Lichens on *Betula* in the Ural Mountains; relationships with bark acidity and element concentrations as indicators of geology and anthropogenic influences

Abstract

Understanding geological, anthropogenic influences and bark pH on epiphytic lichen species richness is important in conservation, pollution monitoring and for assessing environmental health. *Hypogymnia physodes* and other lichens were found abundantly colonising birch twigs in June 2011 at Nyazminsky Ridge, 40 km SW (downwind) from Karabash, South Urals of Russia and 6 km north of Zlatoust city. A biogeochemical lichen study was carried out along a SW-NE transect centred in Karabash valley in 2001 and a second transect running WNW – ESE established in September 2011 within intermediate forests between taiga and forest-steppe zone. *Hypogymnia* is well known to be sensitive to a range of factors, including bark pH, but the extent to which bark acidity and element concentrations influence *H. physodes* and other lichen species richness on twigs is unknown. As a first step towards understanding its occurrence, the present study aims to investigate the links between *Hypogymnia* frequency and lichen species richness on *Betula* twigs and trunks (recorded in 2001) and indicators of geology and anthropogenic impacts. Impacts were assessed from selected element content, notably Sr (an analogue for Ca analysed previously) and S (a potential pollutant) and bark pH. *Hypogymnia* frequency and twig lichen richness confirm a point source influence The study highlighted the sensitivity of bark pH to assess S deposition from smelter-derived aerosols in response to weather over short (<3 month) time periods. The high Ca content recorded in *Hypogymnia* at Novoandreevka suggests geology also influences species assemblage composition on *Betula* here. The outlier appears to represent a lichen 'oasis' and provides a refugium enabling future lichen re-colonisation elsewhere under favourable atmospheric conditions.

Introduction

Developments in analytical techniques and understanding of lichen-mineral and atmospheric interactions have come a long way since pioneering studies carried out in and around Newcastle, North East England linked SO_2 produced from fuel combustion and smelting, with sulphur concentrations in thalli of *Parmelia saxatilis* (L.) Ach. and *Hypogymnia physodes* (L.) Nyl. (Purvis 2010). Areas impacted by point sources are natural laboratories to investigate the effects of pollutants and geology on vegetation. The effects of smelters as a 'point source' in creating 'industrial barrens' and recovery in response to emission reductions and other factors are well-documented. Studies investigating *H. physodes* frequency on *Betula* trunks highlight its sensitivity to SO_2 and metal deposition (Mikhailova & Vorobeichik 1999). Where insufficient natural thalli are present, *Hypogymnia* has frequently been successfully transplanted from where it is abundant to monitoring stations to assess deposition

(Mikhailova 2002). Lichens colonising twigs are excellent indicators of atmospheric deposition (Wolseley 2002). Soil and bark chemistry (pH, element concentrations and ratios) also influence epiphytic lichen assemblage composition (Gilbert 1976; Gauslaa 1985; Hauck & Paul 2005). Studies in boreal woodlands identified pH gradients in relation to canopy height associated with changes in the composition of lichen assemblages (Marmor *et al.* 2010). Further work in other areas is required to apply this knowledge e.g. in conservation. Understanding element sources and cycling and distinguishing natural biogeochemical cycles from human interference (Bargagli & Mikhailova 2002; Reimann & Caritat 2000, 2005) is important.

Frontasyeva *et al.* (2004) compared soil and moss element composition in the South Urals of Russia, including the area adjacent to Karabash copper smelter in close proximity to monitoring stations in this study. Factor analyses identified Karabash as a main pollution source for Cu, Zn, As, Ag, Cd and Sb. Cu and As deposition was mainly limited to within 30-40 km from the smelter. Evidence for significant surface soil contamination for As, Zn and Ni was also provided. Lichen transplant studies, involving removing thalli from *Betula* trunks with bark attached, were carried out over the period 2001-02 around Karabash. Transplant stations were set up and a range of environmental media assessed and sampled along a 58 km NE - SW transect centred on the smelter (reviewed in Purvis *et al.* 2013). Not all elements showed a curvilinear relationship, with increasing concentrations being recorded in samples towards the point source, emphasising biogeochemical aspects. High SO_2 levels, measured at concentrations up to 20,000 μgm^{-3} (spot measurement taken within the smelter plume, 1 km downwind), severely affected lichen richness (Udachin *et al.* 2003). However, *Usnea* species sensitive to SO_2 air pollution were found 13.25 km south of Karabash at Novoandreevka (Udachin *et al.* 2003). In the UK, *Usnea* and other sensitive species have survived high SO_2, as on *Quercus* 4.65 km from Consett Steel works, County Durham (Purvis, 2010) and in the remote limestone Derbyshire Peak District (Hawksworth 1974). Whilst epiphytic lichens under pollution stress have been observed to be restricted to particular regions of tree trunks and upper branches, the extent to which bark acidity and element concentrations influence lichen species richness on twigs has, to our knowledge, not previously been investigated.

During June 2011, *Hypogymnia physodes* and other lichens abundantly colonising *Betula* twigs were unexpectedly discovered at Nyazminsky Ridge, 40 km downwind from Karabash and 6 km north of Zlatoust, around which high anthropogenic pollutant loadings were previously identified (Frontasyeva *et al.* 2004). This outlier of the NE - SW transect centred on Karabash, was selected as a transplant site. Selected trace element data in *Hypogymnia* was considered in relation to modelling data (Pollard *et al.*, 2015). The aims of the current study were (i) to assess *Hypogymnia* frequency on *Betula* trunks as an indicator of anthropogenic activities; (ii) to investigate possible links between geology and anthropogenic activities as assessed from bark pH and element content (sulphur, a potential pollutant, and strontium, a calcium analogue) and epiphytic lichen richness colonising twigs.

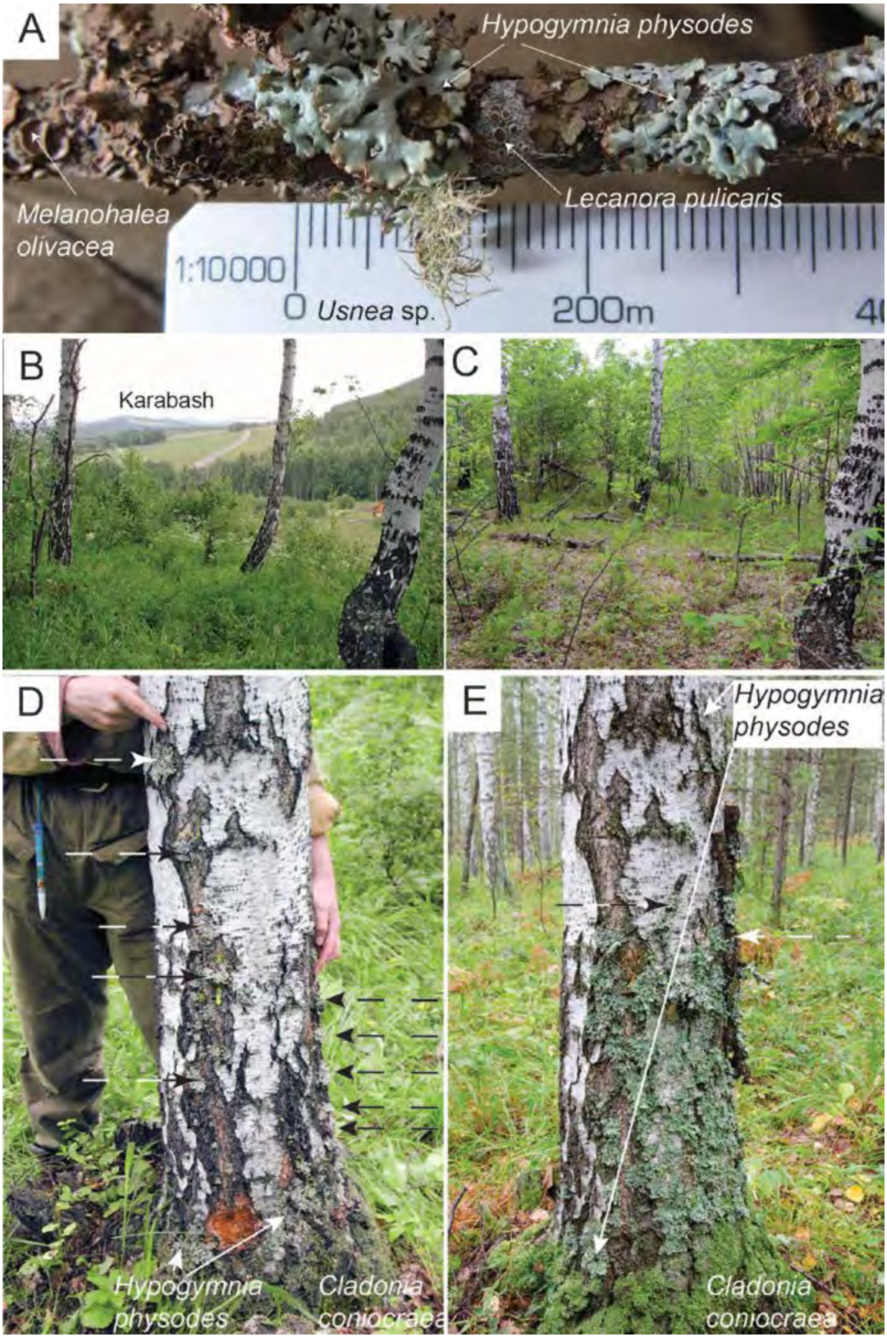

Figure 1. Sites monitored in June to September 2011 included a new outlier and transplant site at Nyazminsky Ridge, 3 June 2011 and some of the same trees previously monitored along a SW - NE transect centred on Karabash (A) *Hypogymnia physodes* colonising young *Betula* trunk at Nyazminsky Ridge, 3 June 2011; (B) View from Site 11, Novoandreevka, 8 July 2001; (C) Same view, 6 June 2011; (D) Monitoring tree no 6, Site 3, Severnye Pechi, 8 July 2001 when thalli-bark samples were glued to transplant station tree bases in two rows of five thalli; (E) two twigs each colonised by at least 5 *Hypogymnia* thalli after 3 months exposure, 13 September 2011.

Materials and Methods

Study area

Karabash lies in Chelyabinsk region within a NE-SW trending flat-bottomed valley. Prevailing winds are from the north-west (Sadykov *et al.* 1992). Phytogeographically, the region lies within intermediate forests between taiga and forest-steppe zone. Characterised by broad-leaved and coniferous forests, *Betula pubescens* and *B. verrucosa* are locally frequent due to forest management and other anthropogenic activities. Two major nature reserves are situated in the region: (i) mineralogical Zapovednik 'Ilmensky' (http://www.rusnature.info/zap/069.htm) and (ii) Taganay with a wide variety of habitats and notable ridges adjoining the northern suburbs of Zlatoust (Richmond *et al.* 2006). Nyazminsky ridge, lies 40 km SW of Karabash and 6 km N of Zlatoust city in mixed coniferous (*Abies*, *Picea*, *Pinus*) / deciduous (*Betula* and *Sorbus*) woodland in herb-rich grassland.

The Ural Mountains form a N-S trending 2000 km long belt extending from the Islands of Novaya Zemlya in the north to the Aral Sea in the south (Pushkarev *et al.*, 2013). Well known for their volcanic-hosted massive sulfide and orogenic lode gold deposits, they also host porphyry and epithermal systems. Soil cover is generally thin and sporadic and mainly consists of luvisols, with minor areas of regosols and cambisols (Udachin *et al.* 2003). Upper soil horizons around Karabash have been influenced by anthropogenic acidification leading to the mobilization of Al and other metals and the formation of soil solutions with anomalously high concentrations of potential toxins (Aminov 2010).

Sites

Sites monitored in June to September 2011 were within *ca* 500 m of 10 monitoring sites (3-12) established in medium-aged birch stands over the period 8-13 July 2001 along a SW - NE transect centred on Karabash. In some cases the same trees were monitored as 10 years randomly sampled previously (Fig. 1B-E). Exceptions were at site 12 established 3.69 km to the North and site 10 was not relocated owing to an incorrect GPS reading made in 2001. In 2011, two ca 60 km transects centred on Karabash (prefixed U) ran from U3, Severnye Peche (ca 25 km S of Karabash), to U8, Kyshtym (ca 33 km NE of Karabash), and from 3 sites up to 20.44 km W (U21) and 4 sites up to 38 km (U20) ESE of Karabash; U0, the outlier and transplant site was located at Nyazminsky Ridge (800 m elevation), 40 km SW of Karabash. Over 150 twigs, each colonised by at least 5 *Hypogymnia physodes* thalli, were collected from *Betula* from Nyazminsky Ridge in June 2011. Twigs were sampled using powder-free gloves and stored in paper capsules prior to transplantation at the outlier and across the NE-SW transect (9 stations). At each station, 6 *Betula* trees were selected and two *Hypogymnia*-colonised twigs tied securely to the tree bases in a similar position to where lichen bark pieces were previously attached using adhesive (reviewed in Purvis *et al.* 2013).

Sites were between 280 and 695 m in elevation apart from site 0 at Nyazminsky Ridge (800 m) and more than 150 m from roads, apart from site 11

(Novoandreevka). All transplanted *Hypogymnia*-covered twigs were randomly collected between 5 and 13 September after 3 months, the exposure period previously determined as being optimal (reviewed in Purvis *et al.* 2013) and stored in clean polythene bags.

Assessment of Lichen Species Richness

Hypogymnia physodes frequency in 2011 was assessed at the base of trunks and at 1.5 m above ground level on each of the 6 *Betula* trees randomly selected for monitoring in each site using a 10-field frequency net (Herzig & Urech 1991). In 2001, all epiphytic lichen species were recorded (Table 1). In 2011, accessible branches were cut at an elevation of 2-4 m and twig lichen richness (presence/absence) assessed on six 1 m length twig samples from each tree and species richness expressed in terms of % frequency for each species at each site. In addition, 2-3 mm thick, smooth and ± flat bark trunk samples measuring ca 4 x 10 cm were collected at each site between 1.5 - 2.5 m above ground level and between 1-6 per site selected for pH measurement. According to accessibility, three *Betula* twigs were sampled (ca 10 x 0.5 - 0.7 mm thick) from 6 trees (i.e. 18 twigs) at sites 0, 3, 4, 5 and 10 and from 1 tree at sites 6, 7, 8, 9, 11-16, 18-21) for pH determination. Lichens were identified, following the lichen nomenclature of Urbanavichus (2010).

pH measurement

The pH of 132 twigs and 85 trunk pieces randomly sampled in September 2011 were measured in triplicate in the laboratory. Twigs were cut into 7 cm lengths, the cut ends sealed with paraffin wax and soaked in tubes filled with 6 cm^3 25 mM KCl. Samples were shaken for 1 hour at room temperature using an automatic shaker, the twigs removed and the pH of the solution measured with a Jenway Model 370 pH/mV/Temp Meter using an epoxy combination pH electrode (924 001) and ATC probe (027 500). Small, ca. 2.5 cm^2 pieces of *Betula* bark (lower surfaces not waxed) were placed into small beakers, immersed for 5 minutes in 2 cm^3 25 mM KCl and surface bark pH measured using a BDH Gelplas Flat Tip combination membrane electrode (cat ref: 309-1070-03). Mean pH values were calculated from H^+ concentrations and average values calculated for twig and bark samples at each site.

Chemical analysis

At the Institute of Mineralogy, Miass, at least five *Hypogymnia physodes* thalli, selected at random, were removed from two twigs exposed at each site using a stainless steel knife and latex powder-free gloves. Species other than *Hypogymnia*, together with bark flakes and foreign matter, were removed under a binocular microscope. Thalli were bulked from each tree at each site (i.e. ca 30 thalli per site), with the exception of 4 stations (corresponding to ‘outlier 2011’ (site U0), 'reference site 2001' (site U3), ‘intermediate’ (site U12) and ‘impact’ (site U5) where samples were bulked from each tree, to provide replicate analyses for five thalli for each tree. Lichen samples were oven dried (40°C) overnight, and up to ca 10 g lichens ground in an agate pestle and mortar under liquid nitrogen to a fine powder. *Betula* bark samples (twigs and trunks) were similarly prepared for analysis. Multi-element analysis was performed via inductively coupled plasma mass spectrometry (ICP-MS) calibrated using commercially available standard solutions for elements, including Sr. Quality control for elements, where published values were available (Sr), was provided through identical analyses of the reference materials BCR 482 Lichen and SRM 1547 Peach Leaves, accepting elements within ±10% of the published reference or information values. Analytical methods employed followed Rusu (2002). Sulphur analysis was performed on samples by ICP atomic emission spectrometry at the University of Sheffield.

Statistics

Multivariate analysis was carried out on *Hypogymnia* frequency and twig lichen richness data using non-metric multidimensional scaling and cluster analysis from the package PRIMER v6. *Hypogymnia* frequency at the base of *Betula* and at 1.5 m above ground level, and for *Betula* trunk and twig, bark pH and element concentrations were plotted as a function of distance from Karabash. The coefficient of determination (r^2) was calculated and Pearson product moment correlation coefficient (r) to assess the strength of the correlations. Student's t tests (assuming unequal variances) were used to investigate differences in sample element concentrations between lichen bark samples.

Results

Hypogymnia frequency and lichen species richness

Frequency ranged from 0-100% on trunk bases and at 1.5 m above ground level, a lower frequency usually being recorded at 1.5 m, compared with at ground level (Fig. 2A-B). In spite of changes in canopy and ground cover across the SW-NE transect over the 10 year period leading to shading and competition (e.g. Fig. 1B-C), statistically highly correlated trends were observed for *Hypogymnia* frequency at both ground ($R^2 = 0.88$, $p<0.001$) and 1.5 m above ground level ($R^2 = 0.77$, $p<0.001$). The classic bell-shaped curves indicate a point source (Karabash) influence consistent with spatial atmospheric SO_2 and metal influences.

18 species were recorded on twigs at Site U0, Nyazminsky Ridge, of which 6 also occurred at Site 3, Severnye Pechi (368 m elevation) (Table 1).The most abundant recorded on twigs was *Melanohalea olivacea* (97%) followed by *Hypogymnia physodes* and *Parmelia sulcata* (both 83%). Five groups were resolved by multivariate analysis (Fig. 3B). Groups corresponded, in order of increasing similarity to:-

(A) Site '0', 39 km from Karabash. The outlier situated at 800 m elevation. Characterised by 18 epiphytic lichen species recorded on *Betula* twigs (Table 1) and the highest recorded frequency of *Hypogymnia* on *Betula* trunks at 1.5 m above ground level (100%) (Fig. 2A).

(B) Site '3', 26 km from Karabash. Southernmost site of SW transect characterised by 6 lichen species recorded on *Betula* twigs: *Cetraria sepincola*, *Hypogymnia physodes*, *Lecanora pulicaris*, *Melanohalea exasperatula*, *Parmelia sulcata* and *Scoliciosporum* sp. and the highest frequency of *Hypogymnia* recorded at the base of *Betula* (100%).

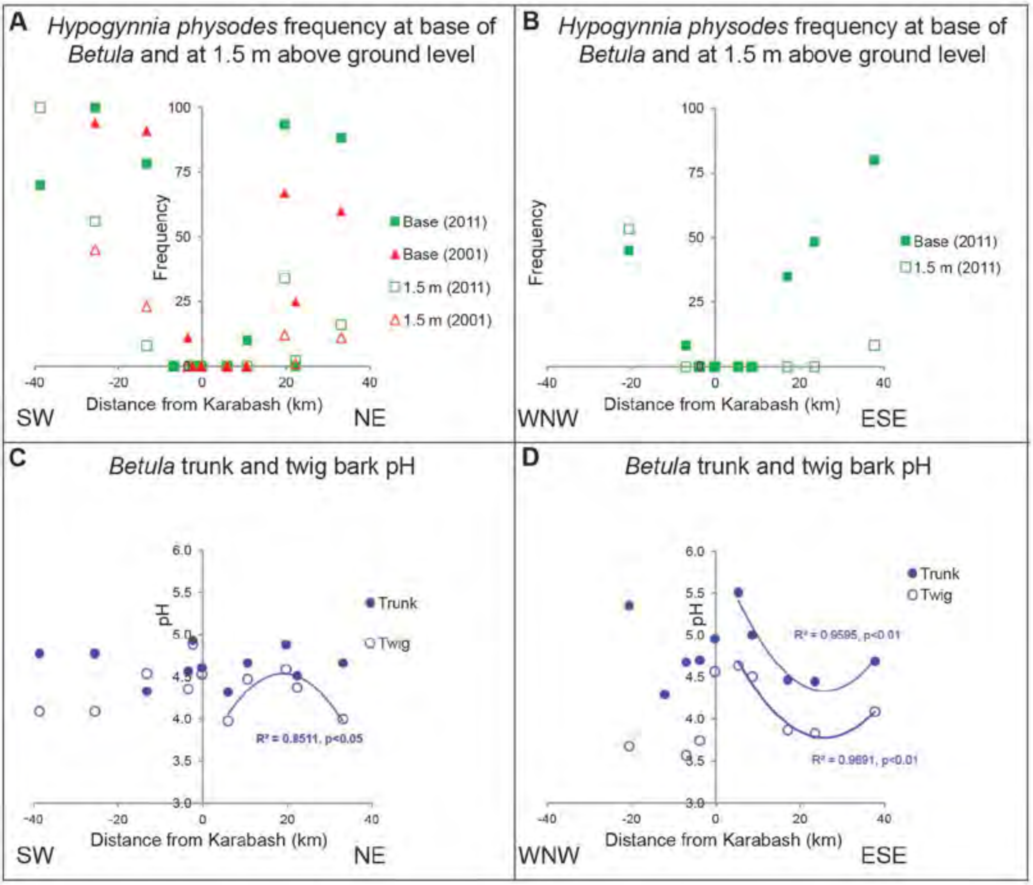

Figure 2. Temporal trends in *Hypogymnia physodes* frequency at base of *Betula* and at 1.5 m above ground level across (A) SW-NE transect in 2001 and 2011, (B) WNW-ESE transect in 2011; *Betula* trunk and twig bark pH recorded in 2011 across (C) SW-NE and (D) WNW-ESE transects.

(C) Sites '21', '8', '9', '11', and '20'. Average site distance from Karabash = 25 km. Outer sites characterised by the absence of lichens recorded on twigs but moderate frequency of average *Hypogymnia physodes* recorded at 1.5 m above ground level (26%) and high frequency at ground level (77%).
(D) Sites '18', '19', '7', '16'. Average site distance from Karabash = 14 km. Inner sites characterised by the absence of lichens recorded on twigs and at 1.5 m above ground level and low frequency at ground level (25%).
(E). Sites '12', '4', 5', '6', '10', '15', '13', and '14'. Average site distance from Karabash = 6 km. Central sites and '10' lying to the NE characterised by the absence of lichens on twigs and *Hypogymnia physodes* on trunks.

Bark trunk and twig pH

Bark pH was acidic throughout, average site values ranging from 3.57 to 4.88 (twigs) and from 4.29 to 5.51 (trunks). The pH of trunk and twig samples was correlated

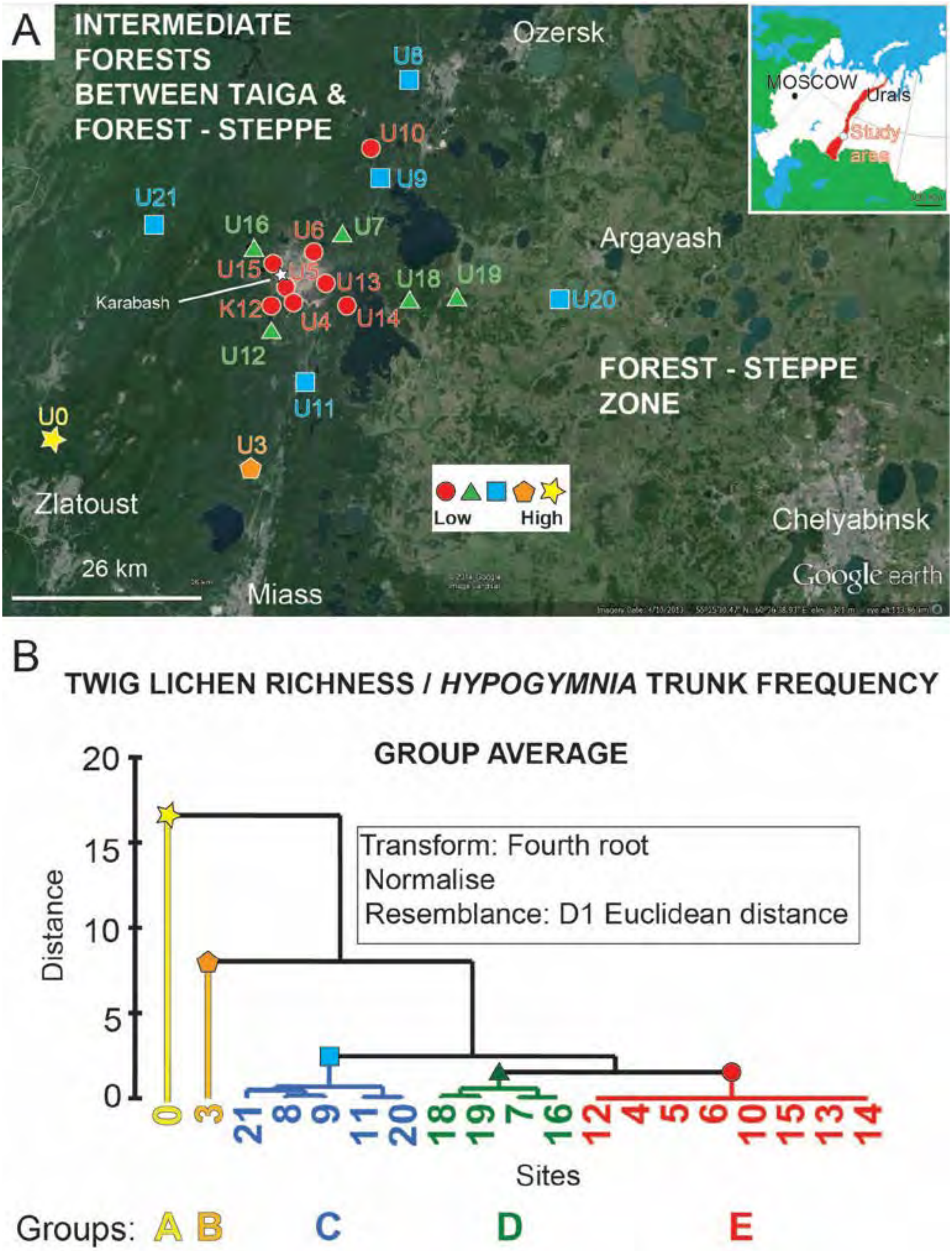

Figure 3. (A) Selected lichen monitoring sites, 2011. Colour coding corresponds to groups resolved by multivariate analysis using 2011 data (see B) Modified GeoEye Image Geocentre Consulting © 2011. Note K12 was monitored in 2001 and U12 in 2011. (B) Dendrogram from cluster analysis (Euclidean Distance, normalised data) showing site separation for twig lichen diversity and *Hypogymnia* frequency on trunks. Plot B was derived from analysing sites according to the variables 'Twig Lichen Richness and *Hypogymnia* frequency' (at ground and 1.5 m above ground level). Twig lichen richness was assessed on six 1 m length twig samples from each tree and expressed in terms of % frequency for each species at each site. These were analysed as separate variables in the CA.

(n=8, R^2 = 0.77, p<0.01) for sites lying along the SW - NE transect, within 25 km of the smelter, and similarly along the WNW - ESE transect (n=7, R^2= 0.66, p=0.05), for sites within 10 km WNW and 25 km ESE of Karabash (Fig. 2C-D). The highest bark pH (pH 5.51) was recorded at site U13, 5.34 km NE of Karabash.

Chemical data for lichens and bark

Strontium concentrations in transplants, naturally occurring *Hypogymnia* and *Betula* trunk bark showed no clear pattern in relation to the point source (Karabash) (Fig. 4A). Recorded concentrations were significantly lower (t=9.5, d.f. = 10, p<0.001) in *Betula* bark (mean 560 $\mu g\ g^{-1}$) than recorded in transplants (mean 1130 $\mu g\ g^{-1}$). Sulphur concentrations in the twigs recorded in 2011 (average 1130 $\mu g\ g^{-1}$) were significantly lower (t=-3.01, d.f. = 13, p<0.05) than recorded in transplants in 2011 (average 1489 $\mu g\ g^{-1}$). Paradoxically, the lowest S concentrations were recorded in *Hypogymnia* transplanted adjacent to the smelter and highest in the sample transplanted furthest away at the 'reference' site U0 (Fig. 4B).

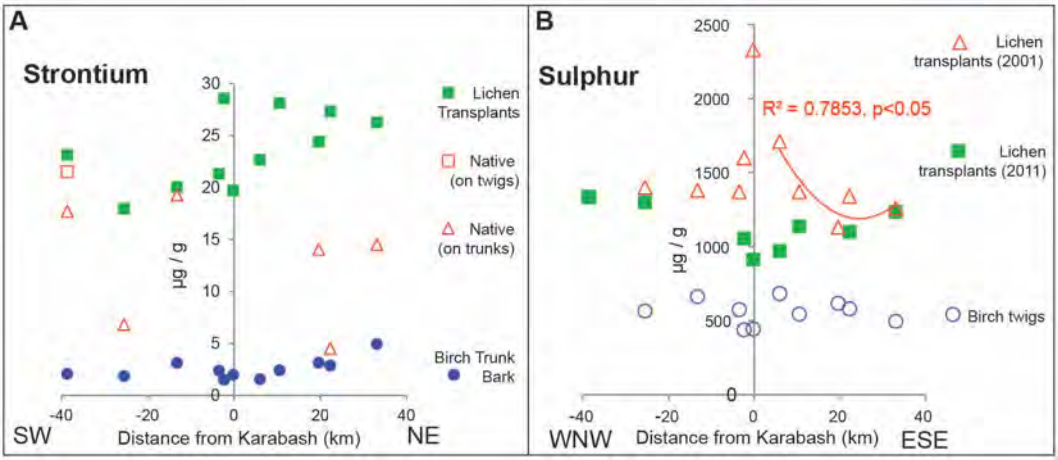

Figure 4. (A) Sr concentrations in transplants, naturally occurring (native) *Hypogymnia* thalli sampled on trunks and twigs, and *Betula* trunk bark across the transect in 2011, with Karabash at the centre; (B) Sulphur concentrations in *Hypogymnia* transplants across the same transect in 2001 and 2011 and birch twigs in 2011.

Discussion

Influence of the point source

Statistically significant *Hypogymnia* frequency bell-shaped patterns recorded at both tree bases and at 1.5 m above ground level over the 10 year period (Fig. 2A), trunk and twig bark pH trends (Fig. 2C-D) and lichen species richness confirm anthropogenic point source influence(s). Statistically significant trends were apparent in both trunk and twig bark pH values sampled E of Karabash, and in twigs sampled WNW and NE of Karabash. This emphasises the importance of weather over the transplant period leading to short term deposition corresponding to the predominant wind direction (i.e. from the SW) over the 3-month transplant period.

Geological influence on lichen assemblage composition

In the 2001 study, Ca reached the highest concentration (60,300 mg kg^{-1}) of all elements analysed in *Hypogymnia* thalli at site 11, Novoandreevka (Purvis *et al.* 2006) where *Usnea* was also recorded (Udachin 2003). *Diploschistes muscorum*, a lichen initially parasitic on *Cladonia coniocraea* squamules, and characteristic of calcareous soils (Smith *et al.* 2009), was recorded here on *Betula* trunks in 2001 (Table 1) and also noted to be present in 2011. Geological influences are implemented, either from terrestrial sources, or aerial deposition. Particles, which if deposited on bark and twig surfaces could significantly raise pH, include limestone or lime (CaO), or its hydrated equivalent (calcium hydroxide $CaOH_2$) (Gilbert 1976) often used as fluxes in smelter operations.

Presence of the outlier at Nyazminsky Ridge

Discovery of 6 species colonising twigs at a lower elevation (365 m) at Site U3 suggests that elevation (and associated factors such as precipitation) is not responsible for the lack of colonisation of twigs by lichens elsewhere. The outlier appears to represent a lichen 'oasis', possibly present due to topographic influences. It provides a refugium enabling future lichen re-colonisation elsewhere under favourable atmospheric conditions. Further study of adjacent areas at higher elevations need to be undertaken in relation to herbarium collections and atmospheric pollution, climatic and geological data in order to fully put the present assemblages into an historical context.

Lichen sulphur contents and spatial patterns of environmental acidification

Sulphur concentrations in transplants sampled furthest from Karabash, at Severnye Pechi and Kyshtym, in 2001 and 2011 were similar (Fig. 4B). However, in 2011 the highest concentration (1335 μg g^{-1}) was recorded in transplants sampled from the outlier, Nyazminsky Ridge and not adjacent to the point source as recorded in 2001 (Fig. 4B). Levels were lower than those reached across transects in Isle Royale National Park, wilderness island in north western Lake Superior, North America (Bennett 1995) and Voyageur's National Park, in Northern Minnesota, North America (Bennett & Wetmore 1997), but higher than recorded in *Hypogymnia physodes* from 'background areas' in Finland (Manninen *et al.* 1991). This contrasts with previous studies in boreal regions which reported curvilinear trends in sulphur contents of *H. physodes* from areas with point sources, the highest levels near the point source corresponding to areas with lower lichen diversity (e.g. Manninen *et al.* 1991; Bennett & Wetmore 1997).

Conclusions

Statistically significant trends in bark pH and *Hypogymnia* frequency confirm a point source relationship. *Hypogymnia physodes* and *Melanohalea olivacea*, the two most abundant species colonizing twigs, were also the most abundant epiphytic lichens on

Betula in northern Fennoscandia near smelters from sea level to the tree line (Aamlid & Skogheim 2001; Bjerke *et al.* 2006). The study highlighted the sensitivity of *Hypogymnia* bark pH to assess sulphur deposition from smelter-derived aerosols over short (<3 month) time periods. Links between *Hypogymnia* frequency and lichen species richness on *Betula* and pH were not made due to the weather over the transplant period. Element cycling via the soil - tree - lichen system must also be considered.

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Table 1. Epiphytic Lichens recorded during quantitative recording along SW - NE transect in 2001 and the same transect and outlier site U0 in 2011.

Lichen Species	on Trunks (2001)	on Twigs (2011)
Candelariella vitellina	+	
Cetraria sepincola	+	+*
Cladonia botrytes	+	
C. cenotea	+	
C. coniocraea	+	
C. digitata	+	
C. fimbriata	+	
C. macilenta	+	

Cladonia rei	+	
Diploschistes muscorum	+	
Evernia mesomorpha	+	+
Flavopunctelia soredica	+	
Hypocenomyce friesii	+	
H. scalaris	+	
Hypogymnia physodes	+	+*
Lecanora chlarotera		+*
Lecanora pulicaris	+	+
Lecanora cf. confusa		+
L. swartzii subsp. *nylanderi*	+	+
L. symmicta s. lat.	+	
Lepraria sp.	+	
Melanohalea olivacea	+	+*
Melanohalea exasperatula		+
Micarea denigrata	+	
M. prasina	+	
Parmelia sulcata	+	+*
Parmeliopsis ambigua	+	
P. hyperopta	+	
Physcia tenella		+
Physconia sp.	+	
Placythiella cf. *dasaea*	+	
P. icmalaea	+	+
P. uliginosa	+	
Pseudevernia furfuracea		+
Ropalospora cf. *viridis*	+	
Rinodina sp.		+
Scoliciosporum chlorococcum	+	+*
Trapeliopsis flexuosa	+	
T. granulosa	+	
Usnea sp.	+	+
Vulpicida pinastri	+	
Sterile crust		+
Fertile sp.		+
TOTAL	**35**	**18**

All lichens on twigs were recorded from Site U0, Nyazminsky Ridge, and *+ additionally recorded from Site U3, Severnye Pechi.

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