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Removal of secondary compounds increases invertebrate abundance in lichens

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ABSTRACT

We investigated how lichen carbon-based secondary compounds (CBSCs) affect abundance of invertebrates in five lichen species growing on the forest floor (*Cladonia rangiferina*, *Cladonia stellaris*) or on tree trunks (*Evernia prunastri, Hypogymnia physodes, Pseudevernia furfuracea*). To do this, CBSCs were removed by rinsing lichen thalli in acetone (which has no adverse effects on the lichens) and the lichens were re-transplanted in their natural habitat. After 4 months there was higher abundance of mites, springtails and spiders in the three epiphytic lichens that had their CBSC concentrations reduced. The increase in predatory spiders following CBSC reduction suggests that the compounds have multitrophic consequences. The acetone treatment reduced the number of nematodes in four of the lichen species. Given that lichens serve as important habitats for a diverse range of invertebrates, increased knowledge of how lichen CBSCs may regulate their abundance helps us to better understand the role that lichens and their defence compounds play in structuring forest food webs.

1. Introduction

Lichens are symbiotic associations between a mycobiont and photobiont that cover trees, rocks or the ground in various, mainly nutrient poor, ecosystems, sometimes in great abundance. As such, lichens are an important food source and serve as shelter for a diverse range of invertebrates (Gerson and Seaward, 1977; Seaward, 1988). Many of these invertebrates are aquatic, and include protozoa, nematodes, rotifers and tardigrades; lichens provide a damp environment for these organisms and protect them from desiccation. Other dominant, non-aquatic invertebrate fauna that occur on lichens include gastropods and micro- and macro-arthropods. There is a growing body of literature on the role of gastropods in shaping lichen species composition via consumption of lichen thalli, as well as the factors that regulate this consumption (e.g. Lawrey, 1984, 1983; Fröberg et al., 2011, 2006; Asplund et al., 2010; Asplund and Wardle, 2013). However, little is known about lichen-arthropod interactions (but see Pöykkö et al., 2005, 2010). Among arthropods, mites (Acari), especially Oribatida and Prostigmata, are an important part of the lichen-associated fauna (Gerson, 1973), and several mite species are reported to feed on lichens (Maraun et al., 2011; Seyd and Seaward, 1984). Further, lichen-feeding springtails (Collembola) can occur in vast numbers on lichens where they may cause significant damage to lichen thalli (Hale, 1972). It has also been shown that the spatial distribution of tree-dwelling arthropods are affected in a large part by the composition of lichen species and growth forms (André, 1985).

Because lichens are sessile and slow-growing and constantly exposed to lichenivorous invertebrates and other lichen-feeding organisms, they have a strong need for anti-lichenivore defence. As such, lichen mycobionts have evolved a wide range of carbon-based secondary compounds (CBSCs), which protects the lichen from various stresses, including lichenivory (Huneck and Yoshimura, 1996; Lawrey, 2009). Lichen CBSCs are commonly weak phenolic acid derivatives that can be effectively and non-

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destructively reduced in concentration from living lichens through the use of acetone (Solhaug and Gauslaa, 1996, 2001). There is now growing evidence from experiments involving the removal of CBSCs that these lichen compounds can deter gastropods (Gauslaa, 2005; Asplund and Wardle, 2013; Černajová and Svoboda, 2014), lepidopteran larvae (Pöykkö et al., 2005), and bank voles (Nybakken et al., 2010). Further, Reutimann and Scheidegger (1987) showed lichen CBSCs to affect two species of oribatid mites though in opposing directions. However, we have limited understanding of how lichen CBSCs affect the abundance and species richness of lichenivorous invertebrates (e.g. springtails and mites), or whether such effects are multitrophic, i.e. affect the abundance of predators that consume lichenivores. However, Pettersson et al. (1995) suggested that lichen abundance increases invertebrate abundance which in turn may increase the abundance of insectivorous birds.

In this study we used five common lichen species, two terricolous and three epiphytic ones, to study the role of CBSCs in regulating lichen-associated invertebrate communities. We tested the following hypotheses: (i) CBSCs in lichens deter lichen-associated invertebrates, and removal of CBSCs will cause an increase in invertebrate abundances and diversity; (ii) the influence of CBSC removal on invertebrate fauna will be greater for those species that otherwise have the highest concentrations of CBSCs; and (iii) increased abundance of particular invertebrates following removal of CBSCs will promote increased abundance of their predators (see Wise, 1979; Chiverton, 1986; Marczak and Richardson, 2007). Lichen-feeding invertebrates potentially play an important role in nutrient cycling by enhancing decomposition and transferring nutrients to higher trophic levels (Denison, 1973; Asplund et al., 2013). In that light, our study aims to improve the knowledge of factors regulating lichenivorous invertebrates, and therefore add to the understanding of the role that lichens play in community and ecosystem processes.

2. Materials and methods

2.1. Study site and species collection

This study was conducted at Mossaberget, Täfteå, Sweden (63°49′N, 20°28′E) in a near coastal forest with rocky ground dominated by *Pinus sylvestris*. *Cladonia stellaris* dominates large flat rocks in the forest while *Vaccinium* spp. and feather mosses (e.g. *Hylocomium splendens* and *Pleurozium schreberi*) occur mainly in the hollows. We used two terricolous fruticose mat-forming lichens: *Cladonia rangiferina* and *C. stellaris*, and three epiphytic species: *Evernia prunastri*, *Pseudevernia furfuracea* (which are fruticose) and *Hypogymnia physodes* (which is foliose). Lichen nomenclature follows Ahti et al. (2013) and Thell and Moberg (2011). Approximately 150 g air-dried material of each species was collected and brought to the lab in May 2012 where it was rinsed from debris and left to air dry for 2 d. After this drying, large numbers of arthropods were found beneath the lichens. As such, this treatment likely drove out most of the arthropods from the lichens, meaning that negligible numbers of arthropods remained in the material that was placed back in the field. Therefore, the arthropod abundances measured at the end of the experiments most likely reflect colonization during the experiment. However, nematodes were unlikely to have been entirely removed by this drying treatment.

2.2. Acetone rinsing

Concentrations of carbon-based secondary compounds (CBSCs) were reduced in lichen thalli using a standard procedure commonly applied to lichens (Solhaug and Gauslaa, 1996, 2001; Gauslaa, 2005; Pöykkö et al., 2005). For each species, half of the rinsed material was placed in excess acetone for three 20 min intervals. To estimate the extraction efficiency, approximately 1 g of acetone-treated and of untreated lichen material for each species was collected from several thalli and ground in a ball mill. All thalli for each species × treatment combination were combined and ground to provide an average value for each combination. Approximately 30 mg of the powder was extracted in acetone for three 45 min intervals. The combined supernatants were evaporated to dryness and dissolved in 1000–2000 μl acetone. The extracted compounds were then quantified by HPLC using an ODS Hypersil column, 50 × 4.6 mm using 0.25% orthophosphoric acid and 1.5% tetrahydrofuran in Millipore (Millipore, Billerica, Massachusetts, USA) water (A) and 100% methanol (B) as mobile phases at 2 ml min^{-1}, and UV detection at 245 nm (following Nybakken et al., 2007). Compound identification was based on retention times, online UV spectra and co-chromatography of commercial standards. The CBSCs were grouped into cortical and medullary compounds according to their position in the lichen thallus (Krog et al., 1994). The CBSCs were again measured, as described above, by the end of the experiment after invertebrate extraction to estimate CBSCs resynthesis.

2.3. Transplantation

All lichen material was fully hydrated with water from a spraying bottle prior to transplantation, on May 18 2012, to avoid fragmentation. The terricolous lichens, *C. rangiferina* and *C. stellaris*, were transplanted in plastic baskets (68 mm height and 87 mm × 87 mm at the bottom) with open tops and 12 mm × 12 mm openings (21 openings per side) on the sides and bottom allowing invertebrate access, and the bottom was lined with 1 mm nylon mesh following Gaio-Oliveira et al. (2006). Baskets were used to keep track of each specimen and thus to avoid the possibility that the wrong thalli were re-sampled. For each of the two terricolous lichens, 20 baskets were set up; 10 of these were filled with approximately 6 g of acetone-rinsed lichen material in an upright position and for the other 10 baskets non-rinsed lichen material was instead used. The baskets were placed in 10 pairs (each pair containing a basket each of acetone-rinsed and non-acetone rinsed lichens) in lichen mats dominated by the respective species. The epiphytic lichens were stapled directly onto the tree trunk, at breast height (i.e., 1.3 m), using plastic Plasstaples (Takkurat®, Dr. Gold & Co. KG, Nürnberg, Germany), and the foliose lichen *H. physodes* was covered with a 2.5 mm nylon mesh to press its thalli close to the substratum. For each epiphytic lichen species, one pair of acetone-rinsed thallus material and non-acetone-rinsed thallus material (each ~ 6 g) was placed on each of ten tree trunks. For *H. physodes* and *P. furfuracea* the tree species used was *P. sylvestris* (on which both species commonly occur), while for *E. prunastri* the tree species used was *Populus tremula* (the only tree species supporting *E. prunastri* in the study site). The lichens were transplanted to the trees and mats from which they were collected. However, because the thalli were randomized each individual thallus was not necessarily transplanted to its original position in the mat or on its original host tree.

2.4. Harvest and microfauna extraction

Lichen material was harvested in two stages due to limitations in invertebrate extraction capacity. On September 19 2012 the epiphytic lichens were quickly collected in a plastic jar which was immediately closed with a lid to prevent arthropods from escaping. *H. physodes* was collected by removing the plastic staples and