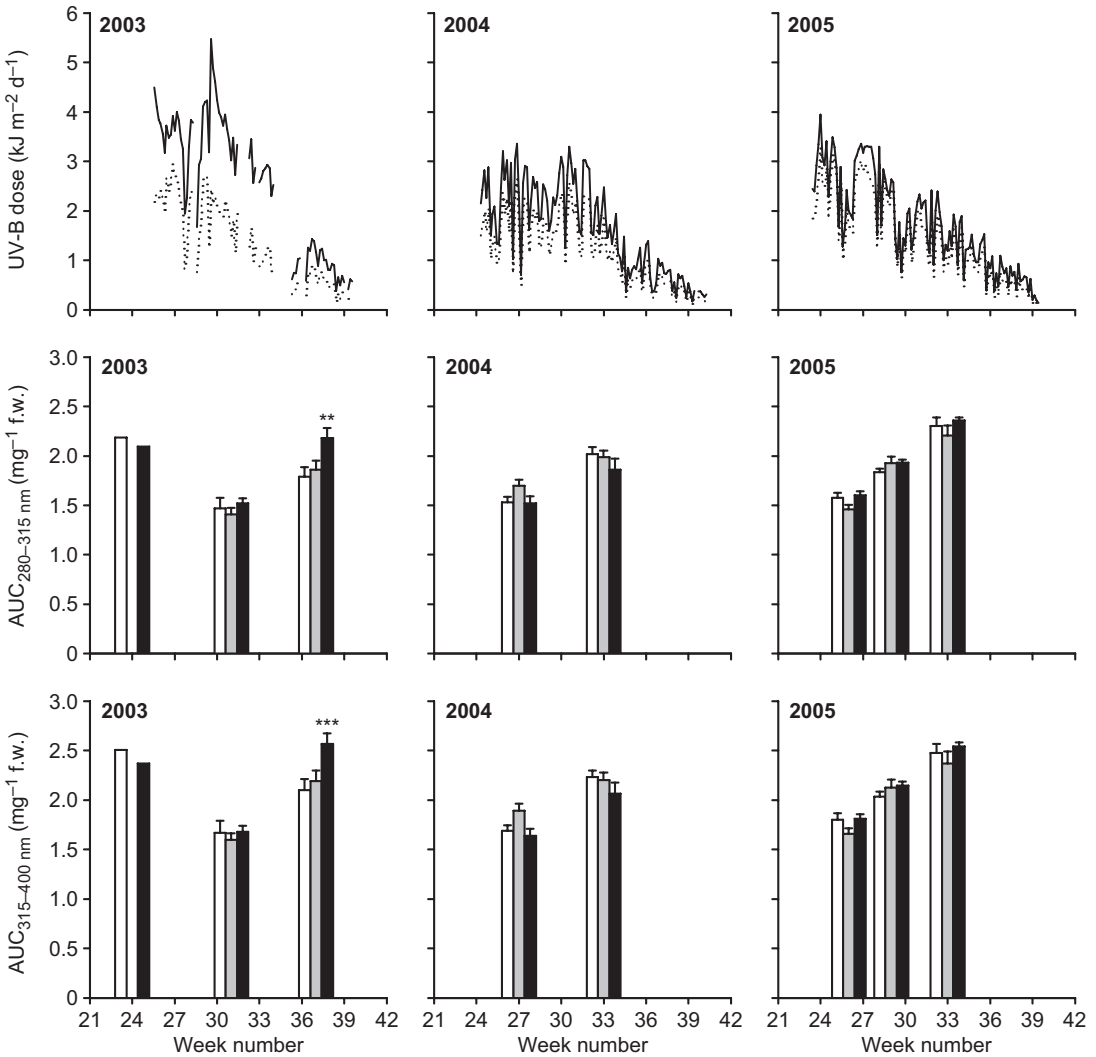


Fig. 1. UV-B dose and cell wall bound (NaOH soluble) UV-absorbing pigments (UV-B_{280-315 nm} and UV-A_{315-400 nm} regions) analysed from *Eriophorum russeolum* leaves (normalized to sample fresh weight) during the three growing seasons (2003–2005). Dotted lines and empty bars represent ambient control, grey bars UV-A control, and black lines and black bars UV-B treatment, respectively. Error bars show the standard errors (SE) of the means ($n = 10$). Asterisks indicate significant differences between the ambient control and the UV-B treatment or the UV-A control within separate measurements. ** $p < 0.01$; *** $p < 0.001$ (linear mixed models, Bonferroni's test).

The precipitation and air temperature data (2003–2005) were obtained from the Arctic Research Centre of the Finnish Meteorological Institute at Sodankylä (H. Suokanerva pers. comm.). During the first growing season (2003), the precipitation (weeks 22–41) was 259 mm, close to the long-term average for the study region (Finnish Meteorological Institute 2003). In the following growing seasons, 2004 and 2005, the precipitation during the same period

was 322 and 314 mm, respectively. The daily mean air temperatures (weeks 22–41) were 11.1, 10.6 and 11.9 °C in 2003, 2004 and 2005, respectively.

Pigment analyses

The amounts of methanol-extractable (soluble) and sodium-hydroxide-extractable (cell-wall

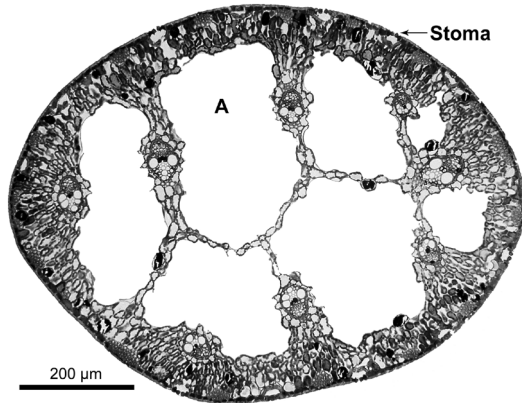


Fig. 2. A cross-section of an *Eriophorum russeolum* leaf. **A:** a gas cavity of aerenchymatous tissue.

bound) compounds in young (length < 12 cm) *E. russeolum* leaves were determined two to three times during each growing season (2003–2005) using a method adopted from Ruhland and Day (2000) and Ruhland *et al.* (2005) with some modifications. A pooled sample was collected from each study plot and the leaf material was frozen in liquid nitrogen. Firstly, MeOH–HCl–H₂O solution (90/1/1, v/v) was used and soluble compounds were analyzed as described in Mörsky *et al.* (2011). The amount of cell-wall bound compounds was analyzed from the same plant material. The leaf pieces were put in an Erlenmeyer flask and 2M NaOH solution was added. The flask was flushed with pure nitrogen for two hours (25 °C) and the reaction was stopped with HCl. Leaf material was removed and MeOH–HCl–H₂O solution (90/1/1, v/v) was added to make soluble and cell-wall bound pigment fractions comparable. Finally, the solution was filtered and the results were calculated as described in Mörsky *et al.* (2011).

Chlorophyll *a*, chlorophyll *b* and carotenoid concentrations in freeze-dried *E. russeolum* leaves were measured using the dimethyl-sulphoxide (DMSO) method (Barnes *et al.* 1992). Two to three times per growing season (2003–2005) pooled samples of juvenile *E. russeolum* leaves (length < 12 cm) were collected from each study plot and frozen in liquid nitrogen. Chlorophyll and carotenoid concentrations of DMSO extracts were analyzed with a spectrophotometer as described in Mörsky *et al.* (2011).

Leaf anatomy and senescence

Four randomly selected, young, current-year *E. russeolum* leaves (length < 12 cm) per study plot were collected two to three times during the growing seasons (2003–2005). First, a tip (3 cm) of the *E. russeolum* leaf was cut and inserted in a chilled glutaraldehyde fixative (2.5% in 0.1 M sodium-phosphate buffer, pH 7.0). Second, a 1.5 mm-long piece from the base of each 3 cm-long leaf tip was cut in the laboratory and prefixed in a glutaraldehyde fixative at 4 °C overnight. After the osmium tetroxide (OsO₄) post-fixation and dehydration in a graded ethanol series, the pieces were embedded in LX SS112 Epon resin (Ladd Research Industries, Burlington, VT, USA). Third, semi-thin cross-sections (thickness ~1 μm) of the *E. russeolum* samples were cut for light microscopy, placed onto microscope slides and stained (for more details see Niemi *et al.* 2002b). The slides were examined under a Zeiss-Axiolab light microscope (Carl Zeiss, Jena, Germany) and digitally photographed (Fig. 2). The areas of the whole leaf cross-section and empty space (aerenchyma) were determined from the pictures using the ImageJ-program (Rasband W.S., ImageJ, U.S. National Institutes of Health, Bethesda, Maryland, USA, <http://imagej.nih.gov/ij/>, 1997–2011). The rest of the sampled *E. russeolum* leaf (3–8 cm from the tip) was used to determine the stomatal density with a super glue method described in Mörsky *et al.* (2008).

In *E. russeolum* shoots, senescence of the two youngest leaves was measured as negative length growth (tip of the leaf is dying and getting brown) during three growing seasons (2003–2005). At the beginning of the growing season, four *E. russeolum* shoots per study plot were randomly selected and marked. Negative length growth of the green part of each leaf was determined with a measurement interval from 2 to 5 weeks. The results were calculated by dividing length reduction (mm) with time (number of days between measurements).

C:N ratio and litter decomposition

Carbon and nitrogen contents in the *E. russeolum* leaves were measured from the freeze-

dried and ground samples collected at the end of July (week 29) in 2005. Three replicates were processed for each sample and analyzed with a CHN-S/O elemental analyzer (Carlo Erba Strumentazione, Milan, Italy). The results with analytical reproducibility better than $\pm 2\%$ were accepted.

The *in-situ* decomposition rate of *E. russeolum* litter was studied during the growing season of 2005. Samples of senescent leaves were collected from the study plots at the beginning of June (week 26) and dried at 25 °C for 7 days. Two replicate leaf-litter samples of approximately 0.7 g (dry weight) were collected from each study plot and from outside the study plots (grown in ambient sun light). The litter samples were packed in 10 × 10 cm polyethylene bags (mesh size 0.5 mm) and placed in the study plots on the peat surface. The litterbags were collected from the field after 3.5 months (week 39). Extraneous plant material and peat particles were carefully removed from the bags and they were dried at 25 °C before weighing.

Statistical analyses

Treatment effects, temporal changes and interaction effects between treatment and time were tested using linear mixed models (LMM) followed by Bonferroni's test, more conservative than LSD, for pairwise comparisons. When analysing a data set that represents several years with repeated measurements, LMM procedure may fail to detect transient significant treatment effects. Thus, Bonferroni's post-hoc test was also conducted when LMM analysis showed no significant treatment effects. In the model, treatment, time and their interaction were regarded as fixed factors. Furthermore, treatment plot was set as a random factor. Water table, measured weekly, was used as a covariate for photosynthesis-related pigments and leaf senescence. The C:N ratio, N concentration and litter decomposition data were tested with one-way ANOVA. Prior to statistical analyses, the data were tested for normality with the Kolmogorov-Smirnov test and log-transformed when needed. Normality tests were verified by inspecting the data distribution diagrams of each variable. The analyses

were conducted with the SPSS 14.0 and 17.0 statistical packages (SPSS Inc., Chicago, IL, USA).

Results

Pigment analyses

Elevated UV-B radiation did not have any overall treatment effects on the amount of the cell-wall bound UV-absorbing pigments ($AUC_{280-315}$) of the *E. russeolum* leaves during the growing seasons 2003–2005 (LMM, Time: $F = 46.328$, $p < 0.0005$; Treatment: $F = 0.472$, $p = 0.627$; Time × Treatment: $F = 2.241$, $p = 0.010$) (Fig. 1). However, elevated UV-B caused a significant increase at the end of the first growing season (2003, week 37: Bonferroni's test, $p = 0.001$). The effect was similar when the absorbance was measured in the UV-A wavelength region ($AUC_{315-400}$). In contrast, enhanced UV-B did not alter the amount of methanol-extractable pigments in *E. russeolum* leaves during the growing seasons 2003–2005 (data not presented).

Total chlorophyll and carotenoid concentrations in *E. russeolum* leaves were not affected by elevated UV-B radiation during the growing seasons 2003–2005 (Table 1). The chlorophyll and carotenoid concentrations were lower in the first growing season (2003) as compared with those in two following ones. Elevated UV-B tended to decrease chlorophyll and carotenoid concentrations during 2003 and 2004, but the differences were not statistically significant.

Leaf anatomy and senescence

The number of stomata in *E. russeolum* leaves did not show any clear response to the enhanced UV-B during the three consecutive growing seasons (2003–2005) (Fig. 3). However, the stomatal density was significantly lower under the UV-A control than the ambient control (LMM: Time: $F = 27.026$, $p < 0.001$; Treatment: $F = 3.459$, $p = 0.033$; Time × Treatment: $F = 1.649$, $p = 0.069$). The difference was significant during the first (2003, week 24: LMM, Bonferroni's test, $p = 0.014$) and the fifth (2004, week 34: LMM, Bonferroni's test, $p = 0.001$) measurements.

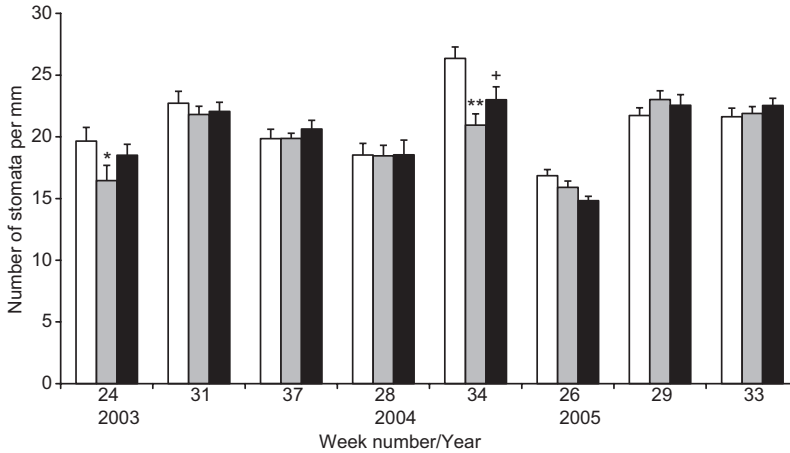


Fig. 3. Numbers of stomata (mean + SE, $n = 10$) in *Eriophorum russeolum* leaves during the three growing seasons (2003–2005). Empty bars represent the ambient control, grey bars the UV-A control and black bars the UV-B treatment. A plus sign and asterisks indicate significant differences between the ambient control and the UV-B treatment or the UV-A control within separate measurements. * $p < 0.1$; * $p < 0.5$; ** $p < 0.01$ (linear mixed models, Bonferroni's test).

UV-B treatment did not affect the cross-section area (LMM: Time: $F = 12.035$, $p < 0.001$; Treatment: $F = 0.029$, $p = 0.971$; Time \times Treatment: $F = 0.853$, $p = 0.611$) in the *E. russeolum*

leaves or the amount of aerenchymatous tissue (LMM: Time: $F = 9.382$, $p < 0.001$; Treatment: $F = 0.133$, $p = 0.876$; Time \times Treatment: $F = 0.882$, $p = 0.579$) of the leaves during any of the grow-

Table 1. Effects of elevated UV-B radiation on chlorophyll (*a* and *b*) and carotenoid concentrations (mean \pm SE, $n = 10$) in *Eriophorum russeolum* leaves during the three growing seasons (2003–2005). The results are normalized to the sample dry weight.

Year	Treatment	Week	Chlorophyll (<i>a</i> + <i>b</i>) (mg g ⁻¹)	Carotenoids (mg g ⁻¹)	Chl <i>a</i> /Chl <i>b</i>	Total Chl/Carot
2003	Ambient control	31	2.93 \pm 0.16	0.85 \pm 0.04	4.24 \pm 0.17	3.42 \pm 0.05
	UV-A control	31	3.69 \pm 0.17*	1.00 \pm 0.04	3.91 \pm 0.09	3.70 \pm 0.07*
	UV-B	31	3.33 \pm 0.16	0.95 \pm 0.02	4.05 \pm 0.12	3.49 \pm 0.13
2003	Ambient control	37	3.19 \pm 0.23	0.86 \pm 0.06	4.88 \pm 0.18	3.70 \pm 0.07
	UV-A control	37	3.18 \pm 0.20	0.87 \pm 0.05	4.63 \pm 0.27	3.63 \pm 0.05
	UV-B	37	3.00 \pm 0.04	0.80 \pm 0.01	4.44 \pm 0.14	3.73 \pm 0.04
2004	Ambient control	28	5.86 \pm 0.06	1.59 \pm 0.02	4.94 \pm 0.03	3.70 \pm 0.02
	UV-A control	28	5.69 \pm 0.16	1.54 \pm 0.03	4.84 \pm 0.05	3.70 \pm 0.03*
	UV-B	28	5.62 \pm 0.08	1.41 \pm 0.03*	4.58 \pm 0.05	4.00 \pm 0.05
2004	Ambient control	33	5.00 \pm 0.14	1.43 \pm 0.05	4.77 \pm 0.08	3.51 \pm 0.02
	UV-A control	33	5.05 \pm 0.14	1.38 \pm 0.02	4.66 \pm 0.06	3.65 \pm 0.05
	UV-B	33	4.87 \pm 0.11	1.34 \pm 0.03	4.49 \pm 0.07	3.64 \pm 0.03
2005	Ambient control	26	5.87 \pm 0.11	1.39 \pm 0.04	4.02 \pm 0.14	4.23 \pm 0.07
	UV-A control	26	6.27 \pm 0.08	1.52 \pm 0.01	4.49 \pm 0.02	4.12 \pm 0.06
	UV-B	26	6.03 \pm 0.06	1.47 \pm 0.01	4.29 \pm 0.12	4.10 \pm 0.03
2005	Ambient control	29	4.76 \pm 0.12	1.30 \pm 0.04	3.97 \pm 0.18	3.67 \pm 0.05
	UV-A control	29	4.97 \pm 0.07	1.34 \pm 0.02	3.79 \pm 0.04	3.71 \pm 0.02
	UV-B	29	4.99 \pm 0.09	1.34 \pm 0.03	3.79 \pm 0.08	3.72 \pm 0.05
2005	Ambient control	33	3.95 \pm 0.09	1.09 \pm 0.04	3.65 \pm 0.13	3.64 \pm 0.10
	UV-A control	33	4.48 \pm 0.12	1.19 \pm 0.03	3.42 \pm 0.06	3.76 \pm 0.06
	UV-B	33	4.25 \pm 0.14	1.15 \pm 0.03	3.57 \pm 0.09	3.71 \pm 0.08

Asterisks (*) indicate significant differences between the ambient control and the UV-B treatment or the UV-A control within separate measurements. * $p < 0.05$ (linear mixed models, Bonferroni's test).

ing seasons 2003–2005 (Table 2; only the 2005 data presented). In the latter part of the last growing season (week 33, 2005), the cross-section area (LMM, Bonferroni's test: $p = 0.070$) and the aerenchymatous tissue area (LMM, Bonferroni's test: $p = 0.032$) of the *E. russeolum* leaves were slightly smaller in the UV-A control as compared with those in the ambient control. Elevated UV-B radiation did not affect the *E. russeolum* leaf senescence rate (data not presented).

C:N ratio and litter decomposition

The elevated UV-B did not change the carbon or nitrogen content of the *E. russeolum* leaves (Table 3). Supplemental UV-B radiation during plant growth and/or litter decomposition did not affect the mass loss of *E. russeolum* leaf litter either.

Discussion

The amount of cell-wall bound pigments in *E. russeolum* leaves, measured in the UV-B and the UV-A regions, increased transiently at the end of the first growing season (2003). This finding is congruent with our hypothesis and supports those reported for *S. papillosum*, but not those for *S. angustifolium* (Niemi *et al.* 2002a). The results by Martz *et al.* (2011) from the Halssiaapa experimental field showed significant UV effects in *E. russeolum* leaves. Total soluble phenolics were higher in the leaves exposed to enhanced UV-A and UV-B radiation than in the control leaves, but

Table 2. Effects of elevated UV-B radiation on cross-section area and aerenchymatous tissue area in *Eriophorum russeolum* leaves (mean \pm SE, $n = 10$) during the 2005 growing season.

Treatment	Week	Cross-section (mm ²)	Aerenchyma (mm ²)
Ambient control	26	0.39 \pm 0.01	0.18 \pm 0.01
UV-A control	26	0.39 \pm 0.03	0.17 \pm 0.02
UV-B	26	0.37 \pm 0.02	0.17 \pm 0.01
Ambient control	29	0.37 \pm 0.02	0.17 \pm 0.01
UV-A control	29	0.38 \pm 0.02	0.17 \pm 0.02
UV-B	29	0.37 \pm 0.01	0.16 \pm 0.01
Ambient control	33	0.45 \pm 0.01	0.21 \pm 0.01
UV-A control	33	0.39 \pm 0.02 ⁺	0.17 \pm 0.01 [*]
UV-B	33	0.44 \pm 0.01	0.20 \pm 0.01

A plus sign and an asterisk (*) indicate statistically significant differences between the ambient control and the UV-A control within separate measurements. ⁺ $p < 0.1$, ^{*} $p < 0.05$ (linear mixed models, Bonferroni's test)

the phenolic composition was not significantly modified. Very similar results were reported for a UV-B exclusion experiment conducted in an oligotrophic tall-sedge *Sphagnum* flark fen in Vuotso, Finland (Soppela *et al.* 2006).

In their meta-analysis Newsham and Robinson (2009) showed that exposure to UV-B radiation in UV enclosure and nonmanipulative studies (simulated ozone depletion varied between 10%–20%) increased the concentrations of UV-B absorbing compounds in plant leaves and lichen thalli, but fluorescent UV lamps failed to elicit a change in the concentration of these compounds. In the present study, we did not see any treatment effects on the concentration of methanol soluble UV-absorbing compounds. We suggest that elevated UV-B radiation might increase concen-

Table 3. Effects of supplemental UV-B radiation on the C:N ratio and N concentration in living *Eriophorum russeolum* leaves and mass loss of senescent *E. russeolum* leaves (mean \pm SE, $n = 10$) during the 2005 growing season. The sampling for C and N analyses was conducted at the later part of July (week 29). The litter decomposition experiment lasted from early June to the end of September (weeks 26–39). For the decomposition experiment, two kinds of plant material were used: plant material collected from the experimental plots (treatment) and plant material grown in ambient UV-radiation outside the experimental plots (ambient).

	C:N ratio	N (mg g ⁻¹ d.w.)	Mass loss _{treatment} (%)	Mass loss _{ambient} (%)
Ambient control	27.1 \pm 0.65	18.0 \pm 0.5	12.5 \pm 0.6	13.1 \pm 1.2
UV-A control	26.5 \pm 0.39	18.3 \pm 0.3	16.7 \pm 2.1	15.2 \pm 1.8
UV-B	27.1 \pm 0.42	17.9 \pm 0.3	13.7 \pm 1.7	14.1 \pm 0.8
F; df; p*	0.413; 2,26; 0.666	0.279; 2,26; 0.759	1.929; 2,27; 0.165	0.593; 2,24; 0.593

* One-way ANOVA.

trations of those compounds more in cell walls than in the methanol-soluble fraction analyzed traditionally, but a significant increase in UV-B (> 60%) is needed to cause this response.

Chlorophyll and carotenoid concentrations in *E. russeolum* leaves, in general, were not affected by elevated UV-B radiation. Earlier Haapala *et al.* (2010) reported that UV-B treatment had no effect on the net CO₂ assimilation rate or the structure of the cell organelles of *E. russeolum* leaves supporting the findings of this study. However, at the end of the first growing season (2003) elevated UV-B tended to decrease the concentrations of the photosynthetically active pigments. During the two following growing seasons, with much lower UV-B radiation levels and higher water table, a similar effect was not apparent.

Contrary to our hypothesis, elevated UV-B did not affect *E. russeolum* leaf anatomy or senescence rate. According to Niemi *et al.* (2002a), elevated UV-B reduced leaf cross-section area and percentage of aerenchymatous tissue in *E. vaginatum* leaves growing in peatland microcosms. The reason for the different UV-B response of these two *Eriophorum* species might be species-related since the supplemental UV-B exposure levels used were similar. The result emphasizes the fact that moderate UV-B enhancement even during the dry summer season does not decrease the vitality of *E. russeolum*.

Supplemental UV-B radiation both during plant growth and litter decomposition has been shown to affect the decomposition process by altering both plant chemistry and decomposer community (Cybulski *et al.* 2000, Moody *et al.* 2001). Moreover, allocation of photosynthesis products and rate of root exudation can be altered by supplemental UV-B, which can affect the activity and composition of the soil microbial community (Rinnan *et al.* 2008). However, the plant species, environmental conditions and experimental setup have major roles in determining the magnitude and direction of the UV-B effect on litter decomposition (Brandt *et al.* 2007, Kotilainen *et al.* 2009). In contrast to our hypothesis, the decomposition rate of *E. russeolum* leaves on the peat surface was not reduced by elevated UV-B. The decomposition rate was unaltered despite the decreased microbial growth

rate in peat pore-water in both UV-A control and UV-B treatments in 2005 (Rinnan *et al.* 2008). However, the UV-B treatment had no effect on the total phenolics concentration in *E. russeolum* leaves in 2005 (Rinnan *et al.* 2008), which points to the lack of clear effects on leaf chemistry. This is in line with the litter decomposition results of the present study.

The target level for the UV-B enhancement was set to 46% above the ambient radiation level. However, due to the technical challenges in humid open-field conditions, the UV-B enhancement levels varied from the target yearly. The UV-B enhancement was highest during the first growing season (2003) and lowest during the third growing season (2005). In general, our results indicate that peatland plants, especially *E. russeolum*, can tolerate realistically (21%–63%) enhanced UV-B radiation levels. Even though there were changes in leaf pigment concentrations under the highest elevated UV-B radiation levels, these changes were reversible. Thus, it is probable that predicted stratospheric ozone depletion periods will not significantly affect carbon sequestration capacity of sedges in northern peatland ecosystems.

Acknowledgements: This study was financially supported by the Academy of Finland (projects 200884 and 200888), the Environmental Risk Assessment Centre — ERAC, the Graduate School in Forest Sciences, the Finnish Cultural Foundation and the Kone Foundation. We are grateful to Hanne Suokanerva and the staff of the Arctic Research Centre of the Finnish Meteorological Institute for maintaining the exposure field throughout the study and for the environmental data. We would like to thank Merja Mustonen, Saara Patronen and Tuulia Venäläinen for their assistance with fieldwork. The English language was revised by James Blande.

References

- Aurela M. 2004. The timing of snow melt controls the annual CO₂ balance in a subarctic fen. *Geophys. Res. Lett.* 31: L16119., doi:10.1029/2004GL020315.
- Austin A.T. & Vivanco L. 2006. Plant litter decomposition in a semi-arid ecosystem controlled by photodegradation *Nature* 442: 555–558.
- Barnes J.D., Balaguer L., Manrique E., Elvira S. & Davison A.W. 1992. A reappraisal of the use of DMSO for the extraction and determination of chlorophylls *a* and *b* in lichens and higher-plants. *Environ. Exp. Bot.* 32: 85–100.
- Brandt L.A., King J.Y. & Milchunas D.G. 2007. Effects of

- ultraviolet radiation on litter decomposition depend on precipitation and litter chemistry in a shortgrass steppe ecosystem. *Global Change Biol.* 13: 2193–2205.
- Carroll P. & Crill P. 1997. Carbon balance of a temperate poor fen. *Global Biogeochem. Cycles* 11: 349–356.
- Cybulski W.J., Peterjohn W.T. & Sullivan J.H. 2000. The influence of elevated ultraviolet-B radiation (UV-B) on tissue quality and decomposition of loblolly pine (*Pinus taeda* L.) needles. *Environ. Exp. Bot.* 44: 231–241.
- Faubert P., Tiiva P., Rinnan Å., Räsänen J., Holopainen J.K., Holopainen T., Kyrö E. & Rinnan R. 2010. Non-methane biogenic volatile organic compound emissions from a subarctic peatland under enhanced UV-B radiation. *Ecosystems* 13: 860–873.
- Finnish Meteorological Institute 2003. *Ilmastokatsaus no. 1–12/2003*.
- Gehrke C., Johanson U., Callaghan T.V., Chadwick D. & Robinson C.H. 1995. The impact of enhanced ultraviolet-B radiation on litter quality and decomposition processes in *Vaccinium* leaves from the Subarctic. *Oikos* 72: 213–222.
- Haapala J.K., Mörsky S.K., Saarnio S., Suokanerva H., Kyrö E., Silvola J. & Holopainen T. 2010. Long-term effects of elevated UV-B radiation on photosynthesis and ultrastructure of *Eriophorum russeolum* and *Warnstorfia exannulata*. *Sci. Total Environ.* 409: 370–377.
- Haapala J.K., Mörsky S.K., Saarnio S., Rinnan R., Suokanerva H., Kyrö E., Latola K., Martikainen P.J., Holopainen T. & Silvola J. 2009. Carbon dioxide balance of a fen ecosystem in northern Finland under elevated UV-B radiation. *Global Change Biol.* 15: 943–954.
- Kotilainen T., Haimi J., Tegelberg R., Julkunen-Tiitto R., Vapaavuori E. & Aphalo P.J. 2009. Solar ultraviolet radiation alters alder and birch litter chemistry that in turn affects decomposers and soil respiration. *Oecologia* 161: 719–728.
- Martz F., Turunen M., Julkunen-Tiitto R., Suokanerva H. & Sutinen M.-L. 2011. Different response of two reindeer forage plants to enhanced UV-B radiation: modification of the phenolic composition. *Polar Biol.* 34: 411–420.
- McKenzie R.L., Aucamp P.J., Bais A.F., Bjoern L.O., Ilyas M. & Madronich S. 2011. Ozone depletion and climate change: impacts on UV radiation. *Photochem. Photobiol. Sci.* 10: 182–198.
- Moody S.A., Paul N.D., Bjorn L.O., Callaghan T.V., Lee J.A., Manetas Y., Rozema J., Gwynn-Jones D., Johanson U., Kyparissis A. & Oudejans A.M.C. 2001. The direct effects of UV-B radiation on *Betula pubescens* litter decomposing at four European field sites. *Plant Ecol.* 154: 29–36.
- Mörsky S.K., Haapala J.K., Rinnan R., Tiiva P., Saarnio S., Silvola J., Holopainen T. & Martikainen P.J. 2008. Long-term ozone effects on vegetation, microbial community and methane dynamics of boreal peatland microcosms in open-field conditions. *Global Change Biol.* 14: 1891–1903.
- Mörsky S.K., Haapala J.K., Rinnan R., Saarnio S., Silvola J., Martikainen P.J. & Holopainen T. 2011. Minor effects of long-term ozone exposure on boreal peatland species *Eriophorum vaginatum* and *Sphagnum papillosum*. *Environ. Exp. Bot.* 72: 455–463.
- Mörsky S.K., Haapala J.K., Rinnan R., Saarnio S., Suokanerva H., Latola K., Kyrö E., Silvola J., Holopainen T. & Martikainen P.J. 2012. Minor long-term effects of ultraviolet-B radiation on methane dynamics of a subarctic fen in northern Finland. *Biogeochemistry* 108: 233–243.
- Newsham K.K. & Robinson S.A. 2009. Responses of plants in polar regions to UVB exposure: a meta-analysis. *Global Change Biol.* 15: 2574–2589.
- Newsham K.K., McLeod A.R., Greenslade P.D. & Emmett A.A. 1996. Appropriate controls in outdoor UV-B supplementation experiments. *Global Change Biol.* 2: 319–324.
- Niemi R., Martikainen P.J., Silvola J., Wulff A., Turtola S. & Holopainen T. 2002a. Elevated UV-B radiation alters fluxes of methane and carbon dioxide in peatland microcosms. *Global Change Biol.* 8: 361–371.
- Niemi R., Martikainen P.J., Silvola J., Sonninen E., Wulff A. & Holopainen T. 2002b. Responses of two *Sphagnum* moss species and *Eriophorum vaginatum* to enhanced UV-B in a summer of low UV intensity. *New Phytol.* 156: 509–515.
- Pancotto V.A., Sala O.E., Robson T.M., Caldwell M.M. & Scopel A.L. 2005. Direct and indirect effects of solar ultraviolet-B radiation on long-term decomposition. *Global Change Biol.* 11: 1982–1989.
- Pancotto V.A., Sala O.E., Cabello M., Lopez N.I., Robson T.M., Ballare C.L., Caldwell M.M. & Scopel A.L. 2003. Solar UV-B decreases decomposition in herbaceous plant litter in Tierra del Fuego, Argentina: potential role of an altered decomposer community. *Global Change Biol.* 9: 1465–1474.
- Rinnan R., Nerg A., Ahtoniemi P., Suokanerva H., Holopainen T., Kyrö E. & Bååth E. 2008. Plant-mediated effects of elevated ultraviolet-B radiation on peat microbial communities of a subarctic mire. *Global Change Biol.* 14: 925–937.
- Rinne J., Riutta T., Pihlatie M., Aurela M., Haapanala S., Tuovinen J., Tuittila E. & Vesala T. 2007. Annual cycle of methane emission from a boreal fen measured by the eddy covariance technique. *Tellus* 59B: 449–457.
- Robson T.M., Pancotto V.A., Flint S.D., Ballare C.L., Sala O.E., Scopel A.L. & Caldwell M.M. 2003. Six years of solar UV-B manipulations affect growth of *Sphagnum* and vascular plants in a Tierra del Fuego peatland. *New Phytol.* 160: 379–389.
- Roulet N., Moore T., Bubier J. & Lafleur P. 1992. Northern fens: methane flux and climatic change. *Tellus* 44B: 100–105.
- Ruhland C.T., Xiong F.S., Clark W.D. & Day T.A. 2005. The influence of ultraviolet-B radiation on growth, hydroxycinnamic acids and flavonoids of *Deschampsia antarctica* during Springtime ozone depletion in Antarctica. *Photochem. Photobiol.* 81: 1086–1093.
- Ruhland C.T. & Day T.A. 2000. Effects of ultraviolet-B radiation on leaf elongation, production and phenylpropanoid concentrations of *Deschampsia antarctica* and *Colobanthus quitensis* in Antarctica. *Physiol. Plantarum* 109: 244–251.
- Searles P.S., Flint S.D. & Caldwell M.M. 2001. A meta analysis of plant field studies simulating stratospheric

- ozone depletion. *Oecologia* 127: 1–10.
- Searles P.S., Flint S.D., Diaz S.B., Rousseaux M.C., Ballare C.L. & Caldwell M.M. 2002. Plant response to solar ultraviolet-B radiation in a southern South American *Sphagnum* peatland. *J. Ecol.* 90: 704–713.
- Smith W.K., Gao W., Steltzer H., Wallenstein M.D. & Tree R. 2010. Moisture availability influences the effect of ultraviolet-B radiation on leaf litter decomposition. *Global Change Biol.* 16: 484–495.
- Soppela P., Turunen M., Forbes B., Aikio P., Magga H., Sutinen M.-L., Lakkala K. & Uhlig C. 2006. The chemical response of reindeer summer pasture plants in a subarctic peatland to ultraviolet (UV) radiation. *Ecol. Studies* 184: 199–213.
- van de Staaij J., de Bakker N. V. J., Oosthoek A., Broekman R., van Beem A., Stroetenga M., Aerts R. & Rozema J. 2002. Flavonoid concentrations in three grass species and a sedge grown in the field and under controlled environment conditions in response to enhanced UV-B radiation. *Photochem. and Photobiol. B* 66: 21–29.
- Tiiva P., Rinnan R., Faubert P., Räsänen J., Holopainen T., Kyrö E. & Holopainen J.K. 2007. Isoprene emission from a subarctic peatland under enhanced UV-B radiation. *New Phytol.* 176: 346–355.