

Contrasting responses of plant and lichen carbon-based secondary compounds across an elevational gradient

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Abstract

1. Vascular plants and lichens often produce a diversity of carbon-based secondary compounds (CBSCs) to protect them against biotic and abiotic stresses. These compounds play important but often compound-specific roles in community and ecosystem processes by affecting herbivore and decomposer activity. However, our understanding of what drives community-level CBSCs among ecosystems or across environmental gradients is limited.
2. We measured concentrations and compositions of CBSCs for all dominant vascular plant and lichen species present across a 500-m alpine elevational gradient. These measurements were combined with data on species composition and abundance to obtain whole-community measures of plant and lichen CBSCs across the gradient.
3. At the whole community level, plant CBSCs had the lowest concentrations while lichen CBSCs had the highest concentrations at the highest elevations. Further, plant CBSCs shifted from those associated with herbivore defence towards those protecting against light and oxidative stress as elevation increased, while lichen CBSCs showed the opposite pattern.
4. *Synthesis.* Our findings that individual compounds show contrasting responses to the same environmental gradient highlight the importance of studying qualitative as well as quantitative changes in CBSCs. Further, the divergent responses between vascular plants and lichens reveal that in systems where both groups are abundant, they need to be considered simultaneously to better understand how future environmental changes may impact on ecosystem-level processes.

KEYWORDS

alpine ecology, defence, Finse Alpine Research Centre, flavonoids, lichens, phenols, tannins, vascular plants

1 | INTRODUCTION

Both vascular plants and lichens have evolved a high variety of secondary compounds, as defence against herbivores (e.g. Coley et al., 1985;

Gauslaa, 2005), pathogens (Witzell & Martín, 2008), oxidative stress and light damage (Close & McArthur, 2002). Plants produce an array of different chemical groups of compounds, but in infertile low-productive systems carbon-based secondary compounds (CBSCs) are

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the predominant type (Coley et al., 1985; Herms & Mattson, 1992). These compounds also have after-life effects in senesced litter as they can inhibit decomposer activity and immobilize nitrogen (N) through protein complexation, and thus reduce plant-available N (Asplund et al., 2013; Fierer et al., 2001). Given the importance of secondary compounds in species-driven ecosystem processes, it is important to understand their effects at the whole community level. Community-level measures of plant functional traits have been widely used for inferring ecosystem processes, but these have mainly focused on leaf nutrient concentrations and morphological and ecophysiological characteristics (Garnier et al., 2007; Mayor et al., 2017; Sundqvist et al., 2013). Meanwhile, only a few studies have considered community-level measures of secondary compounds and how they may vary among ecosystems or across environmental gradients (Kichenin et al., 2013; Siefert et al., 2015; Sundqvist et al., 2013).

Those studies that have explicitly considered community-level measures of secondary compounds (e.g. Asplund & Wardle, 2014; De Long et al., 2016; Sundqvist et al., 2012) have only quantified total amounts of broad classes of compounds like total phenolics or tannins, and not the chemical diversity within these broad classes. However, different compounds and compound groups within these broad classes can have very different biological functions and efficacy. For instance, in vascular plants, some flavonoids such as quercetin and luteolin have a much stronger antioxidant capacity than do other flavonoids, such as kaempferol and apigenin (Agati et al., 2012). In lichens, phenolic compounds such as usnic acid and atranorin, which are both situated in the cortical layer, are considered to have a photo protective role, while other phenolics such as fumarprotocetraric acid and lecanoric acid are more involved in protecting the lichen from other stressors such as herbivory (Solhaug & Gauslaa, 2012). Further, these protective phenolic compounds in lichens can vary greatly in their efficacy (Asplund & Wardle, 2013). This highlights the importance of characterizing individual compounds or specific compound groups, rather than simply total amounts of very broad classes of compounds, such as total phenolics, in plant and lichen material.

At high latitudes and elevations, temperature and low nutrient availability limit plant growth, and the primary producer communities are often dominated by lichens (Matveyeva & Chernov, 2000). Vascular plants and lichens are predicted to invest more into carbon-based secondary defences because of a surplus of fixed carbon (C) when low nutrient availability limits C use for growth (Herms & Mattson, 1992; Stamp, 2003), which is often the case at higher elevations. In addition, lower temperatures with increasing elevation potentially lead to higher light damage and thus more oxidative stress, which could result in further induction of phenolic antioxidants (Close & McArthur, 2002). In contrast, as invertebrate herbivore pressure often decreases with increasing elevation, vascular plants and lichens might allocate less to secondary defence compounds (Descombes et al., 2017; Galmán et al., 2017; Moreira et al., 2018). As such, Zidorn (2010) suggested that while there should be an increase in radical scavenging and UV-B protective secondary compounds with increasing elevation, there should also be a decrease in allelopathic and anti-herbivory secondary compounds such as tannins and stilbenes.

In this study, we investigated the concentrations of CBSCs of individual plant and lichen species along an elevational gradient spanning 500 m in the Norwegian alpine zone. These measurements were combined with data on species composition and abundance to obtain whole community measures of plant and lichen CBSCs. We used this system to test the following two hypotheses: (a) With increasing elevation, there will be a shift towards overall higher concentrations of plant and lichen CBSCs when quantified at the whole community level; and (b) The increase in CBSCs with elevation will be most pronounced for those compounds associated with light protection and antioxidative capacity. Because of the variable role that different CBSCs play in driving ecosystem processes, understanding how they individually respond to a strong natural gradient at the whole community level will advance knowledge of how vascular plants and lichens may contribute to ecological processes across contrasting environmental conditions.

2 | MATERIALS AND METHODS

This study was performed on five sites positioned across an elevational gradient near Finse, alpine Norway (N 60°33'–60°38'; E 7°35'–7°42'), ranging from approximately 1,120 to 1,600 m a.s.l., and with 120 m of elevation between consecutive sites (Roos et al., 2019). These sites are all on south-facing slopes on acidic granite and gneiss bedrock. The air temperature in July decreases on average by 0.9°C with each level (120 m) of increasing elevation, and the growing season is almost half the length at the highest site than at the lowest site (Roos et al., 2019). The study sites are on exposed ridges and have a mixed cover of vascular plants, lichens and bryophytes, but are dominated by ericaceous dwarf shrubs and fruticose mat-forming lichen species. The lowest site is situated approximately 150 m above the nearest tree line, which is dominated by *Betula pubescens* ssp. *czerepanovii*.

To quantify species composition along the gradient, vascular plant and lichen cover were estimated in five 1-m² plots for each of the five elevations between 11 and 24 July 2016. The plots were selected within a 100-m radius at each elevation by haphazardly throwing an object, and were chosen provided that all functional groups were present (vascular plants, lichens and bryophytes). The median distance between plots within elevations was c. 43 m, which is sufficient to ensure adequate independence among plots, considered the small-scale spatial heterogeneity in these communities (Björk et al., 2007; Opedal et al., 2015), and is in line with previous studies along elevation gradients in tundra environments (e.g. Veen et al., 2015). The cover of each species of vascular plant and lichen was estimated visually for each of four 50 × 50 cm quadrats per plot; the whole-plot cover was then calculated as the average across the four quadrats. One quadrat per plot was destructively harvested between 28 July and 18 August 2016: all above-ground material was collected and sorted to species. In case insufficient material was available for a given species, extra material was harvested from the other quadrats or the immediate surroundings of the same plot (maximum 2 m). For both the vascular plants and lichens, the most

abundant species that we collected from each plot collectively comprised at least 80% of the total vascular plant or lichen cover for that plot (Pakeman & Queded, 2007).

For each vascular plant species in each plot, 30 young but fully developed (i.e. current growing season) and undamaged leaves were sampled from 15 shoots, except for small leaves species (leaf length <0.5 cm) for which 150 leaves were used. For each lichen species in each plot, 10 intact thalli of each species were selected and cleaned. The material was air-dried and stored at -18°C until analysis of CBSCs. All samples were ground to powder with a ball mill (Retsch MM400, Retsch).

For extraction of the CBSCs of the leaves from each vascular plant species of each plot (vascular plant CBSCs), approximately 10 mg plant powder was added to 600 μl methanol and homogenized on a Precellys homogenizer (Bertin Technologies, Montigny-le-Bretonneux) for 20 s at 5,400 rpm. The samples were then left for 15 min on ice and centrifuged for 3 min at 1,500 rpm (Eppendorf centrifuge 5417C, Eppendorf), before the supernatant was poured off and the residue re-extracted three times (excluding the 15 min on ice).

For extraction of the CBSCs of the thallus material from each lichen species in each plot, we extracted approximately 15 mg of lichen powder in 2 ml acetone for 3×30 min. The combined supernatants were evaporated and re-dissolved in 1,000 μl methanol.

For each of the lichen and plant extracts, secondary compounds were then quantified by HPLC (Agilent Series 1200, Agilent Technologies) with an ODS Hypersil column, 50×4.6 mm using 0.25% orthophosphoric acid and 1.5% tetrahydrofuran in Millipore water (A) and 100% methanol (B) as mobile phases at 2 ml/min, following the gradient described in Nybakken et al. (2007). When analysing Lichen CBSCs, we used UV-detection at 245 nm, while plant CBSCs were detected at 270 or 320 nm, depending on where they showed highest UV-absorption. Compound identification was based on retention times, online UV spectra and co-chromatography of commercial standards ((+) catechin, epicatechin, neochlorogenic acid for phenolic acids, quercetin3glucuronide for quercetins, kaempferol3glucoside for kaempferols, myricetin3rhamnoside for myricetins, apigenin7glucoside for apigenins, luteolin7glucoside for luteolins, neochlorogenic acid for phenolic acids [all from Sigma], atronorin [Apin Chemicals Ltd]; rhamnetin [Santa Cruz Biotechnology]; batatasin III [ChemFaces Biochemical Co., Ltd.] for batatasin III and other stilbenes; diffractaic acid, fumarprotocetraric acid [Gaia Chemicals]; usnic acid [Sigma]; protocetraric acid and tenuiorin provided by Prof. Harrie Sipman [Botanical Museum]). The compound concentrations were calculated based on response curves of the above-mentioned external standards. Peak-alignment between samples of the same species and with external standards were done using the overlay-function in the Agilent ChemStation-software.

Due to the high diversity of plant CBSCs both within and between species, including the glycosylated flavonoids, not all compounds were identified down to the specific compound. Further, because some compounds (e.g. chlorogenic acid) appear in many plant species while others in only one, it was necessary to perform

some grouping of compounds to allow for comparisons of plant CBSCs across species and across communities. As such, we firstly grouped plant CBSCs by their chemical classes, which is reflective of molecular complexity, molecule size and the position at which they are synthesized along the phenylpropanoid pathway; the groups we used were phenolic acids (including all chlorogenic acid derivatives, hydroxycinnamic acids and unclassified phenolic acids), flavonoids (with the subgroups flavonols and flavones), stilbenes and condensed tannins. The rationale for this grouping is the assumption that chemically similar compounds have similar ecological functions. Phenolic acids are a wide group of small phenolic compounds that appear early in the biosynthetic pathway. Some of these compounds have been shown to have a particular ecological function, but they also serve as precursors for more specific and complex compounds. Further down the pathway, stilbenes are separated from flavonoids. Stilbenes are frequently induced by pathogen attack and are therefore assumed to play a role in protection against fungi (Ganthaler et al., 2017).

Flavonoids are known to vary greatly with regards to their antioxidant capacity, and this capacity is primarily determined by the number of hydroxy (-OH) groups on the B-ring (the right ring of the three phenol-rings that form the typical flavonoid structure; Agati et al., 2012). This means that dihydroxy B-ring substituted flavonoids (e.g. luteolin and quercetin derivatives) are stronger antioxidants than monohydroxy substituted ones (e.g. apigenin and kaempferol derivatives). Therefore, we chose to further classify flavonoids by their aglycon (i.e. the noncarbohydrate part of the glycoside), regardless of the type of glycosyl group, and look at the differences between these; as such, all quercetin glycosides were classified as 'quercetins', all luteolin glycosides as 'luteolins' etc. Condensed tannins comprise a broad group of large and complex compounds that are built up from flavonoid units. They precipitate proteins and are considered important for herbivore deterrence, but they also have antioxidant capacity although this has been little studied (Salminen & Karonen, 2011).

Concentrations of both MeOH-soluble and MeOH-insoluble condensed tannins (CTs) were identified using the acid butanol assay for proanthocyanidins described in Hagerman (2002). MeOH-soluble CTs were analysed from the HPLC extract, while the amount of MeOH-insoluble CTs were analysed from the residues left after the extraction process. Purified tannins from spruce needles were used as standards to calculate concentrations.

Lichen CBSCs were grouped according to their placement in the lichen thallus as given in Ahti et al. (2007, 2013) and Thell and Moberg (2011). This grouping was made because lichen CBSCs in the cortical layer are assumed to primarily function as light screeners while the lichen CBSCs in the medulla defend against other stressors such as herbivory, pathogens and harmful metals (Solhaug & Gauslaa, 2012). This is because the cortical layer, and thus the CBSCs within it, are positioned above the photobiont layer which they protect. Meanwhile, CBSCs in the medullary layer are positioned beneath the photobiont and therefore cannot function as light screeners. We further grouped lichen CBSCs according to their chemical structure

into aliphatic compounds, depsides, depsidones and dibenzurans. Depsides and depsidones are the most common groups and are composed of two (or three) aromatic rings joined by ester linkages. Unlike depsides, depsidones have ether linkages in addition to the ester linkages and are considered to be weaker antioxidants (Atalay et al., 2011; Brisdelli et al., 2013; Hidalgo et al., 1994; Lohézic-Le Dévéhat et al., 2007). *Cladonia* sp. and *Gowardia nigricans* produced one and two compounds, respectively, which we did not manage to characterize (Table S4). These are therefore not included in the abovementioned groups. Further, some lichen species produce melanic compounds (instead of cortical lichen CBSCs) to serve as sunscreens. However, these compounds are difficult to extract from lichens, and previous quantitative estimates have been based on indirect methods, for example, through measuring browning reflectance index of the thallus (McEvoy et al., 2007). This does not yield a concentration but merely a quantification of the colour, which is difficult to directly compare with the concentrations of CBSCs measured with HPLC. In order to account for the importance of melanic species at the community level, we therefore calculated the relative abundance of melanic species.

2.1 | Data analysis

All collected species (collectively composing >80% of total lichen or plant cover) within each plot were weighted according to their relative abundance in that plot. For each individual secondary compound, we calculated community-weighted average for each plot following Garnier et al. (2007) and Fortunel et al. (2009):

$$\text{CBSC}_{\text{weighted}} = \sum_{i=1}^n p_i \times \text{CBSC}_i,$$

where p_i is the cover of species i as a proportion of the total cover of all collected plant or lichen species, and CBSC_i is the secondary compound concentration of species i . For each plot and CBSC, we calculated the 'specific' community average CBSC concentration using the CBSC concentration recorded on the specific plot (this includes both inter- and intraspecific effects), and 'fixed' community average CBSC concentration using the mean concentration for all plots (which removes the intraspecific variability effect). Intraspecific community averages were then calculated by subtracting the fixed community average from the specific community average (which removes the interspecific variability). For each CBSC, the relative importance of intra- and interspecific variability effects in explaining variation in specific community-weighted averages across the five elevations was assessed using sum of squares (SS) decomposition following the procedure of Lepš et al. (2011), using the R package *CATI* (Taudiere & Violle, 2016). We ran three one-way ANOVAs, one for each of the 'specific', 'fixed' and 'intraspecific' community average measures with elevation as factor, and extracted the SS for each of the three response variables (SS_{specific}, SS_{fixed} and SS_{intraspecific}) explained

by elevation. Finally, we subtracted SS_{fixed} and SS_{intraspecific} from SS_{specific} to yield SS_{cov}, which is the effect of covariation between interspecific CBSC concentration variability and intraspecific CBSC concentration variability. A positive covariation means that intra- and interspecific effects work in the same direction and therefore that the inclusion of intraspecific variability increases the total effect. By contrast, when there is negative covariation, intra- and interspecific effects respond in opposite directions and therefore potentially cancel each other out. We fitted and tested the ANOVAs with permutation tests (5,000 iterations) using the *aovp* function of the R package *LMPERM* (Wheeler & Torchiano, 2016), when residuals were not normally distributed.

To depict the composition of CBSC at both the vascular plant and lichen community levels, we used non-metric multidimensional scaling (NMDS) on the basis of a Bray-Curtis distance matrix, using the *metaMDS* function in the R package *VEGAN* (Oksanen et al., 2016). Trait data used in the NMDSs were community-weighted averages as described above. For these analyses, we used two dimensions which yielded satisfactory low stress values. We chose not to use three dimensions because adding a third dimension only slightly decreased the stress value while making the interpretation more difficult (Dexter et al., 2018). For both plant and lichen CBSCs, we performed two separate PERMANOVAs with a Bray-Curtis distance matrix and 999 permutations, using the *adonis* function in the R package *VEGAN* to test for the effect of elevation on 'specific' community-weighted CBSC (both inter and intraspecific effects) and 'fixed' community-weighted CBSC (only species turnover effects). All analyses are at the community-level and were performed in R 3.5.0 (R Core Team, 2018).

3 | RESULTS

3.1 | Species community composition

Vascular plant community composition changed with elevation (Figure S1a). Specifically, at lower elevation the community was dominated by ericaceous shrubs while graminoids were relatively more important at higher elevations. Even though there was a clear shift in species composition, most species were found at more than one elevation (Table S1). Similarly, the lichen community composition changed with elevation (Figure S1b), where various Cladoniaceae species dominated lower elevation while members of Parmeliaceae were relatively more abundant at higher elevations. However, most of the species occurred at all elevations (Table S2).

3.2 | Plant carbon-based secondary compounds

All plant CBSC groups varied significantly with elevation (Figure 1). The community-weighted concentration of total phenols was higher at 1,240 m than at the two highest elevations

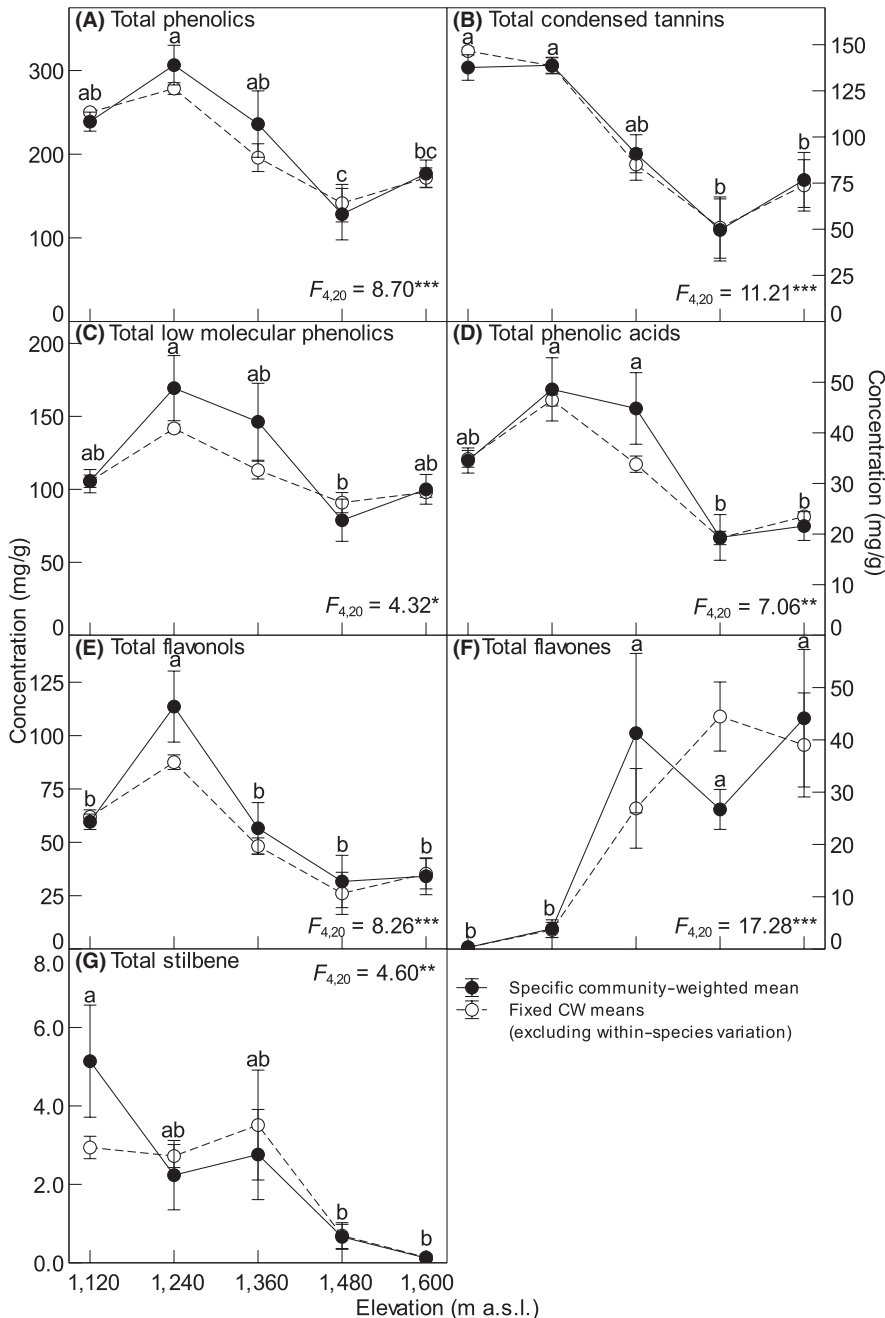


FIGURE 1 Community-weighted (CW) averages (\pm SE) of plant carbon-based secondary compounds divided into (A) total phenolics, (B) condensed tannins and (C) low molecular phenolics. The low molecular phenolics include: (D) phenolic acids, (E) flavonols, (F) flavones and (G) stilbenes. Specific community averages (which include species turnover and intraspecific metabolite variability) and fixed community averages (which do not take intraspecific metabolite variability into account) are calculated from plot-specific and fixed (i.e. plot-independent) metabolite values respectively (see Section 2). F values are derived from ANOVAs testing for the effect of elevation. F values for fixed CW means are not presented. *, ** and *** denotes significant effects of elevation at $p < 0.05$, < 0.01 and < 0.001 respectively. Within each panel, dots topped with the same letter are not significantly different according to Tukey's test at $p = 0.05$

(Figure 1A). Condensed tannins were 1.8 times greater at the lowest elevation than at the highest (Figure 1B), and this was almost entirely driven by a shift in species composition (Figure 2). For total low molecular phenolics, total phenolic acids and total flavonols, the 1,240-m site had significantly higher concentrations than some of the higher elevations, while concentrations at 1,120 m were not significantly different from those at the higher elevations (Figure 1B–D). For these compounds, the responses were stronger when within-species variation was included, as shown by the strong positive covariation of within and between species variability (Figures 1 and 2). Total flavones responded differently to the other types of plant CBSCs, with a strong increase with increasing elevation. Further, we found a significant

within-species effect of flavones, but in the opposite direction to the effect of species turnover; this yielded a negative covariation (Figures 1F and 2). Total stilbenes decreased with elevation and were 42 times higher at the lowest elevation than at the highest (Figure 1G). Concentrations of individual compounds in all species are given in Table S1.

There was a significant shift in the composition of plant CBSCs with elevation at the whole community level (Table 1; Figures 3A and 4A). The PERMANOVAs showed a significant effect of elevation on the composition of community-weighted plant CBSCs both when accounting for within-species variation and when only accounting for species turnover (Table 1). Flavones (luteolins and apigenins) increased in concentration with increasing elevation while

FIGURE 2 Decomposition of the total variability of (A) plant and (B) lichen secondary metabolites explained by elevation into interspecific (species turnover), intraspecific effects and total variability (specific average), depicted using the style of Lepš et al. (2011). Covariation strength is represented by the interval between the total variability and the sum of inter- and intraspecific variability effects. Covariation is negative when total variability is lower than the sum of interspecific variation and intraspecific variation and positive when the total variability is higher than the sum of interspecific variation and intraspecific variation. Lichen secondary metabolites are divided into those present in the cortical layer and those present in the medullary layer. *, ** and *** denotes significant effects of interspecific variability, intraspecific variability and total variability at $p < 0.05$, < 0.01 and < 0.001 respectively

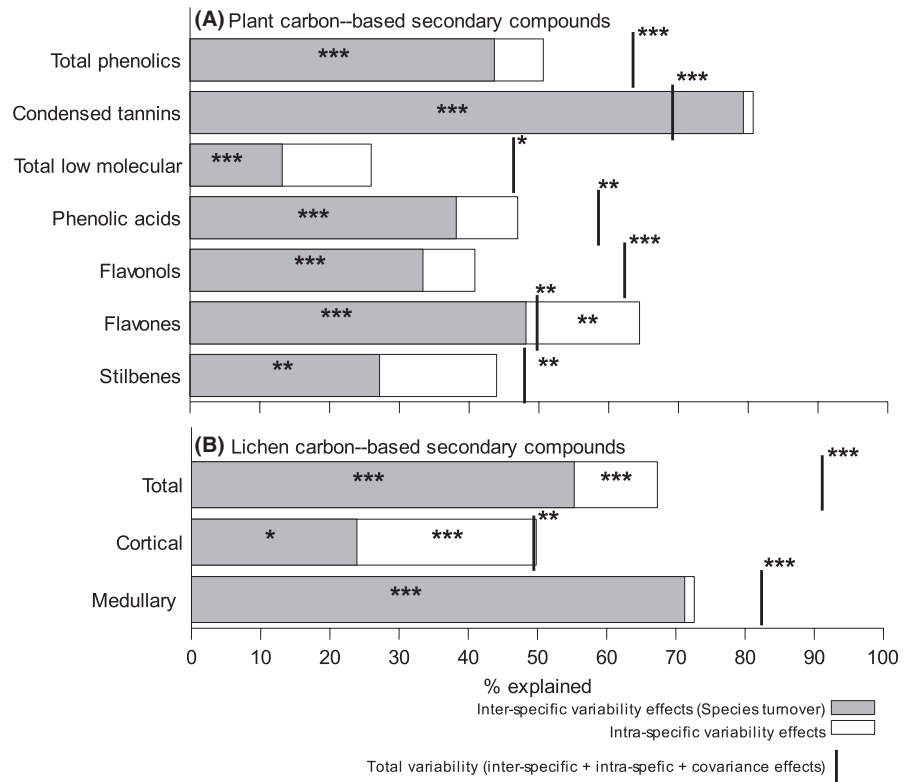


TABLE 1 Permutational multivariate analyses of variance (PERMANOVA; using 999 permutations) testing for the effect of elevation on the composition plant and lichen secondary metabolites at the community level. Specific community-weighted (CW) values include both species turnover and within-species variability while Fixed CW values assume that there is no within-species variation

	SS	MS	$F_{4,20}$	R^2	p
Specific community-weighted plant secondary metabolites					
Elevation	0.45	0.11	9.13	0.646	<0.001
Residuals	0.25	0.01			
Fixed community-weighted plant secondary metabolites					
Elevation	0.44	0.11	14.41	0.742	<0.001
Residuals	0.15	0.01			
Specific community-weighted lichen secondary metabolites					
Elevation	0.84	0.21	4.39	0.468	0.001
Residuals	0.96	0.05			
Fixed community-weighted lichen secondary metabolites					
Elevation	0.60	0.15	4.60	0.479	0.001
Residuals	0.65	0.03			

Abbreviations: MS, mean sum of squares; SS, sum of squares.

most other plant CBSCs decreased (Figure 4A; Table S3). The ratio of concentrations of flavonoids to condensed tannins at the whole community level increased with elevation (Figure S2a). As such, the concentrations of condensed tannins were higher than flavonoids at the two lower elevations and less than flavonoids at the higher sites.

3.3 | Lichen carbon-based secondary compounds

The community-weighted concentration of total, cortical and medullary CBSCs varied significantly with elevation (Figure 5). As such, medullary and total concentration were 3.4 times and 2.0 times greater, respectively, at the highest than at the lowest elevation (Figure 5A,C). Meanwhile, cortical CBSCs peaked at the 1,480-m elevation (Figure 5B). The variation in medullary compounds with elevation was almost entirely driven by species turnover (Figure 2B). By contrast, intraspecific variation was almost equally as important as species turnover in explaining the change in community-weighted cortical CBSCs, and it also contributed significantly to the change in total lichen CBSCs (Figure 2B). The relative abundance of species with melanin, a dark non-phenolic compound functioning as sunscreen instead of cortical lichen CBSCs, increased significantly with elevation (Figure S3). Concentrations of individual compounds in all species are given in Table S2.

The NMDS ordination of the community-weighted averages of lichen CBSCs showed a separation between the three lower elevations and the two upper elevations on the primary axis (Figure 3B). The PERMANOVAs showed a significant effect of elevation on the composition of community-weighted lichen CBSCs both when accounting for within-species variation and when only accounting for species turnover (Table 1). The change in composition of lichen CBSCs with elevation was partly driven by a strong increase of several depsides (e.g. squamic acid, alectorialic acid, baeomycesic acid and barbatolic acid) with increasing elevation (Figures 3B and 4B; Table S4). As such, the ratio of concentrations of depsides to

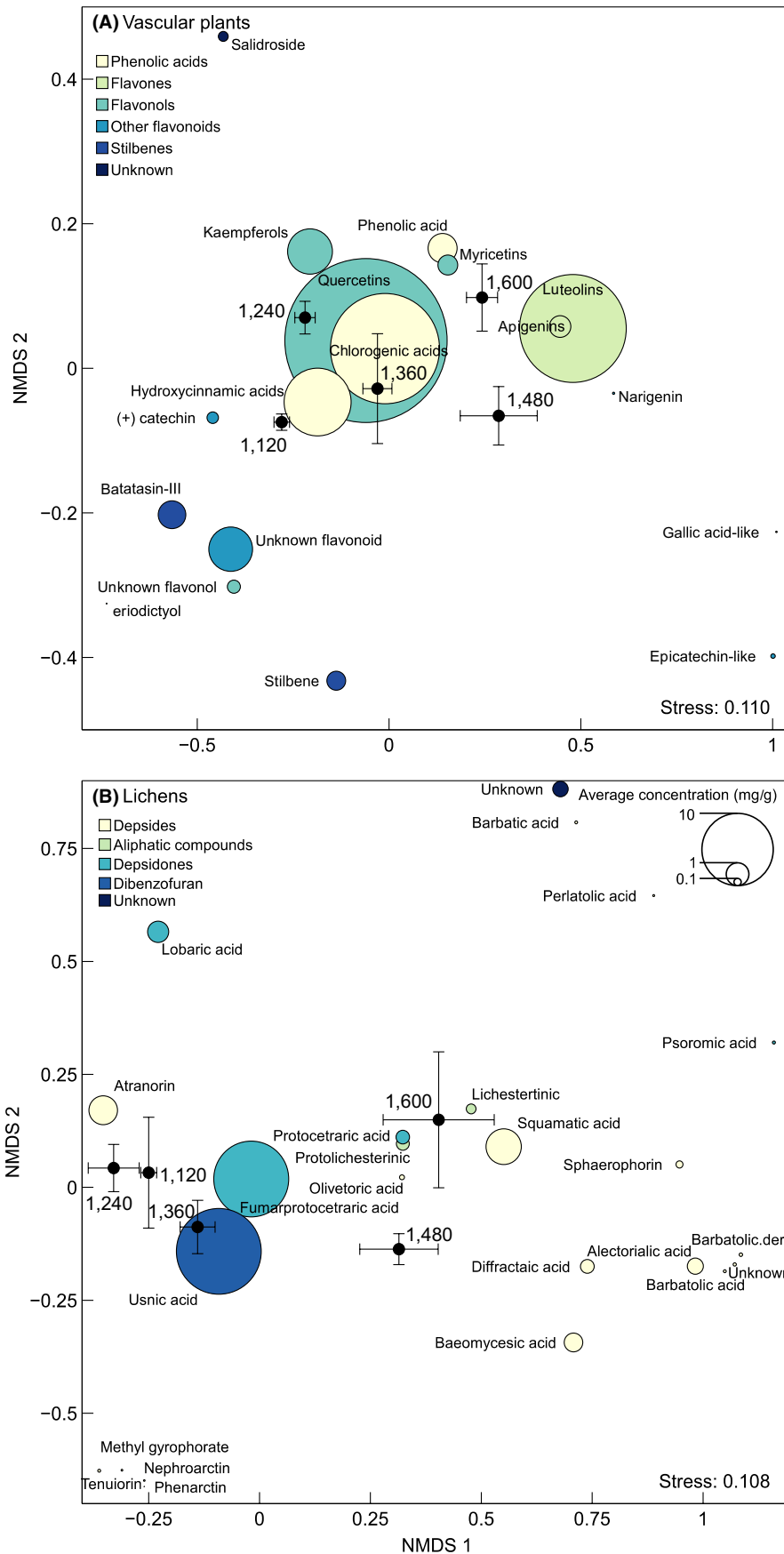
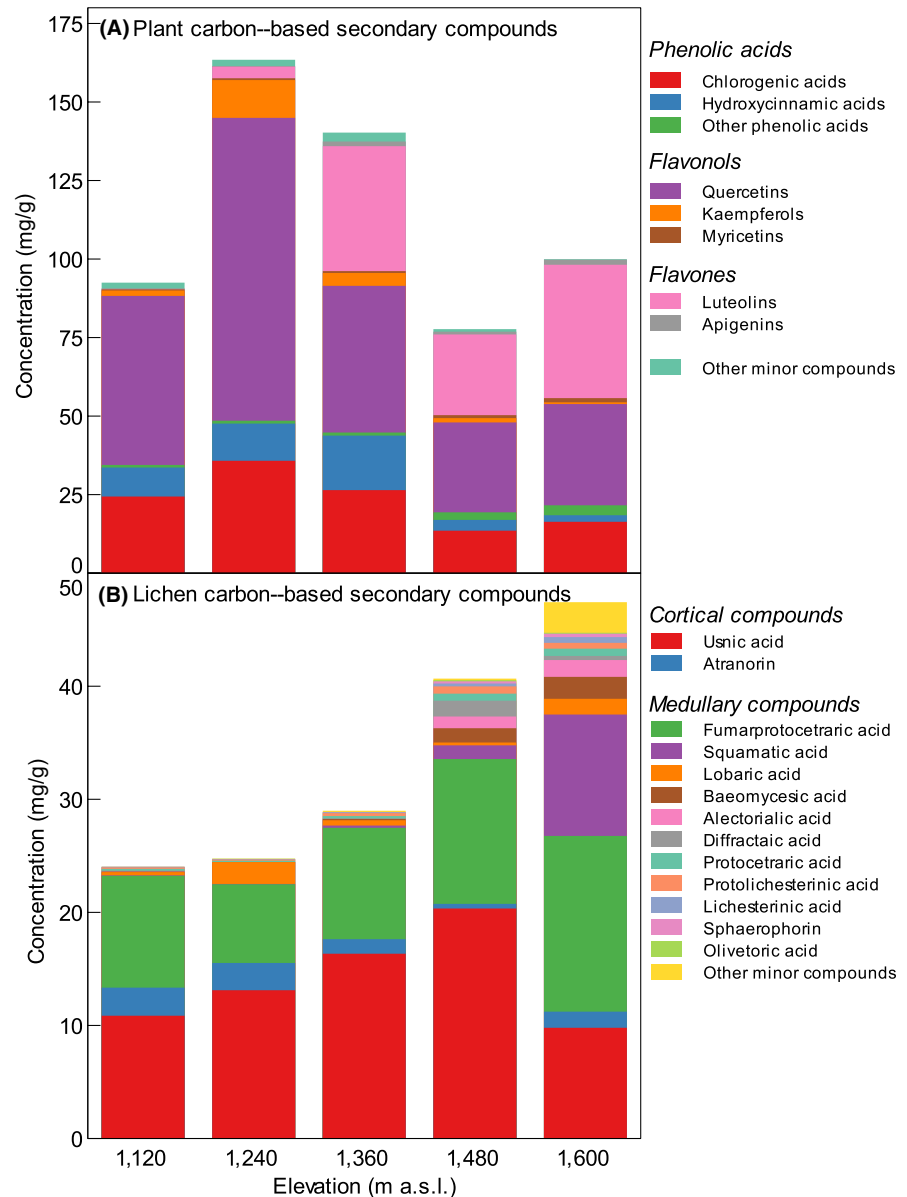


FIGURE 3 Ordination plot derived from a non-metric multidimensional scaling (NMDS) of community-weighted averages of (A) low molecular plant secondary metabolites and (B) lichen secondary metabolites, across an elevational gradient from 1,120- to 1,600- m a.s.l. The area of the circles indicate the community-weighted concentration of each secondary metabolite averaged across the entire gradient. Black dots and error bars represent centroids (\pm SE) of plots at each elevation. Stress values indicate goodness of fit

FIGURE 4 Mean values of individual (A) plant (excluding tannins) and (B) lichen secondary carbon-based secondary compounds across elevation. See Table S1 and S2 for mean \pm SE values and relative contributions of within-species variability and species turnover



depsidones at the whole community level increased significantly with elevation (Figure S2b).

4 | DISCUSSION

We found mixed support for our hypotheses. As such, our first hypothesis predicting a shift towards overall higher concentrations of CBSCs with higher elevation was supported for lichens but not for vascular plants. Meanwhile, our second hypothesis that with increasing elevation CBSCs associated with light protection and antioxidative capacity was supported for plants (e.g. higher flavone concentrations), but for lichens, the story is more complex. We now explore the mechanisms behind the contrasting behaviour of CBSCs in vascular plants and lichens and then discuss possible implications.

Our finding that community-level concentrations of plant CBSCs were lower at higher elevations than at some of the lower

elevations was most pronounced for condensed tannins and stilbenes. This result was largely driven by a switch from a plant community dominated by ericaceous shrubs (which generally have high concentrations of plant CBSCs) to one dominated by graminoids (with lower CBSC concentrations) as elevation increased and as conditions become too harsh for woody plants. In contrast to our results, De Long et al. (2016) found increasing concentrations of total phenols and condensed tannins at the community level with increasing elevation, but in their study ericaceous shrubs increased with higher elevation. In our study, the increasing ratio of flavonoids to condensed tannins at the whole community level with elevation, driven by a relative decrease in ericaceous shrubs, suggests a shift from protection against herbivory (e.g. by tannins and stilbenes) at lower elevations to protection against photodamage (by antioxidants) at higher elevations. This is in line with our second hypothesis predicting an increase in abiotic stressors (e.g. oxidative stress) with increasing elevation, and similar to other studied plant

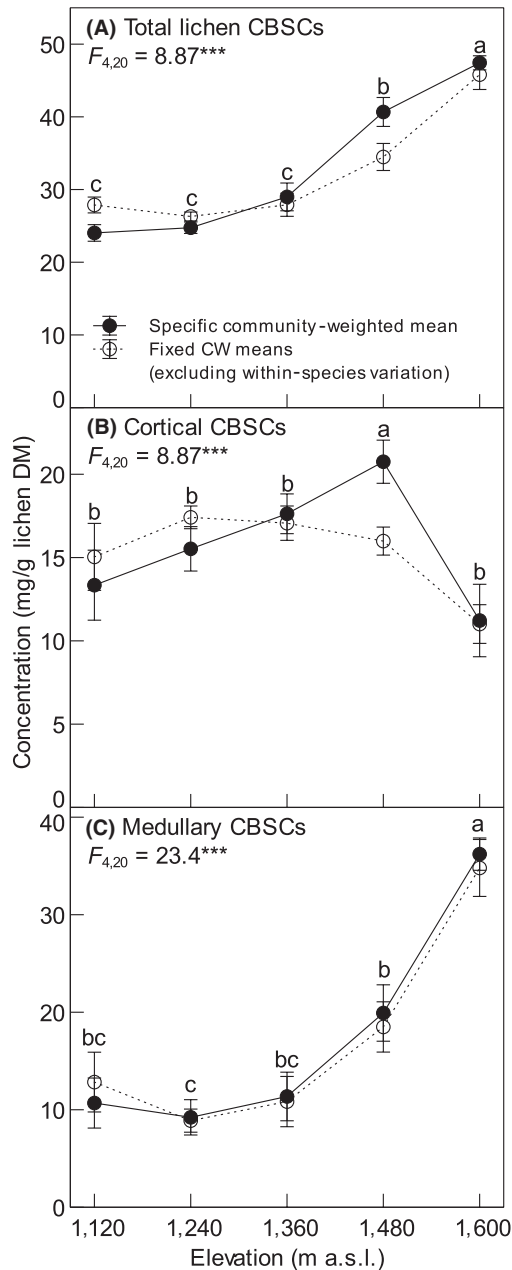


FIGURE 5 Community-weighted (CW) averages (\pm SE) of (A) total lichen carbon-based secondary compounds (CBSCs), (B) CBSCs in the lichen cortex and (C) CBSCs in the lichen medulla. Specific community averages (which include species turnover and intraspecific metabolite variability) and fixed community averages (which do not take intraspecific metabolite variability into account) are calculated from plot-specific and plot-independent metabolite values respectively (see Section 2). F values are derived from ANOVAs testing for the effect of elevation. F values for fixed CW means are not presented. *, ** and *** denotes significant effects of elevation at $p < 0.05$, < 0.01 and < 0.001 respectively. Within each panel, dots topped with the same letter are not significantly different according to Tukey's test at $p = 0.05$

communities (Moreira et al., 2018). The increase in antioxidants with increasing elevation was driven in part by a relative increase in species containing luteolins (i.e. graminoids); these compounds have two hydroxyl groups on the B-ring, and are much stronger

antioxidants than are flavonoids with only one hydroxyl group such as kaempferols and apigenins (Agati et al., 2012). However, quercetins, which are also strong antioxidants with two hydroxyl groups, decreased with elevation. Changes in plant CBSCs were mainly driven by species turnover, suggesting that the harsh environmental conditions favour species with high antioxidant capacity. Meanwhile, we do not have information on herbivore abundance across the gradient and are therefore unable to conclude whether the decrease in species with high tannin concentration is driven by selective grazing. For several compounds, there were also strong effects of covariation between turnover and variation within species, pointing to a role of intraspecific variation also contributing to the overall community-level response.

The increase in total lichen CBSCs with increasing elevation that we observed is consistent with lower temperatures slowing down growth, resulting in a surplus of carbon that can be used for producing CBSCs. This is consistent with plant defence theories suggesting that environmental constraints are more limiting to growth than to defence (Herms & Mattson, 1992; Stamp, 2003). Our findings, which included an increase in concentrations of usnic acid with elevation, are in line with Bjerke et al. (2004) who also found increasing usnic acid in lichens at higher elevations, and who attributed this to declining temperature and increasing frost-sums. Further, Nybakken et al. (2011) ran an open top chamber-experiment in the same area as in our study and showed that warming led to lower concentrations of usnic acid in *Cladonia arbuscula*, but no changes in other compounds in that species or any compounds in other lichen species. In our study, the community level increase in usnic acid with increasing elevation was primarily driven by changes within species, which indicates physiological responses of species to lower temperatures. The changes in other lichen CBSCs were primarily driven by species turnover.

The increase in lichen CBSC concentration with elevation was more pronounced for compounds in the medullary layer that are known to deter herbivores, than for cortical compounds that are recognized as protecting the lichen against damage by sunlight. This is in contrast to our second hypothesis (Solhaug & Gauslaa, 2012). However, even though medullary compounds do not screen light, they do protect against light-induced oxidative stress. Thus, the very large increase in medullary compounds with elevation suggests that the lichen community is increasingly investing in protection against oxidative stress. Still, we did not find support for a shift towards antioxidants, because lichen communities at higher elevations were characterized by an increase in depsides, which are considered to be less efficient scavengers of radicals than are depsidones such as fumarprotocetraric acid (Atalay et al., 2011; Brisdelli et al., 2013; Hidalgo et al., 1994; Lohézic-Le Dévéhat et al., 2007). The low concentrations of cortical lichen CBSCs at the highest elevation can in part be explained by an increase in the relative abundance of brown melanic species such as *Cetraria islandica*, which do not produce cortical CBSCs. These lichens instead rely on melanins for light protection, which leads to a higher surface temperature due to absorbance of visible and near-infrared light (Gauslaa, 1984), and thus greater

susceptibility to heating by excess light (Gauslaa & Solhaug, 1999). Because of their light absorbance, these lichens can also melt snow during winter, causing hydration and thus activation of photosynthesis (Coxson & Coyle, 2003), and they are therefore often restricted to colder environments. As such, the high elevation lichen communities are well defended against high solar radiation despite low concentrations of phenol-based sun-screens.

In this study, we chose to focus on carbon-based phenolic secondary compounds. However, while this covers most of the lichen defence arsenal (except melanins as discussed above), it is well recognized that vascular plants produce an array of different compounds. For instance, some plant species produce nitrogen-based compounds (e.g. alkaloids), but these are more common in productive and nutrient-rich and communities (Coley et al., 1985; Herms & Mattson, 1992). Therefore, in our study system, which is nitrogen limited and comparatively infertile, photosynthetic organisms are less likely to invest in nitrogen-based defences rather than the phenols that we chose to focus on. Further, focusing on a group of secondary compounds that are common to both plants and lichens (i.e. phenols) facilitates the comparisons between them. However, a next step in studying plant defence compounds across elevational gradients could be to include all carbon-based compound groups (i.e. also including terpenes) and compare responses with those of nitrogen-based defence systems.

5 | CONCLUSIONS

In this study, we show that responses of plant and lichen CBSCs to elevation at the whole community level is compound-specific. These compound-specific changes can to some extent be understood from a functional perspective, that is, the shift from those associated with protection against herbivory at lower elevations to those associated with protection from oxidative stress at higher elevations, although this pattern was less pronounced for lichens. As such, our findings highlight the importance of studying qualitative as well quantitative changes in CBSCs in response to environmental factors or gradients.

The fact that with increasing elevation, the vascular plant community has lower levels of herbivore defence compounds while the lichen community has more, suggests a possible shift with increasing elevation in the relative palatability of these two groups. This has potentially important trophic implications in that generalist herbivores may switch from a lichen-based diet to a vascular plant-based diet with increasing elevation. The changed relative palatability of vascular plants and lichens with increasing elevation may also promote a shift in herbivore community composition from those that specialize on lichens to those that specialize on vascular plants. Furthermore, the changes in plant and lichen CBSCs composition may also impact carbon and nutrient fluxes. For instance, the decrease in tannin concentration in the vascular plant community with increasing elevation could potentially result in decreased carbon sequestration and increased N mineralization (Hättenschwiler & Vitousek, 2000). In contrast, the large increase in CBSCs in the lichen community

may reduce decomposition rates (Asplund & Wardle, 2013). Finally, through highlighting the effect of elevation on plant and lichen CBSCs, our results suggest that climate warming in mountain regions could have important effects not only on community composition of primary producers but also on their secondary chemistry at the whole community scale, which in turn will affect the structure and functioning of communities and ecosystems.

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AUTHORS' CONTRIBUTIONS

J.A. designed the study in consultation with K.v.Z., R.E.R., T.B., K.K., S.I.L. and D.A.W.; Field and lab work were conducted by K.v.Z. and R.E.R.; Data analyses were done by J.A. and L.N.; J.A. led the writing in collaboration with K.v.Z., R.E.R., T.B., K.K., S.I.L., D.A.W. and L.N.

DATA AVAILABILITY STATEMENT

Data available from the dataverse.no repository <https://doi.org/10.18710/IGOMMH>.

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SUPPORTING INFORMATION

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