Available online at www.sciencedirect.com

SCIENCE DIRECT®

Science of the Total Environment 343 (2005) 155–167

Science of the Total Environment
An International Journal for Scientific Research into the Environment and its Relationship with Humankind

www.elsevier.com/locate/scitotenv

Effects of season and low-level air pollution on physiology and element content of lichens from the U.S. Pacific Northwest

H.S.Y. Ra[a], L.H. Geiser[b], R.F.E. Crang[a,*]

[a]*Department of Plant Biology, University of Illinois, Urbana, IL 61801, USA*
[b]*USDA Forest Service, Pacific Northwest Regional Air Program, Corvallis, OR 97339, USA*

Received 22 February 2004; received in revised form 7 October 2004; accepted 15 October 2004

Abstract

Lichens were collected from three low-elevation sites in the western Cascade Range: HJ Andrews, OR (clean) and Bull Run, OR, and Pack Forest, WA (moderately enhanced nitrogen and sulfur deposition). The latter sites were within 50 km of Portland and Seattle/Centralia urban-industrial areas, respectively. Tissue concentrations of sulfur, nitrogen, and other macronutrients; rates of net carbon uptake; concentrations of photosynthetic pigments; and thallus density were correlated with season and seasonal changes in *Platismatia glauca*. Ion concentrations in precipitation and total wet deposition were measured from natural settings. Concentrations of depositional ions in precipitation, including NO_3^- and NH_4^+, were generally highest at Bull Run and Pack Forest; SO_4^{2-} concentrations and acidity were highest at Pack Forest. Total wet deposition was higher in the winter rainy season than the dry summer season at all three sites. Lichens adapted physiologically and morphologically to the higher light intensity and the warm, dry climate of summer through decreased optimal water content for CO_2 uptake, increased concentrations of carotenoids and increased thallus density. Compared to the clean site, the sites with enhanced deposition were associated in *P. glauca* with year-round higher tissue concentrations of N, S, K, and Na; higher concentrations of total chlorophyll and carotenoids; higher $OD_{435/415}$ ratios; higher CO_2 uptake and lower thallus density in summer; and a general absence of other sensitive lichens. These results indicate that moderate levels of fertilizing air pollutants can stimulate carbon uptake and provide protection against chlorophyll degradation in air pollution-tolerant lichens of the Pacific Northwest, especially during the dry summer season.

Keywords: Lichens; Air pollution; Field studies; Fertilizing effect

1. Introduction

In western North America, air quality gradients occur between moderately to strongly polluted urban areas and nearly pristine remote sites (Fenn et al., 2003a). As human energy, transportation, and food needs grow, the transitional, low pollution zones

* Corresponding author. Tel.: +1 217 333 0616; fax: +1 217 244 7246.
E-mail address: r-crang@life.uiuc.edu (R.F.E. Crang).

0048-9697/$ - see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.scitotenv.2004.10.003

between urban and pristine airsheds may increasingly include critical natural resources such as forests. Therefore, it is of interest to know how low-level air pollution affects biodiversity and plant community composition in forested ecosystems and, specifically, the mechanisms by which tolerant species may be favored over sensitive species. Lichens provide a useful case study.

In western Oregon and Washington (Fenn et al., 2003b), conspicuous air pollution-sensitive macrolichens characterize remote, clean sites whereas urban sites typically support only the smaller tolerant and nitrophilous species. Tissue concentrations of nitrogen and sulfur are significantly higher in urban lichens than lichens from clean sites. Low pollution zones have intermediate tissue concentrations and community composition. Lichen growth is seasonal; the majority of growth occurs during the late fall and spring when temperatures are above freezing, and light and precipitation are plentiful; drought stress and high temperatures limit growth in summer (Muir et al., 1997; Matthes et al., 1987).

Gaseous air pollutants to which lichens are sensitive, such as SO_2, NO_x, and ozone, generally do not occur at harmful levels outside major urban areas of the Pacific Northwest. Since 1990, mean SO_2 levels inside the Portland and Seattle metropolitan areas have ranged from 2 to 36 ppb, just high enough for potential adverse effects (Johnson, 1979; DeWit, 1976) and maximum 1 h NO and NO_2 levels have been <0.27 ppm (OR DEQ, 2002; WA DEQ, 1999). Most studies reporting adverse effects of ozone on lichens in situ occurred under conditions with regular, multiple episodes exceeding 90–120 ppb (Sigal and Nash, 1983; Zambrano et al., 2000). However, peak 1 hr ozone levels in Pacific Northwest urban areas rarely reach 120 ppb (OR DEQ, 2002; WA DEQ, 1999). Therefore, air pollution effects on lichens in urban/remote transition zones presumably result from secondary pollutants such as acidic and depositional compounds. Depositional pollutants and acidity can strongly affect lichen growth and survival and exacerbate adverse effects of other air pollutants (Farmer et al., 1992). For example, alkalinization and fertilization by ammoniacal nitrogen has been shown to dramatically influence lichen community composition in the Netherlands (Van Dobben and Ter-Braak, 1999). In fact, in many nitrogen-limited natural settings, it has been proposed that increasing nitrogen availability can potentially improve the growth of some lichens (Crittenden et al., 1994; Palmqvist, 2000).

In this study, we hypothesize that low-level air pollution associated with improved physiological integrity in air pollution tolerant lichens of Pacific Northwest forests, and that the magnitude of the response will be affected by season. To test this hypothesis, we collected two pollution tolerant, epiphytic lichens, *Parmelia sulcata* Taylor and *Platismatia glauca* (L.) Culb. and C. Culb. from three low-elevation, forested sites in the western Cascades of Oregon and Washington (a clean site, and two polluted sites) in winter and summer. We used wet deposition, rainfall acidity, macronutrient concentrations in lichen tissue, and presence/absence of sensitive lichens to assess air quality. We also used standard indicators of physiological condition including carbon uptake, chlorophyll *a* concentration, chlorophyll *a*/*b* ratios, and chlorophyll/phaeophytin ratios to show that physiological and nutritional status was generally better at polluted sites, and that most of the enhancement occurred in summer.

2. Materials and methods

2.1. Selection of study sites

The three study sites were: HJ Andrews (HJA) Experimental Forest in Lane County, OR, Bull Run watershed in Mt. Hood National Forest, Clackamas County, OR and University of Washington's Pack Experimental Forest in Pierce County, WA. All sites are located at low elevations in Douglas-fir (*Pseudotsuga menziesii*) dominated forests in the western foothills of the Cascade Range (Fig. 1) and were intentionally selected to minimize climate and elevation differences that could confound the responses to air pollution and season (Table 1). Winters at the study sites are cool and rainy with the lowest temperatures around freezing; summers are dry and moderately warm with maximum July temperatures of about 25 °C. Bull Run and Pack Forest are <50 km downwind of Portland, OR and the Seattle/Tacoma, WA metropolitan areas, respectively, the two major urban centers in the bi-state region. Pack Forest is also <50 km northwest of Centralia, WA, where a coal-fired power plant is the

Fig. 1. Topographic map of the U.S. Pacific Northwest showing locations of the three field collection sites in the western Cascades: Pack Forest, Bull Run, and H.J. Andrews.

largest single source of SO_2 emission in Oregon or Washington states (EPA, 2004). In contrast, HJA Experimental Forest is remote from the most important pollution sources in the region. Regional air pollution sensitive lichens in the genera *Alectoria*, *Bryoria*, *Lobaria*, *Nephroma*, *Pseudocyphellaria*, and *Usnea* (McCune and Geiser, 1997) are plentiful at HJA, but sparse to absent at Bull Run and Pack Forest.

2.2. Air quality measurements

2.2.1. Climate and wet deposition data

The study sites were colocated with wet deposition monitors (NADP/NTN, 2003). Weekly precipitation volume and concentrations of SO_4^{2-}, NO_2^-, NH_4^+, H^+, Ca^{2+}, Mg^{2+}, and K^+ are measured at each site. Mean concentrations and total deposition of these ions are reported seasonally. Data for the years 1994–2000 that met all four data quality criteria recommended by NADP were analyzed. Modeled climate data for the monitor coordinates were obtained from the PRISM 4-km grid (Daly and Taylor, 2000).

2.2.2. Element concentrations in lichen tissue

Twenty-gram (dry weight) samples of *P. glauca* were collected from branches of Douglas-fir at six, randomly selected, one-acre circular plots within a 1.6 km of each of the three NADP monitors in June, 1999, and again in December, 2000. Samples were analyzed for dry weight concentrations of S, N, Ca, Mg, K, and Na at the University of Minnesota Research Analytical Laboratory as described by Geiser (2004).

2.3. Physiological measurements

2.3.1. Collection and storage of lichens for physiological measurements

Lichens for physiological measurements were collected within 0.8 km of the monitoring stations during summer (June, 1999) and winter (January, 2000). *P. sulcata* was collected from branches of Garry oak (*Quercus garryana*), alder (*Alnus rubra*), and vine maple (*Acer circinatum*); *P. glauca* was collected from branches of Douglas-fir (*P. menziesii*) and vine maple. Samples were collected 1–2.5 m above ground from exposed microsites, stored in paper bags at room temperature, and moistened three or four times daily. Measurements of carbon uptake were made within 5 days of collection from the field site, after which the material was kept frozen at −80 °C.

2.3.2. Measurement of net carbon uptake

Lichen samples were packed in cooled chambers and flown to Illinois for analysis within two days of collection. After 1 day of exposure to similar light and temperature conditions to that of the field sites, net carbon uptake in the samples was measured at 15 °C under 400 μmol m^{-2} sec^{-1} photosynthetically active light using an infrared gas analyzer (Licor 6262) and the CO_2 depletion technique of Larson and Kershaw (1975). The infrared gas analyzer was calibrated each day of analyses with 0.5, 1, 1.5, 2, 2.5, and 3 ml of 356 μmol mol^{-1} CO_2. Four and six replicates of each species were analyzed from summer and winter collections, respectively. For each replicate, net carbon uptake was measured at

Table 1
Physical characteristics of the three field collection sites

Parameter	H.J. Andrews	Bull Run	Pack Forest
Location	H.J. Andrews Experimental Forest	Bull Run Watershed	Pack Experimental Forest
Administration	Willamette National Forest	Mt. Hood National Forest	University of Washington
County	Lane	Clackamas	Pierce
State	Oregon	Oregon	Washington
Latitude	44°12′44″ N	45°26′52″ N	46°50′07″
Longitude	122°15′21″ W	122°08′53″ W	122°17′12″
Elevation (m)	450	267	617
NADP station number	OR 10	OR 98	WA 21
Mean annual precipitation (cm)	242 (51)	205 (37)	115 (20)
Mean summer precipitation (cm)	11 (2.0)	16 (3.2)	11 (1.7)
Mean winter precipitation (cm)	122 (11.4)	85 (7.4)	42 (6.1)
Mean annual number of days with measurable precipitation	164 (1.7)	175 (1.0)	161 (2.5)
Mean annual relative humidity (%)	68.1	69.1	73.2
Fog (percent (%) of hours in July with <1.6 km visibility or <100 m cloud ceiling)	0	5.2	9.9
Mean temperature at dew point (°C)	3.0	4.7	4.8
Mean minimum Dec. temperature (°C)	−1.0	1.1	0.9
Mean maximum Aug. temperature (°C)	26.7	25.8	24.8

Annual, summer, and winter precipitation values are means for the period 1994–2000 reported by the National Atmospheric Deposition program (NADP/NTN, 2003); standard errors are in parentheses. Other climate data are modeled values from the Climate Data Source (see Method).

three to six different water contents. Fresh samples were moistened for 1 h with a distilled water mist at room temperature and blotted with paper to remove excess surface water. Lichens were weighed for water content, and sealed in 10-ml glass cuvettes for measurement of CO_2 production for 15 min. Before and after each set of measurements, CO_2 concentration in the cuvette was measured by injecting a 2-ml sample from the cuvette into the infrared gas analyzer. The initial CO_2 concentration was maintained at a nearly constant level (370±20 μmol mol^{-1} CO_2). Lichens were weighed after each measurement and allowed to dry for a short period before the next measurement. Maximum net carbon uptake was obtained from the highest rate or from an average of the two or three highest rates if uptake and water content were similar. Maximum net carbon uptake was calculated based on thallus oven-dried mass (odm) from six to eight thalli.

2.3.3. Quantification of photosynthetic pigments

Photosynthetic pigments were quantified from the same samples used for net carbon uptake measurements. Fragments (20–40 mg) from the margins and central portions of each thallus were placed in a 15-ml test tube and washed for 1 min with 3 ml of filtered, $CaCO_3$-saturated acetone to remove substances that could degrade photosynthetic pigments during extraction (Barnes et al., 1992). The wash was repeated five times before extracting with 5 ml $CaCO_3$-saturated dimethylsulfoxide (DMSO). Extracts were placed in an oven at 65 °C for 40 min to minimize chlorophyll degradation by chlorophyllase (Ronen and Galun, 1984) and were then cooled to room temperature (in the dark to prevent photolysis of photosynthetic pigments) and diluted with 5 ml of $CaCO_3$-saturated DMSO. Light absorbance at 400 to 750 nm was measured using a Beckman DU-40 spectrophotometer calibrated with a $CaCO_3$-saturated DMSO blank. $OD_{435/415}$ is used as a measure of chlorophyll integrity. The wavelengths 435 and 415 nm are the peak absorption wavelengths of chlorophyll *a* and its degradation product, phaeophytin *a*, respectively. Therefore, a high ratio indicates little chlorophyll degradation. Calculations of chlorophyll *a*, chlorophyll *b*, carotenoid concentrations, and $OD_{435/415}$ were made using Wellburn's (1994) equations.

2.3.4. Mass analysis

The area of five thalli of each species at each site in summer and winter was measured using an Analytical

Imaging Station (AIS). AIS digitally quantifies the area of a scanned lichen image in relation to a standardized area of paper. The oven-dried mass (odm) of each sample was divided by the thallus area to obtain a mass/unit area.

2.4. Statistical analyses

Tukey's Honestly Significant Difference tests at the 95% confidence level were used to detect differences in maximum net carbon uptake, pigment concentrations and ratios, mass analysis, tissue concentrations of elements, and deposition among the sites. In addition, correlation coefficients were calculated for net carbon uptake and water content percentage. Analyses were run using Statistix 7 (Analytical Software, Tallahassee, FL, USA). Multiple pair-wise comparisons were performed to detect significant differences due to seasons, collection sites, and species.

3. Results

3.1. Wet deposition

3.1.1. Ion concentrations in precipitation

Ion concentrations in precipitation were generally higher in summer than winter at all sites (Table 2), consistent with longer periods between precipitation events and lower total volume of precipitation in summer. Concentrations of Mg^{2+}, Na^{+}, Cl^{-}, and the pH of precipitation were higher in winter than in summer, consistent with the origination of winter storms over the Pacific Ocean. Mg^{2+}, Na^{+}, and Cl^{-} tended to be highest year-round at Bull Run, located in close proximity to the Columbia River. The Columbia River Gorge (separating Oregon and Washington as shown in Fig. 1) experiences strong diurnal winds that transport salt-laden air from the Pacific Ocean; various marine lichens can be found substantially east of Portland in the Gorge. Of the anthropogenic ions, mean concentrations of SO_4^{2-} and acidity were highest at Pack Forest year-round, although not significantly higher than Bull Run in winter when concentrations at all sites were more dilute. Concentrations of NO_3^{-} were higher at Bull Run and Pack Forests than at HJA year-round, and NH_4^{+} concentrations were higher at Bull Run relative to other sites in summer. Concen-

Table 2
Mean ion concentrations (mg l^{-1}) and pH of summer and winter wet deposition from 1994–2000 at the three field sites

Season	Site	Ca^{2+}	Mg^{2+}	K^{+}	Na^{+}	NH_4^{+}	NO_3^{-}	TinorN	Cl^{-}	SO_4^{2-}	pH
Summer	H.J. Andrews	$0.03^{a}\pm0.005$	$0.015^{a}\pm0.0012$	$0.020^{a}\pm0.0063$	$0.109^{a}\pm0.0111$	$0.07^{a}\pm0.017$	$0.33^{a}\pm0.048$	$0.13^{a}\pm0.022$	$0.18^{a}\pm0.023$	$0.27^{a}\pm0.028$	$5.14^{b}\pm0.037$
	Bull Run	$0.04^{a}\pm0.002$	$0.027^{b}\pm0.0049$	$0.038^{a}\pm0.0135$	$0.224^{b}\pm0.0404$	$0.15^{b}\pm0.009$	$0.46^{b}\pm0.014$	$0.22^{b}\pm0.0091$	$0.38^{b}\pm0.068$	$0.46^{b}\pm0.025$	$5.09^{b}\pm0.021$
	Pack Forest	$0.03^{a}\pm0.003$	$0.016^{a}\pm0.0018$	$0.023^{a}\pm0.0039$	$0.126^{b}\pm0.0209$	$0.08^{a}\pm0.012$	$0.46^{b}\pm0.054$	$0.16^{ab}\pm0.021$	$0.21^{a}\pm0.028$	$0.81^{c}\pm0.073$	$4.75^{a}\pm0.030$
Winter	H.J. Andrews	$0.02^{a}\pm0.002$	$0.020^{ab}\pm0.0030$	$0.008^{a}\pm0.0014$	$0.196^{ab}\pm0.0256$	$0.02^{a}\pm0.003$	$0.09^{a}\pm0.008$	$0.03^{a}\pm0.003$	$0.33^{a}\pm0.045$	$0.12^{a}\pm0.012$	$5.39^{b}\pm0.006$
	Bull Run	$0.02^{a}\pm0.002$	$0.033^{b}\pm0.0038$	$0.013^{ab}\pm0.0014$	$0.301^{b}\pm0.0319$	$0.03^{a}\pm0.003$	$0.16^{b}\pm0.007$	$0.06^{b}\pm0.003$	$0.52^{b}\pm0.058$	$0.18^{ab}\pm0.007$	$5.34^{b}\pm0.010$
	Pack Forest	$0.02^{a}\pm0.004$	$0.019^{a}\pm0.0031$	$0.020^{b}\pm0.0046$	$0.184^{a}\pm0.0211$	$0.03^{a}\pm0.006$	$0.17^{b}\pm0.012$	$0.06^{b}\pm0.007$	$0.31^{a}\pm0.036$	$0.23^{b}\pm0.036$	$5.21^{a}\pm0.029$

Small case letters indicate Tukey's significant differences within seasons by ion; ±values are standard errors. TinorN is the total elemental N from NH_4^{+} and NO_3^{-}. Data Source: NADP/NTN (2003).

trations of SO_4^{2-}, nitrogen, and nitrogen-containing ions and amount of acidity were always lowest, or in the lowest group, at HJA.

3.1.2. Total deposition

Total deposition (kg ha^{-1}) is higher in winter, when most of the annual precipitation occurs, than in summer (Table 3). In summer, when the amount of precipitation is similar at all three sites, deposition of all ions except H^+ was higher at Bull Run compared to the clean site, HJA. At Pack Forest, only SO_4^{2-} was significantly higher than HJA. In winter, there were no significant differences between Bull Run and HJA, although the highest values for NH_4^+, NO_3^-, total N, and SO_4^{2-} among the three sites did occur at Bull Run; Bull Run receives about 70% as much precipitation as HJA. Pack Forest, which receives only about 35% as much precipitation as HJA in winter, had significantly lower deposition of Ca^{2+}, Mg^{2+}, Na^+, and Cl^- compared to HJA and Bull Run, and a lower deposition of H^+ compared to HJA.

3.2. Macronutrient concentrations in P. glauca

Within sites, there was little difference between summer and winter concentrations of elements in lichen tissue, except that Ca^{2+} in lichen tissue from Pack Forest was much lower in winter than summer (Table 4). Winter concentrations of S, N, K, and Na in lichen tissue from Bull Run and Pack Forest were higher than HJA, and above suggested thresholds for N (0.59%) and S (0. 073%) for *P. glauca* from clean sites in the Pacific Northwest. Values above the threshold are associated with absence of the region's most sensitive species (USDA-FS, 2004). The same pattern was observed in summer, although Bull Run was not statistically different from HJA and S and N concentrations were just within clean site thresholds. In summer, Pack Forest lichens had higher S, N, K, and Na tissue concentrations than HJA lichens, higher S and K than Bull Run lichens, and S and N concentrations were above Pacific Northwest clean site thresholds.

3.3. Net carbon uptake and water content

Net carbon uptake (Fig. 2) increased with increasing water content (hydration) of the lichen thalli to

Table 3
Mean total wet deposition of ions (kg ha^{-1}) in summer and winter from 1994–2000 at the three field collection sites

	Site	Ca^{2+}	Mg^{2+}	K^+	Na^+	NH_4^+	NO_3^-	TinorN	Cl^-	SO_4^{2-}	H^+
Summer	H.J. Andrews	$0.03^a \pm 0.007$	$0.017^a \pm 0.0031$	$0.021^a \pm 0.0068$	$0.128^a \pm 0.0338$	$0.07^a \pm 0.018$	$0.35^a \pm 0.0699$	$0.13^a \pm 0.027$	$0.21^a \pm 0.059$	$0.30^a \pm 0.073$	$0.012^a \pm 0.0017$
	Bull Run	$0.07^a \pm 0.013$	$0.048^b \pm 0.0086$	$0.056^a \pm 0.0178$	$0.380^b \pm 0.0737$	$0.26^b \pm 0.050$	$0.82^b \pm 0.150$	$0.38^b \pm 0.072$	$0.65^b \pm 0.131$	$0.84^b \pm 0.157$	$0.017^a \pm 0.0033$
	Pack Forest	$0.04^a \pm 0.006$	$0.018^a \pm 0.0031$	$0.024^a \pm 0.0049$	$0.140^a \pm 0.0297$	$0.09^a \pm 0.023$	$0.54^{ab} \pm 0.131$	$0.19^{ab} \pm 0.048$	$0.24^a \pm 0.045$	$0.89^b \pm 0.150$	$0.018^a \pm 0.0031$
Winter	H.J. Andrews	$0.21^b \pm 0.031$	$0.251^b \pm 0.0434$	$0.103^a \pm 0.0177$	$2.427^b \pm 0.3842$	$0.20^a \pm 0.034$	$1.04^{ab} \pm 0.079$	$0.39^{ab} \pm 0.038$	$4.04^b \pm 0.665$	$1.43^a \pm 0.224$	$0.050^b \pm 0.0038$
	Bull Run	$0.19^b \pm 0.014$	$0.280^b \pm 0.0380$	$0.113^a \pm 0.0152$	$2.577^b \pm 0.3670$	$0.23^a \pm 0.023$	$1.39^b \pm 0.117$	$0.49^b \pm 0.034$	$4.47^b \pm 0.620$	$1.53^a \pm 0.150$	$0.038^{ab} \pm 0.0040$
	Pack Forest	$0.08^a \pm 0.009$	$0.078^a \pm 0.0147$	$0.078^a \pm 0.0111$	$0.766^a \pm 0.1413$	$0.12^a \pm 0.033$	$0.70^a \pm 0.075$	$0.25^a \pm 0.043$	$1.28^a \pm 0.205$	$0.90^a \pm 0.085$	$0.025^a \pm 0.0029$

Small case letters indicate Tukey's significant differences within seasons; ±values are standard errors. TinorN is the total elemental N from NH_4^+ and NO_3^-. Data Source: NADP/NTN (2003).

Table 4
Mean summer 1999 and winter 2000 element concentrations in thalli of *Platismatia glauca* collected from the three field sites

Season	Site	S (%)	N (%)	Ca (μg/g)	K (μg/g)	Mg (μg/g)	Na (μg/g)
Summer	H.J. Andrews	$0.052^{a}\pm0.0025$	$0.38^{a}\pm0.035$	$2450^{a}\pm249$	$2090^{a}\pm138$	$666^{a}\pm47.2$	$63^{a}\pm4.5$
	Bull Run	$0.062^{a}\pm0.0078$	$0.55^{ab}\pm0.032$	$1903^{a}\pm121$	$2250^{a}\pm107$	$686^{a}\pm26.6$	$112^{ab}\pm11.3$
	Pack Forest	$0.103^{b}\pm0.0131$	$0.75^{b}\pm0.128$	$2030^{a}\pm150$	$2950^{b}\pm143$	$691^{a}\pm42.6$	$144^{b}\pm29.5$
Winter	H.J. Andrews	$0.046^{a}\pm0.0022$	$0.37^{a}\pm0.031$	$2080^{b}\pm167$	$1990^{a}\pm116$	$614^{a}\pm69.8$	$67^{a}\pm6.0$
	Bull Run	$0.079^{b}\pm0.0039$	$0.64^{b}\pm0.047$	$1860^{b}\pm70$	$2730^{b}\pm62$	$689^{a}\pm27.1$	$155^{b}\pm10.4$
	Pack Forest	$0.093^{b}\pm0.0051$	$0.73^{b}\pm0.024$	$1280^{a}\pm52$	$3040^{b}\pm96$	$540^{a}\pm22.3$	$148^{b}\pm5.8$

Small case letters indicate significant within-season differences using Tukey's HSD multiple-comparison test; ±values are standard errors.

optimal water content and decreased at higher water contents. (Net carbon uptake declines gradually above optimum water contents due to increased resistance to CO_2 diffusion (Green et al., 1994; Palmqvist, 2000)). Highest carbon uptake in both species was achieved at water contents of 100–150% in summer and at 150–250% in winter.

In summer, maximum net carbon uptakes were highest at polluted sites (Fig. 3). Maximum uptakes of *P. sulcata* and *P. glauca* were highest in samples from Pack Forest and Bull Run, respectively.

Within site, maximum net carbon uptake was higher in winter than summer (Fig. 3) and the largest winter/summer difference occurred at HJA. Within species, CO_2 uptake was similar at all sites in winter. Maximum net carbon uptake was generally higher in *P. sulcata* than in *P. glauca* year-round.

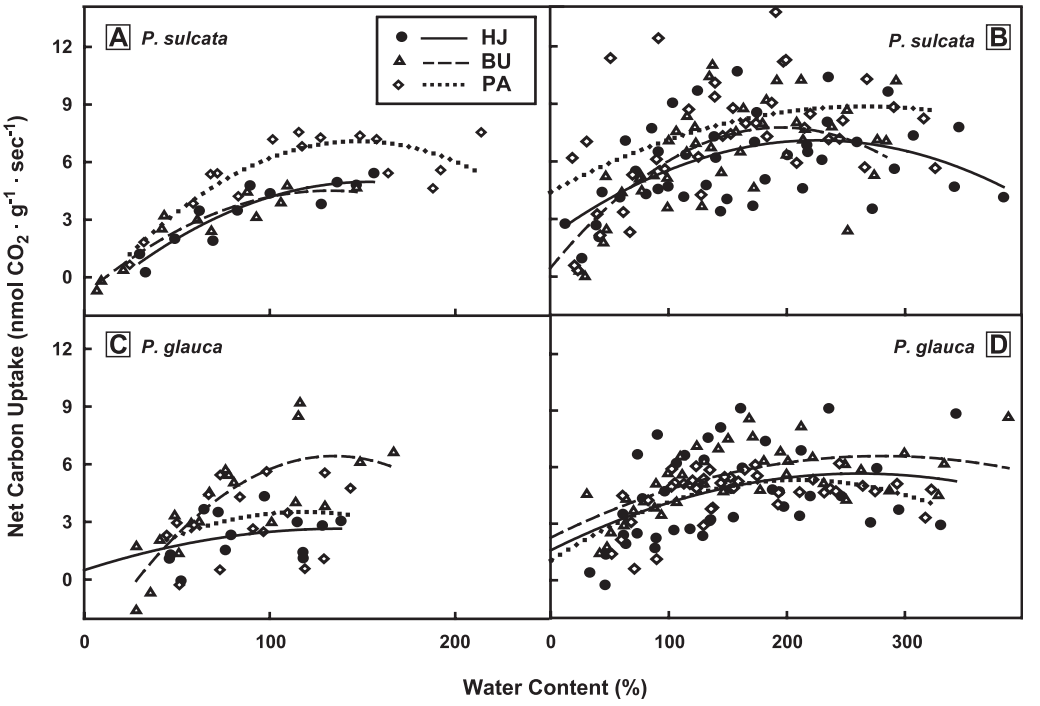

Fig. 2. Scatter plots showing the relationship between water content (percent dry weight) and net carbon uptake (nmol CO_2 g odm^{-1} sec^{-1}) in the lichens, *Parmelia sulcata* (A and B) and *Platismatia glauca* (C and D), collected from the three field sites. Curved lines demonstrate the best fit for each of the three sites. The r^2 values are as follows: In summer for *P. sulcata:* HJ (0.845), BU (0.895), and PA (0.759); in summer for *P. glauca*: HJ (0.466), BU (0.654), and PA (0.067). In winter for *P. sulcata*: HJ (0.283), BU (0.550), and PA (0.306); in winter for *P. glauca*: HJ (0.266), BU (0.521), and PA (0.507). HJ: H.J. Andrews (●); BU: Bull Run (△); PA: Pack Forest (◇).

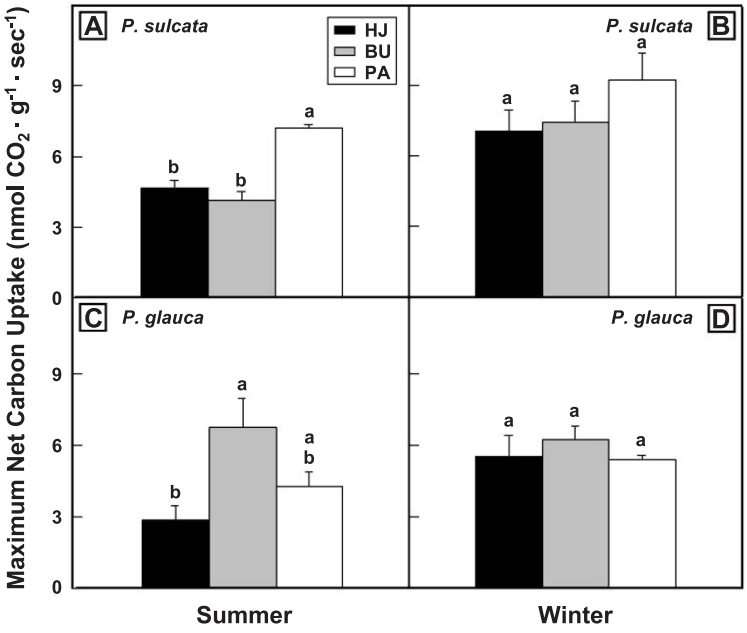

Fig. 3. Maximum net carbon uptake (nmol CO_2 g odm^{-1} sec^{-1}) by the lichens, *Parmelia sulcata* (A and B) and *Platismatia glauca* (C and D) collected from the three field sites in summer 1999 and winter 2000. Bars indicate standard errors. HJ: H.J. Andrews; BU: Bull Run; PA: Pack Forest.

3.4. Pigment analysis

Concentrations of total chlorophyll, chlorophyll *a*/*b*, and carotenoids, and $OD_{435/415}$ ratios were generally higher in samples from the polluted sites compared to the clean site (Fig. 4).

3.4.1. Total chlorophyll

Total chlorophyll concentrations of both species in summer and winter were generally higher in samples from the polluted sites than the clean site (Fig. 4A). Chlorophyll concentrations were highest in summer when lichen thalli were densest and growth rates (i.e., net carbon uptake) were slowest.

3.4.2. Chlorophyll a/b

Summer chlorophyll *a*/*b* ratios were higher in *P. sulcata* from Bull Run than Pack Forest and the clean site (Fig. 4B); otherwise, there were no within site differences in summer or winter. Chlorophyll *a*/*b* ratios were higher in *P. glauca* than *P. sulcata* year-round.

3.4.3. Carotenoids

Carotenoid concentrations were somewhat higher in summer, when light intensity is higher, than in winter (Fig. 4C). In summer, lichens from Pack Forest had the highest carotenoid concentrations. In winter, carotenoid concentrations were highest in lichens from Pack Forest and Bull Run.

3.4.4. Chlorophyll degradation

Summer $OD_{435/415}$ ratios were higher in *P. sulcata* from Bull Run, and *P. glauca* from Pack Forest compared to the clean site (Fig. 4D). In winter, *P. sulcata* from Pack Forest and *P. glauca* from Bull Run had higher ratios than corresponding samples from the clean site.

3.5. Mass analysis

Lichens were generally denser in summer, when carbon uptake was lowest, than in winter (Table 5 and Fig. 3), e.g., *P. sulcata* at Pack Forest and *P. glauca* at Bull Run.

4. Discussion

4.1. Seasonal effects

Most lichens rely on a scarce supply of inorganic nitrogen through wet or dry deposition on the thallus (Palmqvist, 2000). Although mobile elements (S, N, Ca, Mg, K, Na) may vary seasonally with throughfall precipitation modified by leaching from the canopy (Knops and Nash, 1991), and some trees are more effective than others in absorbing ions from the atmosphere (Cantu and Gonzalez, 2001), for the most part, we did not see strong seasonal differences in lichen tissue concentrations of these elements within study sites.

Maximum net carbon uptake of both lichens was higher during the winter rainy season than the dry summer season. We attribute this result to shorter dehydration periods (lichens are essentially dormant when dehydrated), higher pH of precipitation, and cooler temperatures that are mostly above freezing (mean December minimums were −1° to +1 °C) in winter. Maximal net carbon uptakes of other Treboux-ian lichens, including *Alectoria*, *Ramalina*, and

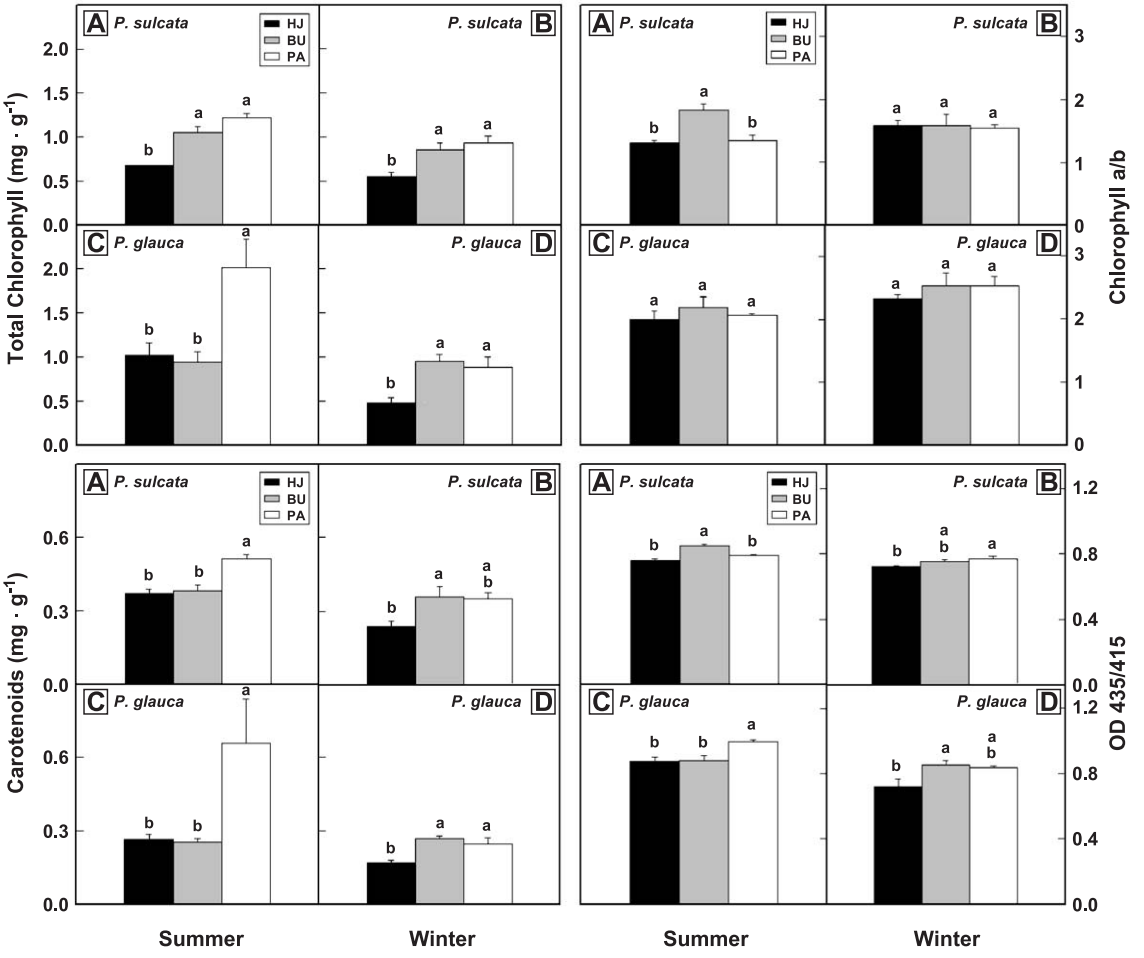

Fig. 4. Total concentrations of chlorophyll (mg g^{-1}) and carotenoids (mg g^{-1}), chlorophyll *a*/*b* ratios, and $OD_{435/415}$ in the lichens *Parmelia sulcata* (A and B) and *Platismatia glauca* (C and D) collected from the three field sites in summer 1999 and winter 2000. Each value represents a mean of four (summer) or six (winter) replicates. Bars indicate standard errors; small case letters indicate Tukey's significant differences. HJ: H.J. Andrews; BU: Bull Run; PA: Pack Forest.

Table 5
Mass per unit area (mg cm^{-2}) of the lichens, *Parmelia sulcata* and *Platismatia glauca*, collected from the three field sites in summer 1999 and winter 2000

Season	Summer			Winter		
Species/Locations	HJ	BU	PA	HJ	BU	PA
P. sulcata	12.54 ± 0.38^{b}	10.43 ± 0.28^{a}	10.70 ± 0.30^{ab}	10.26 ± 0.50^{a}	9.17 ± 0.39^{a}	9.64 ± 0.64^{a}
P. glauca	8.09 ± 0.42^{a}	7.27 ± 0.44^{a}	10.33 ± 0.55^{b}	5.90 ± 0.12^{a}	6.24 ± 0.34^{a}	6.51 ± 0.22^{a}

Each value represents a mean±standard error of five replicates. Small case letters indicate significant differences between sites. HJ, H.J. Andrews; BU, Bull Run; PA, Pack Forest.

Xanthoria spp., are also higher in winter than summer (Boonpragob and Nash, 1991; Brown and Kershaw, 1984). Because the volume density of algal cells extrapolated from cross-sections, constitutes only about 8–10% in both species (Ra et al., 2004), net carbon uptake is quite low on a total mass basis compared to higher plants.

4.2. Pollution effects as related to seasonal effects

Integrating results, we observed that lichens from the two sites with enhanced concentrations of depositional ions and higher total annual deposition had higher year-round tissue concentrations of N, S, K, and Na, higher concentrations of total chlorophyll and carotenoids, larger $OD_{435/415}$ ratios, and higher CO_2 uptake and lower thallus density in summer than lichens from the "clean" site. The absence or sparsity of regionally common, sensitive lichens at the polluted sites is in marked contrast to the apparent improved physiological status of the two pollution tolerant lichens, but is consistent with the high tissue loading of nitrogen and sulfur at these sites.

Higher net carbon uptake at Bull Run and Pack Forest in summer may be a response to greater nutrient availability at the moderately polluted sites. Net carbon uptake and chlorophyll content of *P. sulcata* from the vicinity of Biel, Switzerland (Von Arb and Brunold, 1990), and northern Switzerland (Von Arb et al., 1990) was greater in samples from polluted urban areas than remote areas. NO_x, SO_2, and O_3 were positively correlated with net carbon uptake and chlorophyll content. In southern Poland, net carbon uptake and chlorophyll content of *P. glauca* were higher in samples collected from sites with higher SO_2, NO and dust deposition (Niewiadomska et al., 1998). These authors suggested a stimulatory effect of pollutants, especially NO_x, in *P. glauca*. Taken together, Figs. 3 and 4 show that photosynthetic capacity increases with the chlorophyll status of the lichens and that chlorophyll content increases with tissue nitrogen availability. This is fully in agreement with a broad-scale study of lichens showing that the photobiont tissue concentration increases with the N-status of lichens, and that photosynthetic capacity is strongly correlated to chlorophyll *a* concentration (Palmqvist et al., 2002).

The insignificant differences in winter CO_2 uptake rates between sites was consistent with smaller differences in nutrient concentrations in precipitation and reduced stress from drought, excess heat or photo-oxidation, i.e., better growing conditions at all sites. In winter, therefore, pollution conferred no measurable advantage.

A correlation between of SO_4^{2-} and rainfall pH, such as at Pack Forest in summer has been shown in other areas (Sorensen et al., 1994). Higher summer net carbon uptake and total chlorophyll content in *P. sulcata* at Pack Forest might be attributable to a tolerance of higher SO_4^{2-} availability and lower pH by this lichen compared to *P. glauca*. In our study, *P. glauca* appeared to be more responsive to enhanced nitrogen availability under more neutral pH conditions, i.e., at Bull Run.

Chlorophyll *a*, *b* and carotenoids are light absorbing pigments that transfer electrons to the photosynthetic reaction center; chlorophyll *a* is most efficient in harvesting light (Whitmarsh and Govindjee, 1999). Carotenoids are photosynthetic pigments and also play a role in reducing photo-oxidation of chlorophyll under conditions of excess light (Adams et al., 1993). Higher total chlorophyll and carotenoid concentrations which were at Pack Forest and Bull Run may have contributed to higher carbon uptake

rates at these sites in summer, relative to HJA. Carotenoid concentrations in *Parmotrema uruguense* (Kremph.) Hale transplanted to polluted sites were also higher after 4 and 6-month exposures (Canas and Pignata, 1998). The chlorophyll *a*/*b* ratio in *P. sulcata* from Bull Run was higher in summer than winter; otherwise, we saw few differences among sites or seasons in regard to this parameter. It appears that the lichens were under different stresses at the various sites from which they were collected—some drying out much less than others. Under those conditions, the individual thalli would be differently adapted. In winter, optimum water content is more variable than in summer in which all the lichens are drought stressed. Thus, r^2 values for winter collections at HJ Andrews are significantly lower (Fig. 2).

The higher $OD_{435/415}$ ratios in our study at the two polluted sites year round indicates a higher chlorophyll integrity at these sites. While chlorophyll degradation is usually correlated with pollution intensity (Garty et al., 2001) and high sulfate-S concentrations (Garty et al., 1997), low-level pollution has been associated with improved $OD_{435/415}$ ratios. For example, *P. glauca* had 26% higher $OD_{435/415}$ at a site with enhanced concentrations of SO_2, NO, and dust deposition, than samples from a clean site (Niewiadomska et al., 1998). Enhanced nitrogen and sulfur availability in lichen tissue may permit higher protein synthesis rates, improving the ratio of intact chlorophyll to degraded forms, overall concentrations of pigments, and CO_2 uptake. Using fumigation chambers, Balaguer and Manrique (1991) studied the interaction between sulfur dioxide and nitrate in three fruiticose lichen species and concluded that there was a synergistic inhibitory effect when both fumigants were used. Nevertheless, they acknowledged that the fertilizing effect of nitrates might have been determined by the sensitivity of lichens to SO_2.

A potential problem with increased N availability for a symbiotic organism such as lichens can be that the assimilated nitrogen is invested in a differential way between the partners. This problem was addressed in a study of *P. glauca* and *Hypogymnia physodes*, which showed that an increased investment in the photobiont occurred in response to an increased N supply, resulting in an increased carbon assimilation capacity (Dahlman et al., 2003). Gaio-Oliveira et al. (2004), using long-term exposures of *Xanthoria parietina* (a pollution-tolerant lichen) to NH_4Cl, showed that the photobiont was more capable of recovering from the negative effects than the mycobiont.

5. Conclusions

Lichens adapted physiologically and morphologically to the higher light intensity and warm, dry climate of summer through decreased optimal water content for CO_2 uptake, increased concentrations of carotenoids and increased thallus density. Some air pollution-resistant lichens of the Pacific Northwest, including *P. sulcata* and *P. glauca*, may have enhanced metabolic performance, detectable as higher net carbon uptake and photosynthetic pigment concentrations, under conditions of low-level air pollution (enhanced nutrient input) detrimental to sensitive lichens. The additional nutrient input may protect these lichens by increasing protein synthesis needed to maintain chlorophyll integrity and carbon fixation. No strong seasonal differences were found in the uptake of pollution-related elements, but both species possessed a lower thallus density in spite of a higher CO_2 uptake in winter. This decrease in density during the winter may have come about due to a greater utilization of photosynthates as indicated by the reduction in unit mass of thalli at the polluted sites for both species.

References

Adams III WW, Demmig-Adams B, Lange OL. Carotenoid composition and metabolism in green and blue-green lichens in the field. Oecologia 1993;94:576–84.

Balaguer L, Manrique E. Interaction between sulfur dioxide and nitrate in some lichens. Environ Exp Bot 1991;31:223–6.

Barnes JD, Balaguer L, Manrique E, Elvira S, Davison AW. 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 1992;32:85–100.

Boonpragob K, Nash III TH. Physiological responses of the lichen *Ramalina menziesii* Tayl to the Los Angeles urban environment. Environ Exp Bot 1991;31:229–38.

Brown D, Kershaw KA. Photosynthetic capacity changes in *Peltigera*: 2. Contrasting seasonal patterns of net photosynthesis in two populations of *Peltigera rufescens*. New Phytol 1984; 96:447–57.

Canas MS, Pignata ML. Temporal variation of pigments and peroxidation products in the lichen *Parmotrema uruguense*

(Kremph.) Hale transplanted to urban and non-polluted environments. Symbiosis 1998;24:147–62.
Cantu SI, Gonzalez RH. Interception loss, throughfall and stemflow chemistry in pine and oak forests in northeastern Mexico. Tree Physiol 2001;21:1009–13.
Crittenden PD, Katucka I, Oliver E. Does nitrogen supply limit the growth of lichens? Cryptogam Bot 1994;4:143–55.
Dahlman L, Persson J, Näsholm T, Palmqvist K. Carbon and nitrogen distribution in the green algal lichens *Hypogymnia physodes* and *Platismatia glauca* in relation to nutrient supply. Planta 2003;217:41–8.
Daly C, Taylor G. Spatial Climate Analysis Service at Oregon State University (SCAS/OSU), Corvallis, Oregon, USA. Arc/INFO and ArcView coverages, 2000.
DeWit T. Epiphytic lichens and air pollution in the Netherlands. Bibl Lichenol 1976;5:1–228.
[EPA] US Environmental Protection Agency. 2004. Criteria Pollutants Data. Final 1999 National Emissions Inventory Version 3. (18 May 2004; www.epa.gov/ttn/chief/net/1999 inventory.html).
Farmer AM, Bates JW, Bell JNB. Ecophysiological effects of acid rain on bryophytes and lichens. In: Bates JW, Farmer AM, editors. Bryophytes and lichens in a changing environment. Oxford: Clarendon Press; 1992.
Fenn ME, Haeuber R, Tonnesen GS, Baron JS, Grossman-Clark S, Hope D, et al. Nitrogen emissions, deposition and monitoring in the western United States. BioScience 2003a;53:391–403.
Fenn ME, Baron JS, Allen EB, Rueth HM, Nydick KR, Geiser L, et al. Ecological effects of nitrogen deposition in the western United States. BioScience 2003b;53:404–20.
Gaio-Oliveira G, Dahlman L, Palmqvist K, Máguas C. Ammonium uptake in the nitrophytic lichen *Xanthoria parietina* and its effects on vitality and balance between symbionts. Lichenologist 2004;36:75–86.
Garty J, Karnieli A, Wolfson R, Kunin P, Garty-Spitz R. Spectral reflectance and integrity of cell membranes and chlorophyll relative to the concentration of airborne mineral elements in lichens. Physiol Plant 1997;101:257–64.
Garty J, Weissman L, Cohen Y, Karnieli A, Orlovsky L. Transplanted lichens in and around the Mount Carmel National Park and the Haifa Bay industrial region in Israel: physiological and chemical responses. Environ Res Section A 2001;85:159–76.
Geiser L, Manual for monitoring air quality using lichens on National Forests of the Pacific Northwest USDA-Forest Service Pacific Northwest Region technical paper, R6-NR-ARM-02-04, 2004, Available online at url <http://www.fs.fed.us/r6/aq>.
Green TG, Lange OL, Cowan IR. Ecophysiology of lichen photosynthesis: the role of water status and thallus diffusion resistance. Cryptogam Bot 1994;4:166–78.
Johnson DW. Air pollution and the distribution of corticolous lichens in Seattle, Washington. Northwest Sci 1979;53:257–63.
Knops JMH, Nash III TH. Mineral cycling and epiphytic lichens: implications at the ecosystem level. Lichenologist 1991;23:309–21.
Larson DW, Kershaw KA. Studies on lichen-dominated systems: XIII. Seasonal and geographical variation of net CO_2 exchange of *Alectoria ochroleuca*. Can J Bot 1975;53:2598–607.
McCune B, Geiser L, 1997. Macrolichens of the Pacific Northwest. Corvallis (OR): Oregon State University Press; 1997. p. 1–386.
Matthes SU, Nash III TH, Larson DW. The ecology of *Ramalina menziesii*: VI. Laboratory responses of net carbon dioxide exchange to moisture, temperature and light. Can J Bot 1987;65:182–91.
Muir PS, Shirazi AM, Patrie J. Seasonal growth dynamics in the lichen *Lobaria pulmonaria*. Bryologist 1997;100:458–64.
NADP/NTN. National Atmospheric Deposition Program (NRSP-3)/National Trends Network. Program Office, Illinois State Water Survey, Champaign, IL. Data summaries and collection protocols available online at url <http://nadp.sws.uiuc.edu>, 2003.
Niewiadomska E, Jarowiecka D, Czarnota P. Effect of different levels of air pollution on photosynthetic activity of some lichens. Acta Soc Bot Pol 1998;67:259–62.
OR DEQ. 2002 Oregon air quality data summaries. State of Oregon Department of Environmental Quality, Air Quality Division. Portland, OR, 2002, Available online at url <http://www.deq.state.or.us>.
Palmqvist K. Carbon economy in lichens. New Phytol 2000;148:11–36.
Palmqvist K, Dahlman L, Valladares F, Tehler A, Sancho LG, Mattsson J-E. CO_2 exchange and thallus nitrogen across 75 contrasting lichen associations from different climate zones. Oecologia 2002;133:295–306.
Ra HSY, Rubin L, Crang RFE. Structural impacts on thallus and algal cell components of two lichen species in response to low-level air pollution in Pacific Northwest forests. Microsc Microanal 2004;10:270–9.
Ronen R, Galun M. Pigment extraction from lichens with dimethylsulfoxide (DMSO) and estimation of chlorophyll degradation. Environ Exp Bot 1984;24:239–45.
Sigal LL, Nash III TH. Lichen communities on conifers in southern California: an ecological survey relative to oxidant air pollution. Ecology 1983;64:1343–54.
Sorensen JA, Glass GE, Schmidt KW. Regional patterns of wet mercury deposition. Environ Sci Technol 1994;28:2025–32.
USDA-FS. US Forest Service Pacific Northwest Region: Lichens and air quality homepage. Provisional element thresholds and sensitivity ratings. (18 May 2004; http://airlichen.nacse.org/cgi-bin/qml/usair/region6/area6.qml).
Van Dobben HF, Ter-Braak CJF. Ranking of epiphytic lichen sensitivity to air pollution using survey data: a comparison of indicator scales. Lichenologist 1999;31:27–39.
Von Arb C, Brunold C. Lichen physiology and air pollution: I. Physiological responses of in situ *Parmelia sulcata* among air pollution zones within Biel, Switzerland. Can J Bot 1990;68:35–42.
Von Arb C, Mueller C, Ammann K, Brunold C. Lichen physiology and air pollution: II. Statistical analysis of the correlation between SO_2, NO and O_3, and chlorophyll content, net photosynthesis, sulphate uptake and protein synthesis of *Parmelia sulcata* Taylor. New Phytol 1990;115:431–7.
WA DEQ. Air Quality Trends in Washington. Washington State Department of Ecology, Air Quality Program. Publication 00-02-011; 1999.

Wellburn AR. The spectral determination of chlorophylls *a* and *b*, as well as total carotenoids using various solvents with spectrophotometers of different resolution. J Plant Physiol 1994;144:307–13.

Whitmarsh J, Govindjee. The photosynthetic process. In: Singhal GS, Renger R, Sopory SK, Irrgang K-D, Govindjee, editors. Concepts in Photobiology: Photosynthesis and Photomorphogenesis. New Delhi, India: Narosa Publishing House; 1999. p. 11–51.

Zambrano GA, Nash III TH, Herrera-Campos MA. Lichen decline in Desierto de los Leones, Mexico City. Bryologist 2000;103: 428–41.