

Mountain birch under multiple stressors – heavy metal-resistant populations co-resistant to biotic stress but maladapted to abiotic stress

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shelter;
trade-off.

Abstract

Stress adaptations often include a trade-off of weakened performance in nonlocal conditions, resulting in divergent selection, and potentially, genetic differentiation and evolutionary adaptation. Results of a two-phase (greenhouse and field) common garden experiment demonstrated adaptation of mountain birch (*Betula pubescens* subsp. *czerepanovii*) populations from industrially polluted areas of the Kola Peninsula, north-western Russia, to heavy metals (HM), whereas no adaptations to wind or drought stress were detected in populations from wind-exposed sites. HM-adapted seedlings were maladapted to drought but less palatable (co-resistant) to insect herbivores, even under background HM concentrations. The absence of adaptations to harsh microclimate and the generally high adaptive potential of mountain birch, a critical forest forming tree in subarctic Europe, need to be accounted for in models predicting consequences of human-driven environmental changes, including the projected climate change.

Introduction

Extreme environmental stress, which causes uneven mortality in a population or otherwise reduces the reproductive fitness of sensitive genotypes, may act as a selecting factor resulting in a local population evolving trait advantageous under the local stressor (reviewed by Kawecki & Ebert, 2004). Adaptations of plants to different habitat types that have been studied since the early 20th century (Turesson, 1922, 1925) remain a 'hot spot' in evolutionary ecology. Stress-adapted genotypes do not usually become fixed in the entire species range due to antagonistic pleiotropy, i.e. they are selected against in nonlocal habitats (Kawecki & Ebert, 2004; Bijlsma & Loeschcke, 2005). These kinds of trade-offs are a part of the very foundation of contemporary evolutionary ecology.

Adaptations to anthropogenic heavy metal (HM) stress provide a well-documented example of rapid evolutionary

change (Bradshaw & McNeilly, 1981; Macnair, 1997; Stockwell *et al.*, 2003), but most of the studies have been conducted on short-lived herbaceous species (Dickinson *et al.*, 1991; Turner, 1994). In addition, HM adaptations often incur a cost of reduced performance in nonlocal conditions (Hickey & McNeilly, 1975; Hoffman & Parsons, 1991; Macnair, 1997). However, in some cases adapting to one HM can result in increased tolerance to other HMs (Watmough & Dickinson, 1995; Utriainen *et al.*, 1997) and even biotic stressors (reviewed by Poschenrieder *et al.*, 2006), a phenomenon known as co-resistance or co-tolerance. Co-tolerance between HMs and natural abiotic stress, on the other hand, has, to our knowledge, only rarely been studied (but see Eränen, 2008).

Mountain birch (*Betula pubescens* subsp. *czerepanovii* [Orlova] Hämet-Ahti) is the tree-line species and one of the main forest-forming species in subarctic Europe. The genus *Betula*, particularly *B. pubescens* of northern Europe, shows exceptionally high genetic variation, which can fuel genetic differentiation (Eriksson & Jonsson, 1986; Howland *et al.*, 1995). Indeed, populations of Kola Peninsula (NW Russia) mountain birch growing in

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industrially polluted areas have been shown to be adapted to HM stress (Kozlov, 2005; Eränen, 2008), but adaptations to natural stressors remain almost unexplored (but see Eränen & Kozlov, 2008b). The Kola Peninsula offers an interesting environment for studies on evolutionary ecology, with the subarctic climate, surrounding Arctic Ocean, mountain ranges and heavy industry creating multiple stress gradients with potential to study adaptations to multiple stressors, including, e.g. high wind stress (Kozlov, 2002; Ruotsalainen *et al.*, 2008) and temperature extremes (Kozlov & Haukioja, 1998; Kozlov, 2001).

In the current paper, we report the results of a two-phase common garden experiment with mountain birch seedlings from the high- and low-stress ends of multiple stress gradients around the Kola Peninsula. The stress gradients were classified as natural, with decreasing shelter and increasing wind stress, evaporation and temperature extremes at the high-stress ends, or pollution, with increasing levels of HM and SO₂ at the high-stress ends. In the first part of the experiment, seedlings were studied in a greenhouse with the aim of finding out: (1) if seedlings from HM-polluted sites are adapted to HM stress, (2) if the putative adaptation has resulted in co-tolerance to drought, (3) whether seedlings from open, exposed, sites tolerate drought or HMs better than seedlings from sheltered sites. In the second part of the experiment, seedlings were planted in the field either in sheltered or open (wind-exposed) sites. The questions we aimed to answer were: (4) are seedlings from open habitats adapted to abiotic (wind) stress and (5) are HM-tolerant seedlings co-tolerant to wind stress. In addition,

leaf damage caused by insect herbivores was measured to check: (6) whether seedling origin affects herbivore damage. In both parts of the experiment, we were also interested (in case of no co-tolerance): (7) whether adaptations to a specific stressor have resulted in trade-offs of reduced performance in pristine conditions or under other forms of stress. Our results indicate HM adaptation of mountain birch populations located in industrially polluted areas, a phenomenon probably linked to their lowered nickel uptake. HM-adapted seedlings were maladapted to drought but were co-resistant to insect herbivory, even under background HM concentrations. No evidence of adaptations to natural abiotic stress was detected.

Materials and methods

Sites of seedling origin and pre-experiment treatments

Mountain birch seeds were collected in October 2002 from 18 source sites (from five mother trees per site) around the Kola Peninsula, NW Russia (Table 1). The stress levels at the source sites were classified according to their pollution loads (polluted or control, called 'pollution at origin' hereafter) and wind exposure (open or sheltered, called 'shelter at origin' hereafter). The polluted source sites were located around the copper–nickel smelters of Nikel and Monchegorsk (Table 1). Both smelters have been among the top European sources of aerial pollution since the 1950s, and their emissions have caused extensive forest decline resulting in secondary

Table 1 Characteristics of the source sites.

Code	Coordinates	Altitude (m a.s.l.)	Site description	Habitat characteristics	
				Pollution at origin	Shelter at origin
N1	69°25'N, 30°17'E	240	1.5 km W of the Nikel smelter, sheltered by pine trees	Polluted	Sheltered
N1'	69°25'N, 30°14'E	110	1 km N of the Nikel smelter, open industrial barren	Polluted	Open
N2	69°24'N, 30°11'E	115	3 km S of the Nikel smelter, bog sheltered by pines	Polluted	Sheltered
N2'	69°24'N, 30°12'E	200	2.5 km SE of the Nikel smelter, open industrial barren on a hillside	Polluted	Open
N5	69°20'N, 30°02'E	130	12 km S of the Nikel smelter, healthy mixed subarctic forest	Control	Sheltered
N6	69°19'N, 29°40'E	140	25 km SE of the Nikel smelter, healthy mixed subarctic forest	Control	Sheltered
T1	69°29'N, 31°26'E	250	Open tundra site along the Pechenga–Murmansk main road	Control	Open
T2	69°28'N, 31°51'E	110	Open tundra site along the Pechenga–Murmansk main road	Control	Open
1252	67°51'N, 32°50'E	270	7 km S of the Monchegorsk smelter, a sheltered site in a small woodland	Polluted	Sheltered
1252'	67°51'N, 32°49'E	260	7 km S of the Monchegorsk smelter, open industrial barren	Polluted	Open
1258	67°54'N, 32°47'E	190	1 km S of the Monchegorsk smelter, a sheltered site in a small woodland	Polluted	Sheltered
M2	67°58'N, 32°50'E	135	4 km NW of the Monchegorsk smelter, open industrial barren	Polluted	Open
R1	67°35'N, 33°44'E	580	Open site on the S facing hill of Rasvumchorr mountain above the timberline	Control	Open
R3	67°34'N, 33°41'E	305	Sheltered site in a mixed subarctic forest near the Rasvumchorr mountain	Control	Sheltered
V1	67°41'N, 33°39'E	470	Open site on the S facing hill of the Vudjavr mountain above the timberline	Control	Open
V3	67°39'N, 33°39'E	355	Sheltered site in a mixed subarctic forest near the Vudjavr mountain	Control	Sheltered
O1	66°28'N, 35°12'E	4.3	Open site 50–100 m from the seashore near the village of Olenitsa	Control	Open
O3	66°28'N, 35°12'E	5.2	Sheltered site in a mixed subarctic forest some 400 m from the seashore	Control	Sheltered

open habitats known as industrial barrens (Kryuchkov, 1993; Kozlov & Zvereva, 2007 and references therein). The emissions have also resulted in the local adaptation of mountain birch populations, with individuals from polluted source sites being more resistant to HMs (Cu and Ni) than seedlings from control environments (Kozlov, 2005; Eränen, 2008). The open source sites were located (in addition to Nikel and Monchegorsk) along the White Sea shore in southern Kola Peninsula (Olenitsa), on the hills of the Lovchorr (Rasvumchorr) and Kukisvumchorr (Vudjavr) mountains in central Kola Peninsula and in an open tundra close to Pechenga in NW Kola Peninsula (Table 1), in sites of very little wind shelter. In relative proximity to each polluted and open source site, seedlings were also collected from low-stress sites, each being either pristine in terms of damage by aerial pollution (control), or sheltered from damage caused by wind and other abiotic factors (sheltered) (Table 1). The tundra and Monchegorsk source sites were the exceptions, with only open sites in the tundra and only polluted sites near Monchegorsk. Altogether there were eight source sites classified as polluted, 10 classified as control, nine source sites classified as open and nine classified as sheltered. For a more detailed description and general information on the source sites see Eränen (2008) and Ruotsalainen *et al.* (2008).

After collecting the seeds, they were sent to the Phytotron of the University of Tromsø, Norway, where they were weighed (mean seed weights for each mother tree calculated from 100 seeds) and stored at a low temperature (3 °C) in dry conditions between 12 November 2002 and 15 January 2003. The seeds were stratified by mixing them with moist sand in 1-L containers for 4 weeks at a temperature of 0.5–3 °C. In 14 February 2003, the seed/sand mixture was spread in a thin layer on moistened soil in trays which were kept at 21 °C under good light conditions (150–200 $\mu\text{mol m}^{-2} \text{s}^{-1}$) day and night. After germination (in about 1 week), the temperature was decreased to 18 °C, but the light conditions remained unchanged. As our seedlings were from very northern provenances, a 24-h photoperiod was important to keep the seedlings growing. The total germination was estimated 3 weeks after sowing, and seedlings were individually replanted into pots with a mixture of 70% standardized peat soil and 30% perlite and grown at 15 °C until the beginning of the experiments.

Phytotron experiment

The experiment was conducted by using a sophisticated greenhouse (phytotron) at the University of Tromsø, Norway. The air temperature is controlled at an accuracy of ± 0.5 °C, and air humidity of $\pm 5\%$ RH, always at a constant water vapour saturated deficit of 530 Pa. We intended to use nine seedlings from each 90 mother trees. However, due to low germination in some prog-

enies, the total number of mother trees used was 79, with 711 seedlings in total. These seedlings were evenly distributed among three growth chambers (with identical climatic characteristics) within the phytotron. Rooms were considered blocks in the data analysis. Seedlings within each block were evenly distributed among three treatments: control (C), application of HMs and drought (D) to study potential adaptations to HM and D stress in seedlings of different origin. The experiment started on 13 May 2003 at 15 °C in natural light conditions.

Five days a week, the seedlings in C and HM treatments were watered with 30 mL of tap water, whereas seedlings in D treatment received 15 mL. The water used to irrigate the seedlings in HM treatment included a solution of nickel and copper sulphates, containing 5 mg L⁻¹ copper and 10 mg L⁻¹ nickel. In the last 2 days of the week, all plants were irrigated with clean tap water in the same quantity as mentioned above. The treatments were continued until 24 June 2003, after which the plants were moved outside and left untreated except for irrigation on dry periods.

Seedling height was measured prior to the experiment (planting height) for use as a covariate. Performance indices were measured in August 2003 prior to leaf senescence. The indices measured were seedling height, number of long shoots, shoot length (max ten long shoots), length of the largest leaf (without the petiole) and chlorophyll fluorescence. Average shoot lengths were calculated for each seedling. Chlorophyll fluorescence was measured with a portable plant stress meter (Biomonitor S.C.I. AB, Umeå, Sweden). The indices measured were the ratio of variable to maximum fluorescence yielded under the artificial light treatment (light level 200 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) (F_v/F_m) and the time needed for the leaf to reach half of its F_m ($T_{1/2}$). For maximum level of fluorescence, the leaves were dark adapted for 15 min with a leaf cuvette prior to measuring. Greater seedling height, number of shoots, shoot length, leaf length and F_v/F_m values and smaller $T_{1/2}$ values were considered signs of higher fitness. Survival was monitored throughout the experiment.

Foliar copper and nickel concentrations were analysed at the end of the experiment from HM-treated seedlings. Leaves from short shoots were cut with scissors so that petioles were not included in the sample. Great care was taken to avoid cross-contamination. Unwashed leaf samples were packed into paper bags, dried for 12–24 h at 80 °C, and preserved for the analysis. Concentrations of Ni and Cu were determined using ICP (Analyst 800; Perkin-Elmer, Waltham, Massachusetts, USA) in the Institute of North Industrial Ecology, Apatity, Russia.

Field experiment

The field experiment was established in two localities in northern Norway, with two study sites at each locality. The sites were selected at contrasting environments, with

an open site (sunny and wind-stressed) and a sheltered site (shaded, wind-protected) selected at each locality ('shelter' hereafter). Each study site was further divided into three blocks that were treated as replicates. The sites ($15 \times 21 \text{ m}^2$) were fenced to prevent large-scale damage of seedlings by mammalian herbivores, mostly reindeer. Shrubs and trees were cut down from the open study sites, including a 1-m buffer zone around the sites. The vegetation in sheltered sites was left intact. At the Skibotndalen locality ($69^\circ 07' \text{N}$, $20^\circ 45' \text{E}$), the sheltered site was established in a birch woodland, formed by old polycormic mountain birches (basal diameter 60–100 mm, height 3–5 m), whereas the open site was selected on a heath nearby. The second pair of study sites was established near Narvik ($68^\circ 31' \text{N}$, $17^\circ 58' \text{E}$). The sheltered site was selected in a swampy valley of a small river, and the open site was established atop a small hill nearby on a swampy heath-like spot.

Phytotron-grown seedlings, different from those used in the phytotron experiment, replanted in 1-L pots and stored outside from the middle of May 2003, were planted on 7 August 2003 at the Skibotndalen locality, and on 10 August near Narvik to study potential adaptations to wind-induced abiotic stress. In each block, we planted one seedling from each of the 79 mother trees, with 237 seedlings at each study site and 948 seedlings in total. The distance between experimental seedlings was 70 cm. The seedlings were well irrigated just before planting.

Wind velocity in each study site was measured five times between 3 and 5 August 2003 by using Kestrel® Pocket Wind Meter (Nielsen-Kellerman, Boothwyn, PA, USA; speed range $0.3\text{--}40 \text{ m s}^{-1}$, accuracy 0.1 m s^{-1} or 3% of reading for velocities exceeding 3.5 m s^{-1}). Measurements at each site were performed five times, with the interval between measurements in two sites within localities not exceeding 5 min. The wind meter was placed at a height of 2 m, with impeller perpendicular to the main wind direction; an average wind speed was recorded for a 10-s interval ten times with 10-s intervals between measurements, and averaged for a session-specific value. Illumination was measured from each study site during 11 and 12 August 2004 with a Panlux electronic luxmeter (Gossen, Germany) and replicated twice. The measurements were conducted next to seedling crowns (isc) and at an open site close by at a height of 2 m (i2m) in an overcast day to avoid the effects of shadows. Relative illumination $[(isc/i2m) \times 100\%]$ was calculated for each pair of measurements.

Soil characteristics (pH, C, N, P, Mg, Ca, K, Zn and Mn) were measured from two samples at each study site. Soil samples were dried at room temperature and put through a sieve with a 1-mm grid. The pH value was measured with the glass electrode method from 1 g of sieved soil mixed with 25 mL of distilled water. Total nitrogen was determined using Kjeldahl digestion using a $\text{H}_2\text{SO}_4\text{--K}_2\text{SO}_4\text{--CuSO}_4$ mix, total carbon was determined using

Tjurin probe digestion in a sulphuric acid- $\text{K}_2\text{Cr}_2\text{O}_7$ mix and phosphorus was determined using molybdate colorimetry. The buffer solution used for metal extraction from soil was 1 M ammonium acetate, pH 4.65. Metal concentrations were analysed using atomic absorption spectrometry.

Seedling height was measured prior to the experiment (planting height) to be used as a covariate. Performance variables were measured twice, in August 2004 and 2006 before leaf senescence. The variables measured were seedling height, number of long shoots, shoot length (max 10 long shoots, per-seedling averages calculated for the analyses), proportion of leaves damaged (leaf damage) by insect herbivores and chlorophyll fluorescence (see the Phytotron experiment section), and we sampled two short-shoot leaves from each seedling for measurements of leaf fluctuating asymmetry (FA, only in 2004). Greater seedling height, number of shoots, shoot length and F_v/F_m values and smaller FA and $T_{1/2}$ values were considered signs of higher fitness, and higher leaf damage percentages were considered to represent more herbivore damage. Survival was monitored at measurement times.

In measuring and analysing leaves for FA calculations, we followed the most recent and rigorous methodology developed by Palmer & Strobeck (2003). For each leaf, we measured (to the nearest 0.5 mm) the width of the left- and right-hand sides from the midrib to the leaf margins (at the midpoint between the base and the tip) perpendicular to the midrib. All measurements were performed twice, by different people, without *a priori* knowledge of either plant origin or the results of previous measurements. As a basic measure of FA, we chose the relative difference between the sides ('FA2', *sensu* Palmer & Strobeck, 2003). A mixed model ANOVA demonstrated that the between-side variation (FA) was significantly ($P < 0.0001$) greater than measurement error in all samples.

Quantitative estimates of phenotypic plasticity were calculated for relative vertical increment (the difference between height in 2006 and planting height divided by planting height) and shoot length (data of 2006) by using the relative distances plasticity index (RDPI), ranging from 0 (no plasticity) to 1 (maximum plasticity) (Valladares *et al.*, 2006). Individual distances for seedlings growing in contrasting environments (sheltered and open) were calculated within mother trees whenever possible (> 90% of cases) and within source sites otherwise.

A subsample of 48 leaves used in the FA analyses were also measured for dry weight and leaf area. The measured leaves were from 12 seedlings in each of the four study sites (sheltered and open in Narvik and Skibotndalen). Each set of 12 leaves included three leaves from each of the four kinds of source sites (control open, control sheltered, polluted open and polluted sheltered). Weight/area ratios were calculated for each leaf.

Statistical analyses

The effect of source site on seed weight and germinability were analysed with ANOVAS, with shelter at origin and pollution at origin as fixed explanatory variables and mother tree (nested within source site) used as a random variable. In the phytotron data, the performance indices were analysed with ANCOVAS (seedling height, number of long shoots and shoot length) and ANOVAS (leaf length, F_v/F_m and $T_{1/2}$) to study stress responses and potential adaptations to stress. In the ANCOVAS planting height was used as the covariate. Treatment (C vs. D vs. HM), shelter at origin (open vs. sheltered), their interaction, pollution at origin (polluted vs. control) and its interaction with treatment were used as fixed explanatory variables. Block, mother tree (nested within source site) and treatment \times mother tree interaction were used as random variables. To see whether pollution at origin affected HM uptake, we performed ANOVAS for foliar Ni and Cu concentrations. Pollution at origin was used as a fixed explanatory variable and mother tree (nested within source site) was used as a random variable.

To check how environmental characteristics varied between localities and shelter, we performed ANOVAS for each measured characteristic (wind speed, relative illumination and soil characteristics). Locality, shelter and their interaction were used as fixed explanatory variables in these analyses. The field data were analysed with ANOVAS (FA), repeated measures ANOVAS (leaf damage, F_v/F_m and $T_{1/2}$) and repeated measures ANCOVAS (seedling height, number of long shoots and shoot length) to study performance differences with respect to stress and stress at origin. In the repeated measures ANOVAS and ANCOVAS, measurement year was used as the repeated factor and planting height was used as the covariate in the ANCOVAS. Shelter (open vs. sheltered), shelter at origin (open vs. sheltered), their interaction, pollution at origin (polluted vs. control) and its interaction with shelter were used as fixed explanatory variables. Block (nested within study site), locality, mother tree (nested within source site) and shelter \times mother tree interaction were used as random variables. In addition, if an interaction between measurement year and one, or a combination of, fixed variable(s) was significant, it was included in the analysis. Both in the phytotron and field data, shoot length was log transformed to achieve normality of residuals. The interactive effects of locality and shelter on the study seedlings were analysed separately to verify whether shelter effects were similar in both localities (Narvik and Skibotndalen). Statistical outliers and seedlings damaged during measuring were removed from all analyses. Pairwise comparisons were analysed with least squares means (LSMeans). Survival in both experiments was 100% and thus not analysed.

Differences in plasticity (quantified with the RDPI, for relative vertical increment and shoot length) with regard

to seedling origin were analysed with ANOVAS, with pollution at origin and shelter at origin as the fixed explanatory variables. To check if seedling origin or shelter affected the weight/area ratio, it was analysed with an ANOVA, with shelter, pollution at origin, their interactions, shelter at origin and its interaction with shelter as fixed explanatory variables. All ANOVAS and ANCOVAS were performed with procedure MIXED in SAS version 9.1. (SAS Institute Inc., Cary, NC, USA) (Littell *et al.*, 1996).

Leaf damage was correlated *a posteriori* with the number of long shoots and $T_{1/2}$ to check whether the differences between pollution origins could be attributed to differences in insect herbivory or solely to population differences. A correlation analysis was also conducted between F_v/F_m and seedling height within the drought treatment, to assess whether the detected differences were due to seedling size or pollution at origin. The correlation analyses were conducted with procedure CORR in SAS version 9.1.

Results

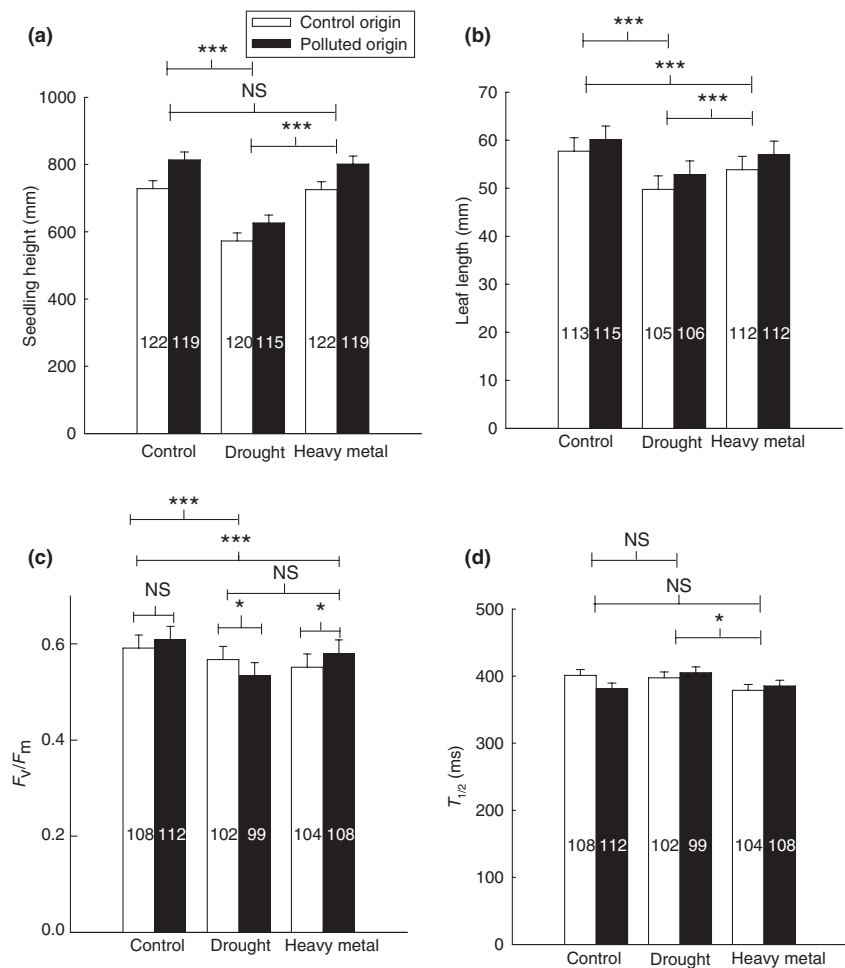
Phytotron data

Seed weight did not vary with pollution ($F_{1,65.3} = 0.66$, $P = 0.42$) or shelter at origin ($F_{1,77.1} = 1.59$, $P = 0.21$). Polluted origin seeds had higher germinability than control origin seeds (52.48 ± 3.68 vs. $37.54 \pm 3.34\%$, respectively, $F_{1,70.2} = 9.03$, $P = 0.0037$, results are given as LSMeans \pm SE throughout the article) and sheltered origin seeds had higher germinability than open origin seeds (59.69 ± 3.53 vs. $30.32 \pm 3.53\%$, respectively, $F_{1,77.3} = 34.32$, $P < 0.0001$).

Treatment had a significant effect on seedling height, leaf length, F_v/F_m and $T_{1/2}$ (Table 2): drought treated (D) seedlings were the shortest and had the shortest leaves, whereas C seedlings had the largest leaves (Fig. 1a,b). C seedlings had the highest F_v/F_m values (Fig. 1c), whereas HM seedlings had lower $T_{1/2}$ values than D seedlings (Fig. 1d). Treatment \times shelter at origin interaction was significant for $T_{1/2}$ (Table 2), with sheltered origin seedling having lower $T_{1/2}$ values than open origin seedlings in D treatment (386.49 ± 8.44 vs. 416.12 ± 9.28 ms, respectively, $t_{220} = 2.76$, $P = 0.0062$). Polluted origin seedlings were taller than control origin seedlings (Table 2; Fig. 1a). Treatment \times pollution at origin interaction was significant for F_v/F_m (Table 2), with polluted origin seedlings showing lower stress in HM treatment, but control origin seedlings being less stressed in D treatment (Fig. 1c). Polluted origin seedlings had lower foliar nickel concentrations than control origin seedlings (7.43 ± 0.71 vs. $9.71 \pm 0.71 \mu\text{g g}^{-1}$, respectively, $F_{1,22} = 5.36$, $P = 0.0303$), whereas foliar copper concentrations did not vary with seedling origin ($F_{1,22} = 2.18$, $P = 0.15$). There was a significant positive correlation between F_v/F_m and the height of seedlings

Table 2 ANCOVA and ANOVA results for factors affecting the measured seedling characteristics in the phytotron data (significant *P*-values bolded, factors not included in analyses are marked with an -- --).

Factor	Explanatory variable	Response variable					
		Seedling height	Nr of long shoots	Shoot length	Leaf length	F_v/F_m	$T_{1/2}$
Fixed	Treatment (= Trm)	$F_{2,166} = 56.52$ $P < 0.0001$	$F_{2,640} = 0.94$ $P = 0.39$	$F_{2,465} = 1.23$ $P = 0.29$	$F_{2,124} = 18.09$ $P < 0.0001$	$F_{2,145} = 18.81$ $P < 0.0001$	$F_{2,145} = 3.73$ $P = 0.0262$
	Shelter at origin (= SO)	$F_{1,81} = 1.33$ $P = 0.25$	$F_{1,80.6} = 2.89$ $P = 0.09$	$F_{1,66.9} = 0.05$ $P = 0.82$	$F_{1,65.2} = 0.43$ $P = 0.51$	$F_{1,74.6} = 0.92$ $P = 0.34$	$F_{1,75.1} = 1.88$ $P = 0.18$
	Trm \times SO	$F_{2,161} = 0.43$ $P = 0.65$	$F_{2,637} = 1.14$ $P = 0.32$	$F_{2,462} = 0.07$ $P = 0.93$	$F_{2,123} = 1.69$ $P = 0.19$	$F_{2,144} = 1.92$ $P = 0.15$	$F_{2,145} = 3.41$ $P = 0.0359$
	Pollution at origin (= PO)	$F_{1,81.4} = 10.17$ $P = 0.0020$	$F_{1,80.7} = 0.39$ $P = 0.53$	$F_{1,68.1} = 0.04$ $P = 0.84$	$F_{1,65.2} = 0.04$ $P = 0.84$	$F_{1,74.4} = 0.25$ $P = 0.62$	$F_{1,75.1} = 0.09$ $P = 0.77$
	Trm \times PO	$F_{2,160} = 0.42$ $P = 0.66$	$F_{2,636} = 0.12$ $P = 0.88$	$F_{2,460} = 0.72$ $P = 0.49$	$F_{2,123} = 0.04$ $P = 0.96$	$F_{2,114} = 8.12$ $P = 0.0005$	$F_{2,144} = 2.48$ $P = 0.09$
Random	Block	$P = 0.21$	$P < 0.0001$	$P = 0.59$	$P = 0.16$	$P = 0.16$	$P = 0.20$
	Mother tree (source site)	$P = 0.0002$	$P < 0.0001$	$P = 0.0002$	$P = 0.0001$	$P = 0.0067$	$P = 0.07$
	Trm \times mother tree	$P = 0.10$	$P = 0.43$	$P = 0.78$	$P < 0.0001$	$P = 0.0253$	$P = 0.0001$
Covariate	Planting height	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$	-- --	-- --	-- --

**Fig. 1** The effects of treatment and pollution at origin on the (a) height, (b) leaf length, (c) F_v/F_m and (d) $T_{1/2}$ values of phytotron-grown seedlings (* $P < 0.05$, *** $P < 0.0001$). For significance of other effects, see Table 2. *N* is given inside bars.

growing in the drought treatment ($r = 0.19$, $P = 0.0072$, $n = 206$).

Field data

Wind velocity in the sheltered study sites was 35% lower than in open study sites ($F_{1,15} = 9.02$, $P = 0.0089$), whereas herbivory was lower in open study sites (Table 3; Fig. 2a). Relative illumination was lower in sheltered than in open study sites (83.8 ± 2.06 vs. $94.0 \pm 2.06\%$, respectively, $F_{1,4} = 12.36$, $P = 0.0246$). The locality \times shelter interaction was significant for pH ($F_{1,4} = 11.37$, $P = 0.0280$), but no within-locality pairwise comparisons were significant. The locality \times shelter interaction was significant also for K ($F_{1,4} = 29.7$, $P = 0.0055$), Ca ($F_{1,4} = 12.41$, $P = 0.0244$), Mg ($F_{1,4} = 13.83$, $P = 0.0205$) and P ($F_{1,4} = 33.91$, $P = 0.0043$), with the Narvik open site having values 3–16 times greater than that of the other sites. Site had a significant effect on total carbon ($F_{1,4} = 10.81$, $P = 0.0303$) and nitrogen ($F_{1,4} = 11.25$, $P = 0.0285$), with Narvik having concentrations of eight to nine times greater than that of Skibotndalen.

Seedlings growing in open conditions were taller (Fig. 2b) and had longer shoots (40.5 ± 2.3 vs. 31.1 ± 2.3 mm) than seedlings in sheltered sites (Table 3). Shelter at origin had a significant effect on fluctuating asymmetry (Table 3), with sheltered origin seedlings showing less asymmetry than open origin seedlings. This

effect was due to the increased asymmetry of open origin seedlings in sheltered conditions (0.099 ± 0.006 vs. 0.087 ± 0.006 , $t_{175} = 2.58$, $P = 0.0106$, respectively, for open and sheltered origin; Table 3). No other pairwise comparisons were significant for fluctuating asymmetry. The shelter \times shelter at origin interaction was significant also for $T_{1/2}$ (Table 3), with open origin seedlings being more stressed than sheltered origin seedlings in open sites (396.98 ± 14.58 vs. 379.25 ± 14.52 ms, respectively, $t_{151} = 2.50$, $P = 0.0136$) and no difference with respect to shelter at origin in sheltered sites (373.34 ± 14.59 vs. 384.53 ± 14.51 ms, respectively, for open and sheltered origin, $t_{151} = 0.95$, $P = 0.34$). Polluted origin seedlings had more long shoots, lower $T_{1/2}$ values and less leaf damage than control origin seedlings (Table 3; Fig. 2). The measurement year \times shelter \times shelter at origin interaction was significant for the number of long shoots (Table 3), with increasing evidence for adaptation to wind exposure between measurement years. In open sites, open origin seedlings had 0.07% more shoots than sheltered origin seedlings in 2004, but in 2006 the difference was 5.74%. In sheltered sites, sheltered origin seedlings had 3.81% more shoots than open origin seedlings in 2004, but in 2006 the difference was 15.77%. However, pairwise comparisons between sheltered and open origin seedlings did not reach statistical significance even in 2006 data. The effects of shelter on the measured performance variables did not vary between the studied localities (data not shown).

Table 3 ANCOVA and ANOVA results for factors affecting the measured seedling characteristics in the field data (significant P -values bolded, factors not included in analyses are marked with an ---).

Factor	Explanatory variable	Response variable						
		Seedling height	Nr of long shoots	Shoot length	Leaf damage	Fluctuating asymmetry	F_v/F_m	$T_{1/2}$
Fixed	Shelter (=S)	$F_{1,9.47} = 5.20$ $P = 0.0479$	$F_{1,10.4} = 4.27$ $P = 0.06$	$F_{1,11.9} = 5.22$ $P = 0.0415$	$F_{1,8.98} = 5.87$ $P = 0.0385$	$F_{1,8.38} = 1.10$ $P = 0.32$	$F_{1,10.9} = 1.00$ $P = 0.34$	$F_{1,13.2} = 0.36$ $P = 0.56$
	Shelter at origin (=SO)	$F_{1,58} = 0.01$ $P = 0.91$	$F_{1,61.2} = 0.46$ $P = 0.50$	$F_{1,64.5} = 0.42$ $P = 0.52$	$F_{1,75.1} = 1.17$ $P = 0.28$	$F_{1,81.5} = 4.24$ $P = 0.0427$	$F_{1,68.3} = 0.51$ $P = 0.48$	$F_{1,64} = 0.35$ $P = 0.55$
	S \times SO	$F_{1,61.1} = 2.43$ $P = 0.12$	$F_{1,61.4} = 2.53$ $P = 0.12$	$F_{1,1137} = 0.10$ $P = 0.75$	$F_{1,1799} = 0.03$ $P = 0.86$	$F_{1,1242} = 3.04$ $P = 0.08$	$F_{1,1155} = 0.12$ $P = 0.73$	$F_{1,1216} = 9.84$ $P = 0.0018$
	Pollution at origin (=PO)	$F_{1,57.7} = 0.31$ $P = 0.58$	$F_{1,60.9} = 8.30$ $P = 0.0055$	$F_{1,63.7} = 0.71$ $P = 0.40$	$F_{1,75.8} = 8.48$ $P = 0.0047$	$F_{1,80.6} = 0.90$ $P = 0.35$	$F_{1,68.4} = 2.17$ $P = 0.15$	$F_{1,63.9} = 11.62$ $P = 0.0011$
	S \times PO	$F_{1,61.2} = 0.00$ $P = 0.99$	$F_{1,61.6} = 2.05$ $P = 0.16$	$F_{1,1143} = 0.32$ $P = 0.63$	$F_{1,1800} = 0.40$ $P = 0.53$	$F_{1,1242} = 0.99$ $P = 0.32$	$F_{1,1158} = 0.26$ $P = 0.61$	$F_{1,1220} = 0.40$ $P = 0.53$
	Block (study site)	$P = 0.0215$	$P = 0.07$	$P = 0.0118$	$P = 0.0200$	$P = 0.13$	$P = 0.0147$	$P = 0.0099$
	Locality	$P = 0.26$	$P = 0.24$	$P = 0.50$	$P = 0.25$	$P = 0.73$	$P = 0.15$	$P = 0.26$
	Mother tree (source site)	$P = 0.37$	$P = 0.0010$	$P = 0.07$	$P = 0.45$	$P = 0.77$	$P = 0.46$	$P = 0.0467$
Covariate	S \times mother tree	$P = 0.0096$	$P = 0.10$	$P = 0.45$	NS	$P = 0.65$	$P = 0.42$	$P = 0.42$
	Planting height	$P < 0.0001$	$P < 0.0001$	$P = 0.0181$	$P = 0.0001$	---	---	---
Repeated	Measurement year	$F_{1,949} = 104.45$ $P < 0.0001$	$F_{1,1380} = 1151.45$ $P < 0.0001$	$F_{1,1052} = 361.57$ $P < 0.0001$	$F_{1,1794} = 29.22$ $P < 0.0001$	---	$F_{1,1203} = 11.11$ $P < 0.0001$	$F_{1,1257} = 363.27$ $P < 0.0001$
	Year \times S \times SO	---	$F_{3,1380} = 2.84$ $P = 0.0370$	---	---	---	---	---

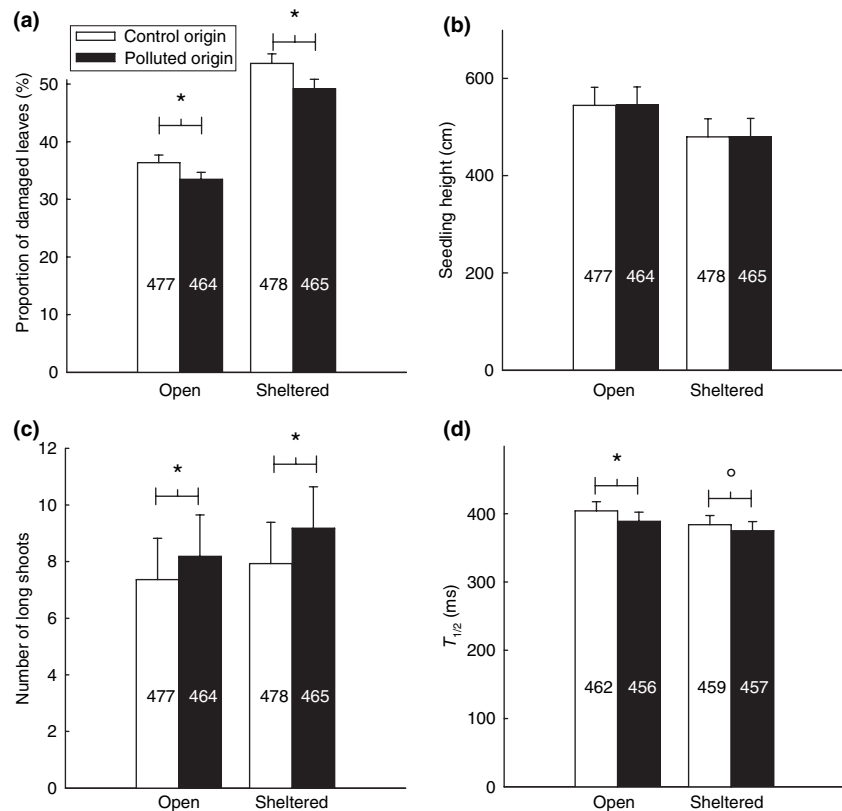


Fig. 2 The effects of shelter and pollution at origin on the (a) proportion of damaged leaves, (b) height, (c) number of long shoots and (d) $T_{1/2}$ values of field-grown seedlings ($^{\circ}P < 0.1$, $*P < 0.05$). For significance of other effects, see Table 3. N is given inside bars.

Neither relative vertical increment nor shoot length showed any variation in phenotypic plasticity between seedlings of different origin (pollution at origin: $F_{1,141} = 2.08$, $P = 0.15$ for vertical increment; $F_{1,150} = 0.00$, $P = 0.95$ for shoot length; shelter at origin: $F_{1,141} = 0.03$, $P = 0.86$ for vertical increment; $F_{1,150} = 0.07$, $P = 0.79$ for shoot length). The RDPI was approximately 0.3 for shoot length and between 0.40 and 0.45 for relative vertical increment.

None of the factors analysed had any effect on the weight/area ratio of the studied leaves ($F_{1,40.5} = 0.12$, $P = 0.73$ for shelter; $F_{1,9.65} = 0.97$, $P = 0.35$ for shelter at origin; $F_{1,10.3} = 0.10$, $P = 0.76$ for pollution at origin). There was a significant positive correlation between leaf damage and the number of long shoots ($r = 0.22$, $P < 0.0001$, $n = 1880$) and a marginally significant positive correlation between leaf damage and $T_{1/2}$ ($r = 0.04$, $P = 0.08$, $n = 1833$).

Discussion

Adaptation to heavy metal stress

We found evidence of HM adaptation in the phytotron data, with polluted origin seedlings showing less stress in the HM treatment than control origin seedlings (Fig. 1c). The result is in accordance with a previous

study by Eränen (2008), who found Kola Peninsula mountain birch from polluted habitats to be HM adapted based on growth and survival data. Lowered nickel concentrations in polluted origin seedlings indicate that the rapidly evolved (60–70 years, i.e. one to two generations) HM resistance in mountain birch populations may be based on reduced HM uptake. This study, together with the study by Eränen (2008), is among the few studies showing HM adaptation in long-lived trees (but see Denny & Wilkins, 1987; Punshon *et al.*, 1995; Utriainen *et al.*, 1997). In the case of Kola Peninsula mountain birch potential pre-adaptation to HMs in natural serpentine soils is discussed elsewhere and is considered extremely unlikely (Eränen, 2008). Moreover, the role of maternal effects (Roach & Wulff, 1987) was minimized by using seeds and a controlled germinating environment. Rather, we suggest that the high genetic variation in Nordic mountain birch (Eriksson & Jonsson, 1986; Howland *et al.*, 1995) gives rise to a small amount of HM-adapted genotypes each generation. In conditions of extremely high mortality, as is the case in the polluted source sites (Kozlov & Haukioja, 1999; Kozlov, 2005; Eränen & Kozlov, 2006, 2007), the adapted genotypes can become fixed in local populations via strong survival selection from standing genetic variation (Hoffman & Parsons, 1991; Barrett & Schluter, 2008).

The greater vitality of polluted origin seeds (as shown by greater germination rate) may have masked the trade-offs in control treatment detected in a previous study (Eränen, 2008). In spite of the higher drought (M.V. Kozlov, unpublished data) and wind stress (Kozlov, 2002) experienced in the polluted source sites, polluted origin seedlings showed no adaptations to these abiotic stressors. Rather, a trade-off was visible in the drought treatment, where HM-adapted seedlings were outperformed by control origin seedlings (Fig. 1c). The effects of drought were not due to population-specific size differences as there was no negative correlation between the variable indicating a trade-off (F_v/F_m) and seedling height. These findings indicate that HM adaptation in mountain birch is stressor specific and does not result in co-tolerance but rather in maladaptation to drought, and possibly other forms of abiotic stress. This is partially in accordance with the theory that adaptation to stress carries a cost, and spending resources on defence or resistance leads to weakened performance in conditions where these traits are not needed (Hoffman & Parsons, 1991; Bazzaz & Grace, 1997; Kawecki & Ebert, 2004; Bijlsma & Loeschcke, 2005).

Contrary to drought stress, the field data showed that seedlings from polluted origin suffer less leaf damage from insect herbivores than control origin seedlings (Fig. 2a), indicating co-resistance to at least one form of biotic stress in the HM-adapted seedlings. Polluted origin seedlings were also less stressed (Fig. 2d), probably reflecting mitigated herbivory (see correlation data). Polluted origin seedlings also had more long shoots (Fig. 2c), in spite of the fact that herbivore damage (greater in control origin seedlings) and the number of long shoots were positively correlated. This would indicate that the bushy growth form of mountain birch growing in polluted sites (Kryuchkov, 1993) has a genetic as well as an environmental background, providing further evidence of genetic differentiation in birch populations with differing pollution at origin.

Increased herbivory resistance of HM-adapted plant populations (mostly, but not exclusively on hyper-accumulators) has been detected on several taxa (see review by Poschenrieder *et al.*, 2006). However, this co-tolerance is always attributed to defence mechanisms derived from HMs. This cannot be the case in our study, as the study seedlings were grown from seeds in pristine, nonpolluted conditions. No difference in leaf weight/area ratio with respect to pollution at origin was detected, hinting that the detected variation in herbivore damage is not the result of leaf physical, but rather physiological or chemical, characteristics. Whether this change is part of the primary mechanism resulting in HM resistance, or a secondary side effect, cannot be ascertained from the current results. Whatever the mechanism, our data give novel evidence that adaptation to HM stress can result in co-resistance to a biotic stressor irrespective of actual HM concentrations in plant tissues or the surrounding environment.

Population differences with respect to wind exposure

Fluctuating asymmetry gave some indication of adaptation to shelter, with open origin seedlings showing the highest stress in sheltered sites. However, open origin seedlings showed no home-site advantage; so, this result does not satisfy the 'local vs. foreign' criterion suggested by Kawecki & Ebert (2004) as proof of evolutionary adaptation. Moreover, the number of long shoots gave some indication of population differentiation due to wind exposure, with increasingly evident home-site advantages with time. However, the result cannot be considered as hard evidence for adaptation, as differences between shelter origins did not reach statistical significance. Moreover, $T_{1/2}$ data showed differentiating responses to abiotic stress with respect to shelter at origin. However, these differences were indicative of maladaptations to source site conditions, with open origin seedlings (that have evolutionary histories of drought and high wind stress) being outperformed by sheltered origin seedlings both in the drought treatment and in the open windy study sites. Considering all this, the differences between open and sheltered source sites cannot be attributed to adaptations towards differences in habitat exposure. Moreover, no co-resistance to HMs was detected. Although population differences with respect to wind exposure were detected from different variables in the field and phytotron studies, they tell the same story, with differences in variables affected attributable to variation in general growth conditions and variables measured.

The lack of adaptation with respect to habitat openness is in contrast with a large number of studies showing home-site advantages in gradients of abiotic stress (Miller & Weis, 1999; Byars *et al.*, 2007; Ohsawa & Ide, 2008). Despite the high mortality of experimentally planted mountain birch in some of the open source sites (Eränen & Kozlov, 2008a, b), gene flow between the open and sheltered source sites, that are often located close to each other in the mosaic of landscapes of the study area, is probably too great for the fixation of locally adapted ecotypes. The lack of differences in phenotypic plasticity with shelter at origin rules out the possibility that the population differences are due to lower plasticity of populations from open source sites (Taylor & Aarssen, 1988; Heschel *et al.*, 2004). This result is partly in conflict with a previous study, where we detected lower amounts of phenotypic plasticity in high stress populations in elevation gradients (Eränen & Kozlov, 2008b). Probably the large amount of different (and possibly contrasting) gradients used in the current study mask possible trends in individual stress gradients. Moreover, our open and sheltered experimental sites varied not only with respect to wind exposure but also insect herbivory (Fig. 2a). The high levels of herbivory in the sheltered study sites may be above the levels occurring in the source gradients, possibly masking potential adaptations. Another thing to consider is the strong positive effect of nurse trees on

mountain birch seedlings growing in exposed habitats (Eränen & Kozlov, 2008a). Refuges created by adult conspecifics might override the need to adapt to wind-induced stress (Odling-Smee *et al.*, 1996; Laland *et al.*, 1999), whereas they probably provide no mitigation against pollution stress (Lukina & Nikonov, 1999; Ginocchio *et al.*, 2004). This disparity might explain why mountain birch populations show no adaptations to natural stress, despite their documented capacity for rapid evolutionary change (Eränen, 2008).

Although we detected significant shelter \times locality variation in soil conditions, the differences in soil chemistry did not explain the variation in birch performance, hinting that the superior performance of seedlings growing in open, wind-exposed sites may rather be explained by the lower herbivore damage (Fig. 2a). It is worth noting, that 2004 and 2005 were outbreak years for the autumnal moth (*Epirrita autumnata* Bkh.) in the study area (Virtanen *et al.*, 2006); so, the effect of herbivory relative to abiotic stress during the experiment was probably above average.

Several performance variables showed significant differences between families (mother trees) with respect to treatment (in phytotron) and shelter (in the field). The result indicates greater variation within populations than between them, suggesting great potential for adaptation, but low levels of actual differentiation between the studied populations. The effect was more significant in the phytotron, probably reflecting the lower amounts of confounding environmental variation. Possibly the great, but regular, annual variation in summer conditions in the Kola Peninsula (Bakka, 1990; Valkama & Kozlov, 2001) promotes genotypic variation in mountain birch populations, with too much specialization resulting in an evolutionary 'dead end' in the varying environmental conditions. The result is interesting in the light of the projected climate change, with the differences between June temperatures (warmest June being 5.1 °C warmer than the June mean *sensu* Bakka, 1990) being in line with the projected anthropogenic increase in temperature of 4–7 °C in the northernmost parts of the northern hemisphere during the next 100 years (ACIA, 2004). With the absence of adaptations to harsh environmental conditions, in combination with its great capacity to respond positively to rising temperatures and the potential for rapid evolution (Ovaska *et al.*, 2005; Eränen, 2008), mountain birch may have good chances of persisting also under human-induced climate change.

Conclusions

Our data are in line with the results of a previous experiment (Eränen, 2008), confirming a rapid evolutionary adaptation to HM stress by mountain birch around the copper–nickel smelters in Nikel and Monchegorsk, NW Russia. No co-tolerance to other forms of abiotic stress was detected but rather, as predicted by

theory (Futuyma & Moreno, 1988; Hoffman & Parsons, 1991), HM adaptation resulted in a trade-off of reduced performance under another form of abiotic stress (drought). Contrary to drought, HM adaptation resulted in co-resistance to biotic stress (insect herbivory). To our knowledge, this is the first example of HM-induced co-resistance to biotic stress where the actual mechanism of resistance is not HM related.

Despite the high abiotic stress in our open source sites, no adaptations to wind or drought stress were detected. We suggest that high genetic variation and gene flow between mountain birch populations act as powerful buffers towards changes in the genetic structure of populations but, given a strong enough selection pressure, the high genetic variation retains a capacity for exceptionally rapid evolutionary leaps. The absence of adaptations to harsh microclimates and the generally high adaptive potential of mountain birch, a critical forest-forming tree in subarctic Europe, need to be accounted for in models predicting the consequences of human-driven environmental change, including the projected climate change.

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