

Canopy settings shape elemental composition of the epiphytic lichen *Lobaria pulmonaria* in unmanaged conifer forests



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ABSTRACT

The importance of forest canopy settings for the elemental chemistry of epiphytic lichens in undisturbed forests is poorly understood despite its impact on epiphytes, understory vegetation and microbial soil communities. Here, we examine the elemental composition in standardized thalli of the cephalolichen *Lobaria pulmonaria* transplanted to the lower branches of *Picea glauca* x *engelmannii* in inland British Columbia. Transplants were attached to nets (avoiding direct contact with tree bark) exposed to natural canopy throughfall in three categories of natural forest stands: 1) within dripzones of *Populus* species in mixed stands always supporting *L. pulmonaria*; 2) outside *Populus* dripzones, yet with *L. pulmonaria* and 3) outside *Populus* dripzones and without *L. pulmonaria*, which is the most common canopy setting. After one year, and with the exception of Fe and Al, the elemental content in lichen transplants changed within and between forest categories. Lichen transplants within *Populus* dripzones had more base cations (Ca, Mg, K) and less Mn than in the two categories outside dripzones. In *Picea* canopies outside *Populus* dripzones, lichen transplants had higher Ca, Na, Mo and lower C, S and Cu in trees with *L. pulmonaria* than without. The pH of *Picea* branches and soil within the rooting zone of trees with transplants were both approximately ten times more acidic outside *Populus* dripzones than within them. However, bark pH did not differ between the two categories of stands outside *Populus* dripzones. Based on our findings we conclude that canopy setting shapes the elemental composition of epiphytic lichen transplants, suggesting that such transplants might be used to monitor spatial variation in throughfall elements from forest canopies in unmanaged stands. We also conclude that elemental concentrations, Ca in particular, is a better predictor than bark pH for the occurrence of *L. pulmonaria* on *Picea*.

1. Introduction

A lichen is a symbiotic consortium of one or more heterotrophic fungal partners and one or more autotrophic photobionts (Spribille, 2018). As such, lichens are self-supporting associations that often rely on airborne minerals to satisfy their requirements for inorganic nutrients (e.g. Nieboer et al., 1978). Lacking roots, lichens depend on efficient concentration mechanisms to secure nutrients from dilute solutions like rainwater, dew and, in the case of forest lichens, canopy throughfall. Epiphytic lichens may affect ground-level deposition of nutrients in canopy throughfall (Knops et al., 1996) through their various nutrient acquisition mechanisms and leaching behaviour. Many air pollution studies have emphasized the ability of lichens to accumulate even minor elements to measurable concentrations (e.g. Ferry et al., 1973; Conti and Cecchetti, 2001; Massimi et al., 2019), but little

is known about the influence of canopy settings on elemental composition in epiphytic lichens.

In many forest settings, scarcity of mineral nutrients impedes the growth of trees (Van Sundert et al., 2018) and their epiphytes (Benner and Vitousek, 2007). By contrast, conifers in the dripzones of *Populus* species are known to support N-fixing cyano- and cephalolichens often absent from adjacent unmixed conifer stands (Goward and Arsenaux, 2000). From this it can be inferred that some lichens are better adapted than others to the leachates of specific tree species (Rambo, 2012; Campbell et al., 2013), i.e., consistent with the species-specific capacity of trees to modify soil chemistry and nutrient cycling (Falkengren-Grerup, 1989; Augusto et al., 2002). In general, elements leached from plants can include all essential minerals (Tukey, 1970) and feed epiphytes, ground vegetation and microbial soil communities (as reviewed by Van Stan and Pypker, 2015). Quantifying these nutrient fluxes by

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direct measurement is difficult (Campbell et al., 2010) owing in part to high elemental variability across time and space (Zimmermann et al., 2007; Zimmermann and Zimmermann, 2014). Rambo (2012) found that leachates among tree species can differ in spring, but not in fall when many leachate elements occur in higher concentration. Recently, Asplund et al. (2015) suggested that the elemental composition of lichen transplants can be used as a surrogate for the chemical composition of stem flow. Similarly, *in situ* lichens have successfully been used to estimate N-deposition on trees in western North America (Root et al., 2013; Hoffman et al., 2019). Here we use the cephalolichen *Lobaria pulmonaria* (L.) Hoffm. to explore the potential of specific canopy settings to shape the elemental composition in lichen transplants in unmanaged conifer forests.

Lobaria pulmonaria is a large wide-ranging foliose lichen known for its ability to capture air-borne elements (Yemets et al., 2014) – a by-product of its broad lobes and pitted surface facilitating capture of water (Phinney, 2019). As a member of the Lobarion (Rose, 1988) – a conspicuous epiphytic community that includes most known N-fixing epiphytic lichens – *L. pulmonaria* may help to fertilize the forest ecosystems in which it occurs (Forman, 1975; Antoine, 2004; Kobylinski and Fredeen, 2015). Like other N-fixing members of the Lobarion, it is unable to colonize the bark of trees with rather acidic bark (Gauslaa, 1985, 1995). On conifer branches with acidic bark (Gauslaa and Holien, 1998), the Lobarion is facilitated by dripzones of *Populus* canopies (Goward and Arsenault, 2000) raising the bark pH and so promote the growth of *L. pulmonaria* (Gauslaa and Goward, 2012).

Although *L. pulmonaria* responds to bark pH, the relationship between its elemental composition and canopy setting remains to be elucidated. Phosphorus is often a limiting nutrient (Benner and Vitousek, 2007; Johansson et al., 2011); it increases N-fixation in *Nostoc* symbionts (Weiss et al., 2005; Benner et al., 2007) and free-living epiphytic cyanobacteria (Stanton et al., 2019). The P-availability peaks at intermediate pH (as reviewed by Penn and Camberato, 2019). In some rainforest ecosystems, the addition of P has been shown to strongly boost the growth of Lobarion (Benner, 2011; McCune and Caldwell, 2009), though this effect is by no means universal (Gauslaa and Goward, 2012; Marks et al., 2015). Furthermore, the micro-nutrients molybdenum (Mo) and vanadium (V), known cofactors for enzymes involved in N fixation (Darnajoux et al., 2019), have also been hypothesized to boost *L. pulmonaria* (Marks et al., 2015).

By transplanting standardized *L. pulmonaria* thalli from a homogeneous source habitat to nets on branches of *Picea glauca* x *engelmannii*, we hypothesize that such transplants without direct contact with the tree bark can capture habitat-specific levels of ecologically relevant elements influenced by e.g. canopy throughfall. At the same time, we also hypothesize that the elemental composition of such *L. pulmonaria* transplants left in place for one year will differ within and outside dripzones of *Populus* canopies, as well as in pure conifer canopies with and without natural occurrences of the Lobarion. Based on these assumptions, this study aims to identify links between elemental concentration in *L. pulmonaria* transplants and the bark pH in *Picea* branches across an array of forest settings.

2. Material and methods

2.1. Study area

The study was carried out in the southern Clearwater Valley in south-central British Columbia, Canada. The study area is humid-sub-boreal, with cool, moist summers and cold, snowy winters (see Goward and Ahti, 1992). Soils are mostly derived from underlying peralkaline basalts laid down in volcanic eruptions 1–2 million years ago (Hickson, 1986). The basalts are predominantly overlain by glacial till deposited during the Pleistocene glaciation, which ended here about 11,000 years b.p. Subsequent leaching or enrichment by water – depending on local topography – caused spatial differences in soil fertility. The study area

belongs in the Interior Cedar-Hemlock Biogeoclimatic Zone (ICH) of Meidinger & Pojar (1991). See Hämet-Ahti (1965) for a more detailed description of the vegetation.

2.2. Categories and habitat characteristics of studied localities

We selected nine mid-successional coniferous forest localities based on their dominant epiphytic vegetation on the branches of *Picea glauca* x *engelmannii*, a dominant tree species. *Pseudotsuga menziesii*, *Abies lasiocarpa*, *Pinus contorta*, *Tsuga heterophylla*, *Thuja plicata*, *Populus tremuloides*, *P. trichocarpa* and *Betula papyrifera* often occurred as sub-dominant or scattered trees (henceforth, trees are referred to by their genus names). These localities were effectively located along a 31-km north–south gradient ranging from warmer and drier in the south to cooler and moister in the north (see e.g., Arsenault and Goward, 2016). Forest in the six southernmost localities dated from a 1926 wildfire (Edwards, 1954), while the three northernmost stands dated from a wildfire in the late 1890s (Goward, unpubl. data).

The sampled localities were divided into three categories:

A: Non Lobarion: Three localities supported only *Picea* with acidophytic chlorolichens belonging to the Parmeliaceae, representing non-Lobarion localities, the most common stand type in the valley:

Non1, north of Nakiska Ranch (N51 55.453-549 W120 00.809-878; 720 m) was a forested fen in a wide U-shaped valley bottom. All trees (only *Picea*, *Abies*, *Pinus*) were on *Sphagnum* peat (no mineral soil) with both bog and forest plants.

Non2, south of the base of Pyramid Mountain (N51 58.362-428 W120 07.091-190; 833–839 m) was a well-drained, slightly undulating forested plateau. Mainly *Picea*, *Pseudotsuga* and *Pinus*; some *Betula*, but no *Populus* trees were present. Stones were rarely seen at the surface, and the soil had brownish-red silt.

Non3, the Edgewood West locality (N51 52.046-116 W120 01.909–960; 707–717 m), was well-drained, slightly undulating terrain including gentle W-SW slopes and some low local ridges. Silty soils had a dense cover of boulders partly above the soil surface, with a whitish layer beneath the organic layer. *Picea* and *Pseudotsuga*-dominated, with a few *Thuja* and *Tsuga*, and some *Betula*. *Populus tremuloides* was common, but no selected trees grew within its dripzone. Trees present in the dripzone (not included) did not support Lobarion here.

B: Dripzone: Four localities supported Lobarion communities characterized by cephalo- and cyanolichen belonging to the Peltigerales. These occurred on *Picea* trees within the dripzone (see e.g. Goward and Arsenault, 2000) of an overarching *Populus* canopy:

DZ1, near Ray Farm Draw (N52 03.860–04.057 W120 09.820–10.247; 654–670 m), was a shallow valley with an open, tall gallery forest of cottonwood and slender *Picea*. It likely represented the bottom of the former lake. Vegetation was within rooting reach of mineral-rich ground water; soils were silty and red-brown, but without clear layers. *Corylus* shrubs were dense. High biomass of *L. pulmonaria* occurred on *Picea* within and outside dripzones, but all selected trees were in dripzones of *Populus trichocarpa*.

DZ2, near the trail to the Majerus Farm south of Murtle River (N51 57.977–58.040 W120 06.665–942; 821–832 m), was a shallow, winding drainage channel which appeared edaphically rich, with dense shrubs of *Cornus*, and formed openings in an otherwise denser forest. The vertical distance between the channel and the flat surrounding forest was < 1 m. In upper parts, the channel was wider, partly transitioning to a wider wetland with more *Spirea* and large *P. trichocarpa*. No stones were seen on the silty soil surface, but there were many boulders below-ground.

DZ3, the Bear Dusky Woods locality (S of Edgewood; dripzone environment; N51 51.778–836 W120 01.389–568; 708–734 m), represented a gently undulating plain of a U-shaped draw with many adjacent small, edaphically rich wetlands. Tree roots accessed rich ground water. Soils were silty with some organic material and many boulders, though these were rarely visible on the surface. The stand was

Picea- and *Pseudotsuga*-dominated with very few *Thuja* and *Tsuga*, and some *Betula*.

DZ4, Poacher Lane (N51 52.772–886 W120 00.271–457; 751–791 m), at the foot of Battle Mountain (dripzone environment) was a well-drained toe slope, W-facing mixed forest, partly some *Thuja*, but many *Picea*, *Pseudotsuga*, *Populus* and *Betula*. Soils had some boulders and were silty with a thin organic layer.

C: Lobarion: Two localities had *Picea* supporting the Lobarion community without *Populus* dripzones. Here, cephalo-, cyano- and chlorolichens often co-occurred.

Lob1, Dragon's Tongue (N52 08.547–661 W120 10.917–979; 747–767 m), was on a flat-bottomed valley \approx 50 m above Clearwater Lake, near the southern end of the lake, east of the Osprey view trail. This stony site had forested lava flows 5000 years bp with thin silt sediments forming lenses. Lobarion was abundant on *Picea* in the absence of *Populus*. Most soils had a thick white layer beneath the organic layer.

Lob2 N of Ray's Farm (N52 03.956–04.032 W120 10.279–373; 650–660 m) was a large and flat silty plateau. Soils were deep, silty and red-brown. The plateau was the bottom of a former lake. *Picea* was a dominant species, with some *Thuja* and *Abies*, a few *Betula*, but hardly any *Populus*. The understory had dense *Corylus* shrubs.

2.3. Habitat parameters

Trunk circumference and tree height were recorded for every tree used for lichen transplants. Basal area ($\text{m}^2 \text{ha}^{-1}$) taken from the trunk position of each tree was recorded using a relascope; the contribution of each tree species was counted separately. In addition, percent open sky seen from a position near the selected transplant branches was visually estimated for each tree. This was done independently at three separate visits to the stand, and the average recording was used to represent the final estimate. Habitat parameters recorded for every tree were given as mean values for individual localities (Fig. 1).

Bark pH was measured as follows. We removed one 7 cm long, 6–9 mm thick, defoliated branch segment for each of the two branches on every selected tree at the time of transplant. Each branch segment was cleaned of lichens and submerged in a vial containing 7 ml 25 mM KCl; an ion exchange method described by Gauslaa & Holien (1998). The corked vials were kept at 20 °C and shaken at regular intervals for 1 h. Afterwards, branch segments were removed, and the pH of remaining solution was immediately taken with a pH probe (VWR SympHony 304 pH/Temp Meter, VWR International, Edmonton, AB, Canada) with two point calibration (buffers at pH 4 and 7) at frequent intervals.

For soil pH, four subsamples of the mineral soil below the litter layer (sampling depth 5–15 cm) were sampled in various parts of the root zone of each tree. The subsamples were combined, air dried at room temperature, and sifted to remove particles larger than 1 mm. Because sites with mineral soils (8 of 9 localities) were well sorted and silty, soil particles were < 0.05 mm. For the mineral soils, 10 g soil was added to 20 ml distilled water. For peat (Non1), 2 g of peat was added to 10 ml distilled water. The vials with soil and water were repeatedly shaken over a period of 1.5 h before pH was measured.

2.4. Lichen material and transplant design

Two hundred healthy *L. pulmonaria* thalli with few or no soralia were collected from a 3 m tall and 10 m long east-facing vertical rock wall inside an open forest (N51 51.950 W120 02.997; 596 m). Each transplant consisted of a branched lobe with intact meristems; the average mass was 288 mg. The basal parts fastened to the rock were not used because their elemental concentrations often differ from more apical portions (Ayrault et al., 2007); the outer freely hanging lobes were cut as transplants, after removing attached debris and after a rapid rinsing in water from a lake. They were then left to desiccate at room

temperature and low light.

At each of the nine localities, ten *Picea* were selected that: 1) had living branches below 3 m; 2) were positioned in rather open canopies (required by *L. pulmonaria*); 3) were spaced more than 30 m from other selected trees to ensure non-overlapping root zones and dripzones; and 4) were located at least three tree-lengths from the nearest forest edge. For each tree, two separate branches were selected to receive lichen transplants.

After randomizing thalli by random numbers, 20 randomized thalli were set aside and placed in a freezer to be used as controls (start level of elemental content). Each of the remaining 180 randomized lichen transplants was fastened onto a strip of fine, pale plastic netting using polyester thread. Only basal parts of *L. pulmonaria* were fastened, thereby allowing the younger tips to hang freely and thus collect canopy throughfall. This protocol permitted the thallus to curl when dry, a response providing photoprotection by self-shading (Barták et al., 2006), and avoided direct contact with the tree bark. Each net with one *L. pulmonaria* thallus, hereafter referred to as transplant, was fastened to the upper surface of the branch with two 10 cm cable ties (the technique is illustrated in Gauslaa and Goward, 2012). In positioning the transplants, we consistently placed them in the outer 1/3 segment of each branch, which represented the branch segments with the richest lichen vegetation. Lichens were transplanted 7–17 September 2017, and harvested one year later on 3–6 September 2018 after having experienced natural canopy throughfall for 353–361 d.

2.5. Elemental analysis

After harvesting, the two transplants from separate branches of each of the 90 trees were combined to one sample. Likewise, the 20 control thalli set aside at the start were randomly combined two by two. These samples ($n = 100$; 90 combined transplants and 10 combined controls) were shipped to the Ministry of Environment, British Columbia Analytical Chemistry Research Laboratory (Victoria, BC, Canada). There the samples were ground using a ball mill to ensure a uniform mixture of both samples for each tree, and then analyzed via combustion methods (C, N, S) or microwave digestion, ICP-OES (Al, B, Ca, Cu, Fe, K, Mg, Mn, Mo, Na, P and Zn).

2.6. Statistical analyses

To test for differences between localities or between categories, we used one-way ANOVA after checking for heteroscedasticity and non-normality. Some parameters were log-transformed to satisfy the ANOVA requirements. We used all pairwise multiple comparison procedures (Tukey post hoc test for most elements), whereas the Welch's Test was used for a few parameters showing unequal variations within groups. Means ± 1 standard error are given in the text, Figs and Table 1. We used a general linear model for bark pH using nine localities nested in the three categories non-Lobarion, Lobarion, dripzone as fixed factors, and with Mg concentration in lichen transplants as a covariate. These statistical analyses were run in Minitab 16 (Minitab Inc., State College, PA, USA).

PCA analysis was conducted using JMP 14.0 (SAS Institutes Inc., North Carolina, USA). Prior to analysis, all data were standardized because the range of the different parameters varied.

3. Results

3.1. Elemental concentrations

From the initial randomization of the thalli, we infer that the mean elemental concentrations measured in our control thalli correspond to the mean initial elemental concentrations in our transplant thalli. If so, then the concentration of all elements apart from Al and Fe significantly changed in one or more stands during the one-year transplant period.

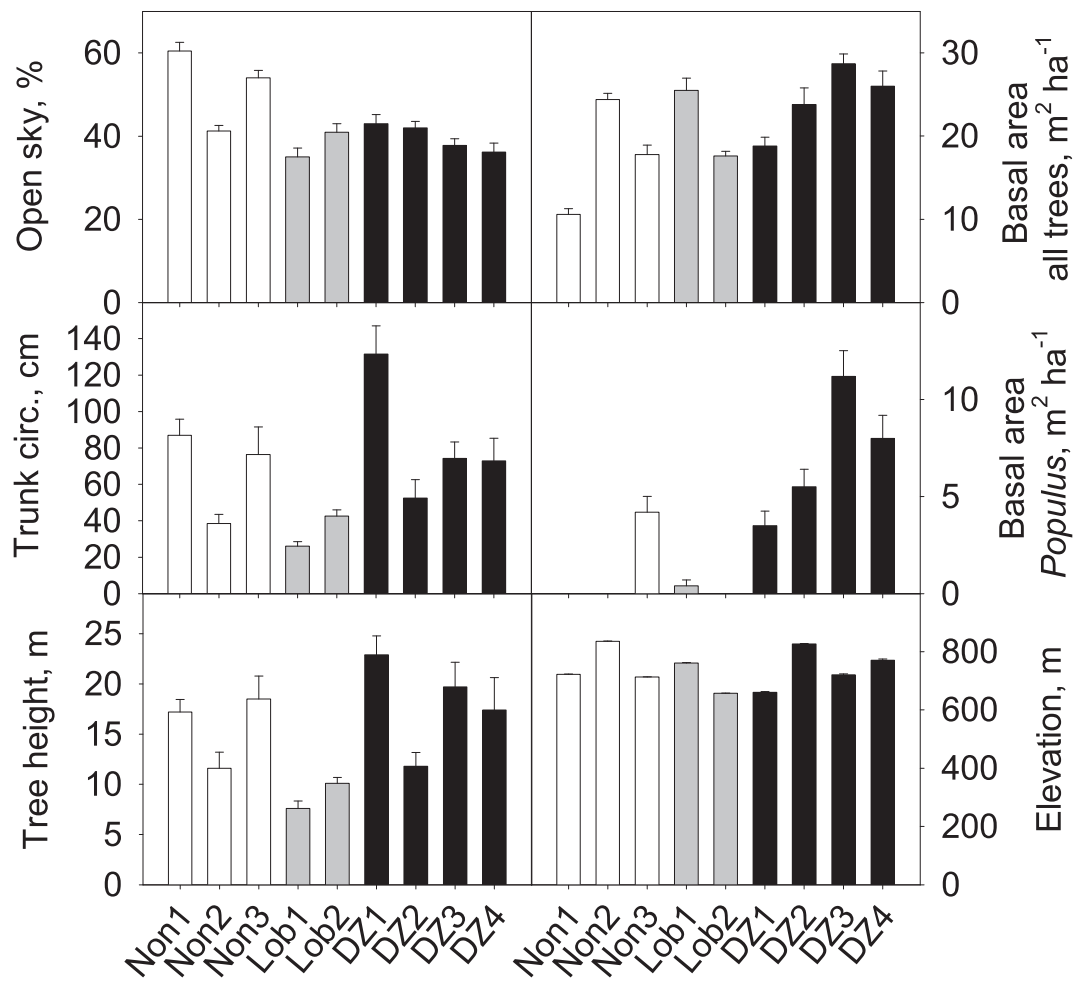


Fig. 1. Habitat and tree parameters (means \pm 1SE; $n = 10$) for *Picea* in each of nine localities used for the transplant of *Lobaria pulmonaria*. White columns: localities with non-Lobarion trees (Non1-3); grey columns: localities with Lobarion trees in the absence of *Populus* dripzone (Lob1-2); black columns: localities with trees in the dripzones of *Populus* (DZ1-4).

Table 1

Elements with significantly different ($P < 0.1$) mean concentrations in *Lobaria pulmonaria* transplanted to Lobarion and non-Lobarion *Picea* outside *Populus* dripzones.

| Element | Lobarion $n = 20$ | Non-Lobarion $n = 30$ | R_{adj}^2 | P |
|---------|-------------------------------------|---------------------------------------|-------------|---------|
| C, % | 46.3 \pm 0.1 | 46.8 \pm 0.2 | 0.095 | 0.017 |
| S, % | 0.0540 \pm 0.0021 | 0.0644 \pm 0.0018 | 0.248 | < 0.001 |
| Cu, ppm | 2.27 \pm 0.08 | 2.88 \pm 0.11 | 0.243 | < 0.001 |
| Ca, % | 0.226 \pm 0.007 | 0.185 \pm 0.008 | 0.202 | 0.001 |
| Na, ppm | 25.5 \pm 1.5 | 20.7 \pm 1.0 | 0.123 | 0.007 |
| Mo, ppm | 0.179 \pm 0.020 | 0.139 \pm 0.013 | 0.046 | 0.072 |

Means \pm 1SE are given; the higher means appear in **bold**. R_{adj}^2 and P computed by a one-way ANOVA. S was log-transformed prior to analysis.

Four elements (Ca, Mg, K and Mn) clearly responded to our predefined stand categories (Fig. 2), indicating highly significant contrast between the non-dripzone (non-Lobarion/Lobarion) and dripzone trees. From the start (control), the base cations (Ca, Mg, K) increased in transplants under *Populus*, whereas Mn decreased. In the two categories of non-dripzone transplants, K remained rather constant (Fig. 2). When contrasting the two non-dripzone categories, S, Cu and C had their highest concentrations in transplants on non-Lobarion trees whereas Ca and Na were highest on Lobarion trees (Table 1). Additionally, transplants on our Lobarion trees had marginally higher Mo concentrations relative to transplants on non-Lobarion trees ($P = 0.072$; Table 1).

According to the PCA-ordination the elemental composition of the

transplants within the dripzone separated well from those of non-dripzone transplants, whereas trees from the two non-dripzone categories were more similar (Fig. 3a). The first axis gave the best separation of the three categories, with overall positive scores for the dripzone trees and the most negative scores for the non-Lobarion trees (Fig. 3a), representing a gradient strongly influenced by base cations and Mn (Fig. 3b). Ca appeared to be the only element that significantly differed between all three predefined categories (one-way ANOVA with a Tukey test; see the graphical abstract). Ca was highest in transplants from dripzone trees ($0.270 \pm 0.010\%$), lower in transplants from Lobarion trees ($0.226 \pm 0.007\%$), and lowest in transplants from non-Lobarion trees ($0.185 \pm 0.008\%$). Mn showed a reverse pattern compared to the base cations (Ca, Mg, K) with approximately two times higher Mn concentration in non-dripzone compared to dripzone transplants (Fig. 2). P partly followed the base cations, but weakly (Fig. 3b) with a high variation between localities (Fig. 2). The common soil elements Fe and Al showed a significant covariation between locations (Fig. 3b; regression analysis of means: $r_{adj}^2 = 0.560$; $P = 0.008$; $n = 9$), but were uncoupled to the predefined categories (Fig. 2). The second PCA-axis accounting for 12.9% of the variation showed a gradient in Fe and Al and a partly inverse pattern of Mo and B (Fig. 3b).

The most abundant elements in the lichen transplants, C and N, as well as the C/N-ratio, showed rather limited variations between localities. Compared to the controls, N tended to decrease in many localities after transplant, whereas the fen transplants increased their C concentration (Fig. 2).

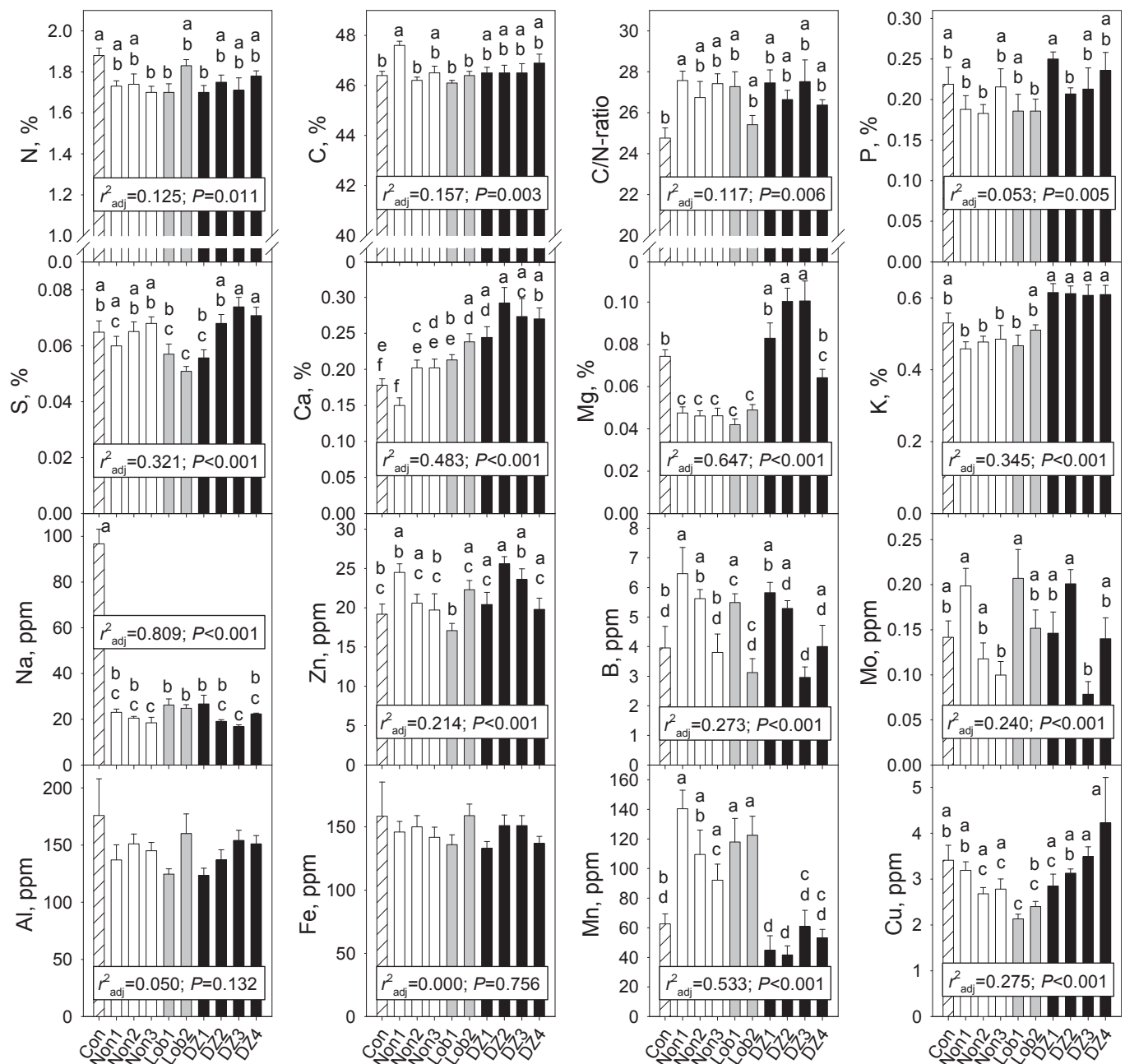


Fig. 2. Elemental concentration (means \pm 1 SE; $n = 10$) in *Lobaria pulmonaria* before (Con = control; hatched columns) and one year after being transplanted onto the branches of *Picea* in nine forest stands. White columns show three stands without cyano- and cephalolichens (Non1-3). Grey columns show two forest stands where cyano- and cephalolichens occurred on *Picea* branches in the absence of dripzones (Lob1-2). Black columns show four stands where *L. pulmonaria* was transplanted within *Populus* dripzones (DZ1-4). The insert in each plot shows the r^2_{adj} and corresponding P -value from a one-way ANOVA. S, Ca, Na, Al, and Mn were log-transformed to satisfy the ANOVA requirements. Means with different letter ranges were statistically different according to all pairwise multiple comparison procedures (Tukey post hoc test for most elements), whereas the Welch's Test was used for C/N and P that had unequal variations within groups.

A comparison of elements in transplants beneath the two dripzone trees *P. tremuloides* and *P. trichocarpa* (one-way ANOVA) showed that only Mg ($P = 0.008$) and B ($P = 0.022$) peaked beneath *P. trichocarpa* ($n = 25$), whereas Al ($P = 0.002$) was highest beneath *P. tremuloides* ($n = 15$).

3.2. Bark and soil pH

pH of *Picea* bark was significantly lower (one-way ANOVA; $P < 0.001$; $r^2_{adj} = 0.691$) on non-dripzone trees (4.55 ± 0.04 ; $n = 50$; mean \pm SE) than on trees within dripzones (5.69 ± 0.07 ; $n = 40$), but was more variable in the dripzones than in the non-dripzone sites

(Fig. 4a). For the non-dripzone *Picea*, the bark pH did not differ between the Lobarion (4.52 ± 0.06 ; $n = 20$) and the non-Lobarion (4.57 ± 0.05 ; $n = 30$).

Soil pH (Fig. 4b) differed between all three categories of trees (one-way ANOVA; $P < 0.001$; $r^2_{adj} = 0.496$). Soil pH was substantially higher for dripzone trees (6.15 ± 0.08 ; $n = 40$) than for non-dripzone trees (5.19 ± 0.08 ; $n = 50$), but among the non-dripzone trees, the Lobarion trees were located in slightly more acidic soils (4.90 ± 0.13 ; $n = 20$) than the non-Lobarion trees (5.39 ± 0.09 ; $n = 30$; $P = 0.002$).

Bark pH in *Picea* beneath the *Populus* dripzones did not depend on the type of *Populus* species forming the dripzone (15 *P. tremuloides* and

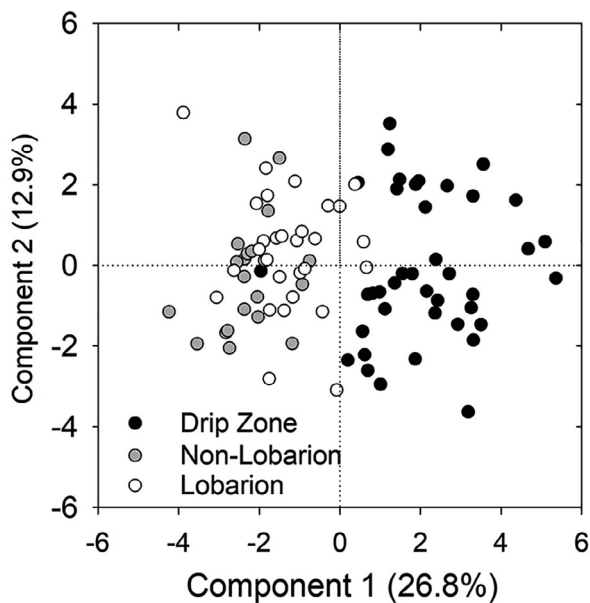


Fig. 3a. Summary plot for principle component analysis using all measured elemental concentrations in *Lobaria pulmonaria* transplants including soil and bark pH. The three categories of trees (dripzone, non-Lobarion and Lobarion trees) are shown. Principle components 1 and 2 explain 26.8% and 12.9% of the variability, respectively.

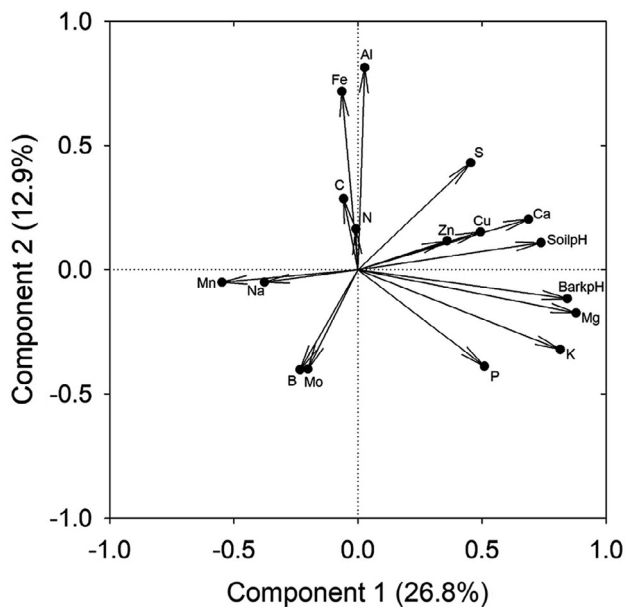


Fig. 3b. Variable correlation plot for the first two principal components in the PCA analysis.

25 *P. trichocarpa*; $P = 0.714$; one-way ANOVA). However, soil pH was slightly, but not significantly higher ($P = 0.059$; one-way ANOVA) beneath *P. trichocarpa* relative to *P. tremuloides*.

Plotting bark pH against soil pH shows that individual dripzone trees separated well from individuals located outside the *Populus* dripzones (including Lobarion and non-Lobarion; Fig. 5). Within categories, there were no correlations between soil and bark pH, but when the less acidic dripzone trees and the more acidic non-dripzone trees were combined, there was a weak, but highly significant relationship between soil and bark pH (Fig. 5).

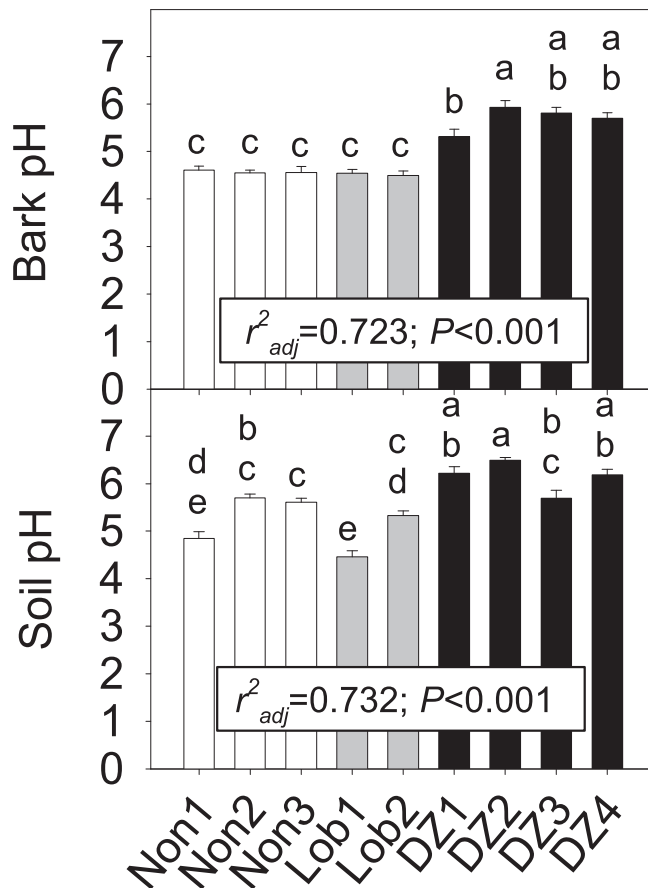


Fig. 4. a: Bark pH (means \pm 1SE; $n = 10$) of *Picea* branches in each of nine localities used for *Lobaria pulmonaria* transplants. **b:** Soil pH in the root zone of these trees. White columns: localities with non-Lobarion trees only (Non1-3); grey columns: localities where Lobarion trees consistently occur outside of *Populus* dripzones (Lob1-2); black columns: localities with trees within *Populus* dripzones (DZ1-4). The insert in each plot shows the r^2_{adj} and corresponding P -value from a one-way ANOVA. Means with different letters were statistically different according to all pairwise multiple comparison procedures (Tukey post hoc test).

3.3. Links between bark pH and elemental concentrations in lichen transplants

The base cations Ca, Mg, K, as well as P, in transplants, were all positively correlated with soil and bark pH (Fig. 3b). However, bark pH was more strongly coupled to Mg (Figs. 3 and 6) than to any other base cations (regression analyses; data not shown). Combining the Mg + Ca + K concentrations as an independent variable for bark pH accounted for 57.1% of the variation in bark pH (regression analysis; $P < 0.001$). This combination was second to Mg (66.2%; Fig. 6) in explaining bark pH variability.

A general linear model for bark pH using nine localities nested in the categories non-Lobarion, Lobarion, dripzone (fixed factors), and with Mg concentration in lichen transplants as a covariate, accounted for as much as 81.3% of the variation in bark pH (Table 2). In this, the strongest model, Mg had a strong influence, although the fixed factor stand category was also highly significant, with a weaker contribution of locality nested in the category.

4. Discussion

All lichen transplants used in our study originated from a homogeneous source population and appeared equally healthy both before and after transplant. From this, we infer that the observed site-specific

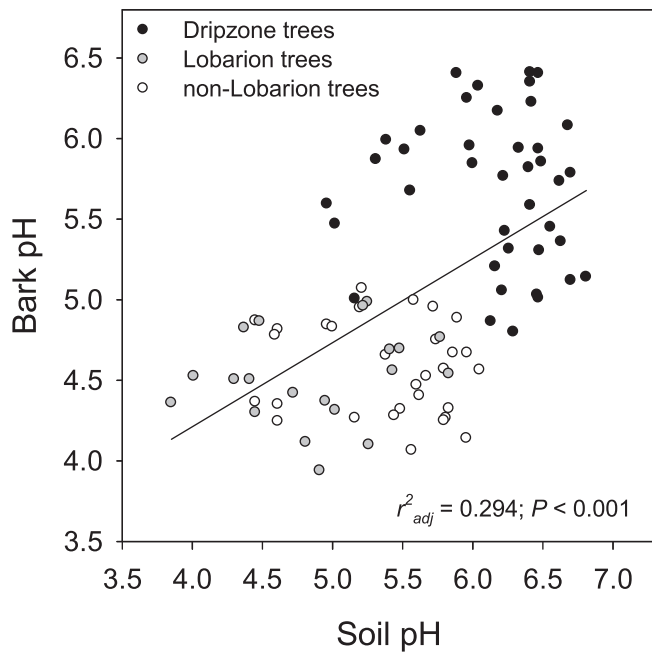


Fig. 5. The relationship between bark pH in *Picea* branches and soil pH in the root zone of the same trees ($n = 90$).

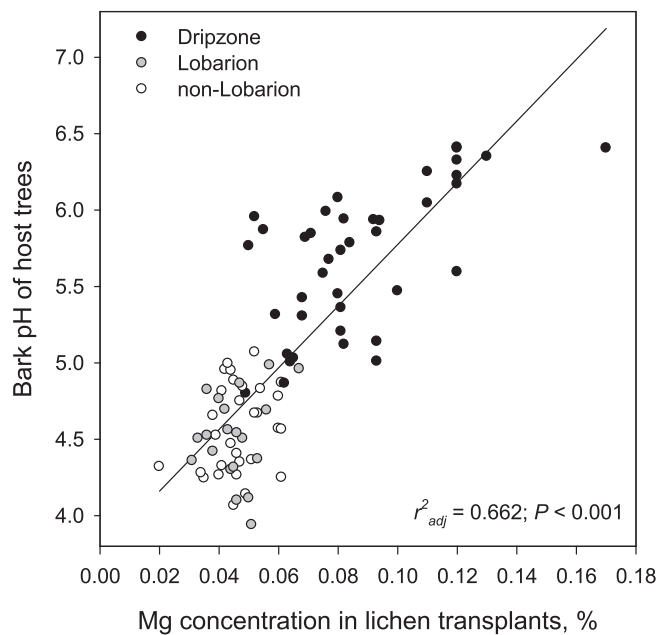


Fig. 6. The relationship between bark pH in *Picea* branches ($n = 90$) and Mg concentration in *Lobaria pulmonaria* transplants (percent of dry matter) following one-year exposure to various canopy settings.

elemental changes in the transplants reflect a balance between elemental uptake on the one hand and natural leaching by rain on the other hand (Fig. 2), although the rate of elemental accumulation has been shown to be higher in element-rich environments than the elemental loss in environments low in elements (Paoli et al., 2018). It seems reasonable to suggest that post-transplant elemental contrasts in our lichen transplants largely mirror the natural spatial and temporal variations in canopy throughfall chemistry, although different canopy settings may also differ in dry deposition. The exceptions are C, which accumulates mainly by means of photobiont photosynthesis, and N, which depends on N-fixation by cyanobacterial photobionts in the transplants themselves. There is no evidence from this study that any of

Table 2

General linear model for the bark pH of *Picea* branches using nine localities nested in the categories non-Lobarion, Lobarion, dripzone (fixed factor), and with Mg concentration in lichen transplants as a covariate.

| Source | DF | F | P |
|------------------------|----|-------|-------|
| Category (C) | 2 | 17.92 | 0.000 |
| Locality (nested in C) | 6 | 3.84 | 0.002 |
| Mg | 1 | 39.61 | 0.000 |
| Error | 80 | | |
| Lack-of-Fit | 71 | 2.28 | 0.090 |
| Pure Error | 9 | | |
| Total | 89 | | |

$r^2_{adj} = 0.813$; $P < 0.001$.

the studied canopy settings improved N_2 -fixation in *L. pulmonaria*. Indeed, N-content decreased in three localities, and did not increase in any locality (Fig. 2). Even for chlorolichens, percent N did not correlate well with throughfall N% (Hoffman et al., 2019). Because the retention of N may vary between species-specific tree canopies (Fenn et al., 2013; Lovett and Lindberg, 1993), it is worth noting that transplants in our three canopy categories did not differ in N.

Among the elements studied by us, K, Ca, Mg and Mn usually leach from plants in greatest quantity (Tukey, 1970). They are likely of local origin, initially taken up by the roots of the host trees. Their concentration in our transplants significantly responded to the predefined stand categories including the presence or absence of *Populus* canopies above the *Picea* trees (Fig. 2) and shaped the first axis in the PCA-ordination. Such data suggest that these ecologically relevant elements were taken up in our lichen transplants from leachates in specific canopy settings. This is true even in the case of Ca and Mg, which readily leach from lichens (Farmer et al., 1991b). Among the mentioned elements at highest concentration in the transplants beneath *Populus* canopies, only K and Mg are recognized as macronutrients (Nash, 2008), whereas Ca (Pitt and Ugalde, 1984) and Mn are micronutrients for algae and fungi. Nevertheless, relatively high Ca is apparently required to support the Lobarion community on oak trunks (Gauslaa, 1985; Farmer et al., 1991a) and may compensate for adverse effects of excess Mn (Hauck et al., 2002; see below). In high concentrations like those occurring in non-dripzone environments, Mn reduces the effective quantum yield of PSII in photobionts of Lobarion species (Hauck et al., 2006) and decreases the production and viability of symbiotic diaspores in the lichen *Hypogymnia physodes* (Hauck et al., 2001; 2002).

High Mn/Ca- and/or Mn/Mg-ratios in tree bark have been hypothesized to exclude the Lobarion species outside *Populus* dripzones in western North America (Hauck and Spribille, 2002). Lichen transplants in our non-dripzone trees had a mean Mn/Mg-ratio of 2.50 ± 0.11 , that is 3.9 times higher than beneath *Populus* dripzones (0.64 ± 0.06). The Mn/Mg-ratio accounted for a higher portion of the contrast ($r^2_{adj} = 0.689$) between dripzone and non-dripzone transplants than did either Mn ($r^2_{adj} = 0.428$) or Mg alone ($r^2_{adj} = 0.542$; one-way ANOVA). These results are consistent with the hypothesis of Hauck and Spribille (2002) suggesting that high Mn/Mg-ratios may exclude the Lobarion community on *Picea* branches outside dripzones from *Populus*. Furthermore, our study supports the view that canopy throughfall in some canopy settings adversely affects the Lobarion community (Goward and Arsenault, 2000); this may help to explain why the Lobarion does not grow everywhere.

For the two categories of trees outside our *Populus* dripzones, Mg, Mn, K and the Mn/Mg-ratio did not explain the establishment of the Lobarion versus that of the acidophytic Parmeliaceae-dominated epiphytic community. We infer from this that the Mn/Mg-ratio hypothesis (Hauck and Spribille, 2002) does not apply in all forest situations. For elements that differed between the two non-dripzone categories (Table 1), the higher concentration of S and Cu in transplants on non-Lobarion trees may suggest detrimental effects of ambient S and Cu

loads for Lobarion species. The high Cu-binding in secondary compounds of *L. pulmonaria* (Gauslaa et al., 2016) did not prevent Cu-induced membrane damage (Cabral, 2002; Yemets et al., 2015). Yet, Cu and S did not occur in higher concentration in the non-Lobarion transplants than in those from dripzones, implying that direct toxic effects alone are unlikely. In contrast, the higher Ca concentration in transplants on Lobarion trees (Table 1) is consistent with the aforementioned positive roles of Ca for Lobarion species. Finally, Mo is an essential cofactor in the nitrogenase enzyme required in N-fixation (Eady, 1996; Einsle et al., 2002; Bellenger et al., 2011). It is interesting that canopy settings outside the *Populus* dripzone adds more Mo to transplants in trees supporting N-fixing Lobarion species than in trees without N-fixing epiphytes - likely an important consideration in northern conifer forests where Mo is often a limiting factor for the N-fixation (Darnajoux et al., 2019). Because, however, this contrast was significant at $P = 0.072$ (Table 1) with high between-locality variation, we are hesitant to conclude a functional relationship of Mo. In a fertilization experiment adding Mo, V and P to *L. pulmonaria* in western Oregon, neither of these elements was found to limit lichen growth (Marks et al., 2015), whereas Mo and P boosted N_2 -fixation in free-living cyanobacteria in tropical forest canopies (Stanton et al., 2019).

Both Ca and K occurred at higher concentration in our transplants than did Mg. In this context, the strong coupling between Mg in transplants and pH in *Picea* bark (Fig. 6), and the poorer fit in multiple regression after adding Ca and/or K, may seem surprising. The poorer fit for K may be due to its intracellular storage in lichens, whereas Ca and Mg are bound mainly at external exchangeable sites (Brown and Slingsby, 1972). Nevertheless, the strong link between Mg and pH is consistent with an earlier study of boreal deciduous trees in Norway spruce (*Picea abies*)-dominated forests, where bark pH beneath the Lobarion community showed a stronger correlation with Mg than with Ca and K. (Gauslaa, 1995). The bark pH beneath the acidophytic lichen community, locally co-occurring in some kind of mosaic on the same trunks, correlated with Mg only, not with Ca or K. By contrast, Ca accounted for most of the variation in bark pH on *Quercus* trunks in temperate oak forests (Gauslaa, 1985). These studies assessed elements in the bark itself, not in the lichens. High Ca rather than high bark pH seems to explain the presence of the Lobarion in stands outside *Populus* dripzones. In our Lobarion trees with relatively low bark pH, the pH is still slightly above the lower pH threshold reported for the Lobarion in Norway spruce forests (Gauslaa and Holien, 1998).

Finally, we note that earlier attempts to assess elemental throughfall using rain gauges have not always been successful (Campbell et al., 2010). Our results suggest that lichen transplants have a potential to yield clear canopy-specific element throughfall profiles. Various factors may account for this: (1) lichens accumulate dry and wet deposition including snow, dewfall and fog (Knops et al., 1991), whereas rain gauges efficiently capture only rain; (2) lichen thalli can easily be used with many replicates covering a patchy throughfall pattern; (3) there is strong seasonal variation in leachates from trees (Rambo, 2012; Van Stan et al., 2012); whereas rain gauges function only in seasons without frost (Campbell et al., 2010); lichens accumulate elements efficiently also in winter (Yemets et al., 2014); (4) elemental variation across small spatial scales can be substantial (Zimmermann et al., 2007); and (5) it is simpler to place lichen transplants on ecologically relevant positions on tree branches than it is to situate gauges. Future studies using the lichen transplant method might be improved if each transplant is divided into two at the outset of the experiment. Transplanting only one of the pieces, and analyzing both chemically, would make it possible to quantify both start and end elemental concentration, thus substantially improving the predictive power. Because different lichen species accumulate elements differently (Bennett, 2008), it is important to consider the species used.

In summary, our study supports the hypothesis that canopy settings with *Populus* dripzones provide cations with a potential to raise the bark pH of conifer branches beneath (Fig. 4a), and is consistent with earlier

bark pH studies on pairs of dripzone and non-dripzone *Picea* trees (Gauslaa and Goward, 2012). These patterns suggest that canopy setting can be an important driver of bark pH, and thereby of the local occurrences of cyano- and cephalolichens that depend on relatively high bark pH (Gauslaa, 1985; Gauslaa and Holien, 1998). Soil pH was also higher within *Populus* dripzones than outside of them (Fig. 5). While it is too early to conclude that *Populus* shapes the higher soil pH in our study sites, past work has shown that Norway spruce can acidify its own soils (Nihlgård, 1971), while *Populus* can enrich local soils with nutrients (Buck and St Clair, 2012). Such opposite effects of these two tree genera likely explain the low level of overlap in realized bark as well as soil pH inside and outside of *Populus* dripzones (Fig. 5).

5. Conclusions

Canopy setting shapes the elemental composition of *L. pulmonaria* transplanted to a range of forest ecosystems, suggesting that lichens have potential as biomonitors of the spatial variation in ecologically relevant throughfall elements in unmanaged forest stands not subjected to anthropogenic pollution. The elemental signature of lichen transplants on *Picea* branches within the *Populus* dripzone is shown to elevate bark pH while at the same time introducing elements known to stimulate the growth of cyano- and cephalolichens. Given their ability to fix atmospheric N_2 , cyano- and cephalolichens - when boosted by base cations apparently originating from *Populus* canopies - may cause cascading effects through increased N-fertilization of natural forest ecosystems. Thus, our results help to shed new light on the importance of elemental composition in forest canopy epiphytes.

Credit authorship contribution statement

Yngvar Gauslaa: Conceptualization, Methodology, Investigation, Formal analysis, Writing - original draft. **Trevor Goward:** Conceptualization, Methodology, Investigation, Writing - review & editing. **Thomas Pypker:** Conceptualization, Methodology, Formal analysis, Funding acquisition, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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