Lichen decline in areas with increased nitrogen deposition might be explained by parasitic fungi

A survey of parasitic fungi on the lichen *Alectoria sarmentosa* after 4 years of nitrogen fertilisation

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Abstract

Nitrogen (N) deposition in Europe has recently increased and is expected to continue to increase in the future. There is a well-documented decline in lichen diversity with higher N availability, although the mechanisms behind this are poorly known. In this study, I tested whether attacks by fungal parasites increase with higher N deposition. This pattern has been found in a number of studies on vascular plants, but it has never been investigated for lichens. I surveyed dark lesions and discolorings caused by fungi on the pollution-sensitive lichen Alectoria sarmentosa, after 4 years of increased N deposition in a whole tree fertilisation experiment in a boreal spruce forest. I found two species of fungi growing on the investigated lichen thalli. One of these species responded positively to increased N deposition. The results show that lichens can suffer from increased parasite attacks under a higher N load. Further studies using multiple lichen species and many years of recording are needed to understand the importance of parasites for the response of whole lichen communities to an increased N load.

Key Words: Parasitic fungi, lichen diversity, Alectoria sarmentosa, nitrogen deposition
Contents

Introduction........................................................................................................................................1
Materials and methods..................................................................................................................2
  Study site....................................................................................................................................2
  Fertilisation experiment...........................................................................................................3
  Sampling.......................................................................................................................................3
  Statistical analysis....................................................................................................................3
Results............................................................................................................................................4
  Nitrogen.........................................................................................................................................4
  Spatial pattern............................................................................................................................4
Discussion.......................................................................................................................................5
Acknowledgements......................................................................................................................8
References......................................................................................................................................9
Introduction

Anthropogenic nitrogen (N) emissions make up half of global emissions and are expected to increase in the future (Vitousek et al., 1997). There is a well documented decline in lichen diversity with increasing N availability, with sensitive lichens being replaced by a few tolerant, nitrophilic species, and lichens have successfully been used as indicators of N pollution (Davies et al., 2007; van Herk, 2009; Rogers et al., 2009). Even small additions of N reduced species richness in an experiment where N was added to an alpine heath (Britton & Fisher, 2007). The increase in N deposition has been unevenly distributed, such as in Northern Europe where the deposition is larger than total emissions (Holland et al., 2005). Measured levels of N deposition in the most polluted areas of Northern Europe are as much as 100 times higher than in unpolluted areas (Vitousek et al., 1997). Most of the N is deposited near the source, e.g. farms (van Herk, 1999) and traffic (Davies et al., 2007), so the effects are mainly concentrated to populated and industrial areas. It can, however, also be transported long distances in the atmosphere. Some parts of Northern Europe, such as coastal Norway, are predicted to receive more long distance nitrogen in the future, because of increased precipitation following climate change (Hole & Engardt, 2008).

Several N-related factors are known to influence lichens negatively, e.g. substrate pH changes (van Herk, 1999), shifts in the photobiont/mycobiont ratio (Makkonen et al., 2007) and cytotoxic effects (Munzi et al., 2009), but which factor that is ultimately responsible for the loss of species is not known. Parasitic fungi are one potential factor which to my knowledge has never been studied in this context. This is surprising considering it is a well known pattern from plants that parasite attacks increase together with increasing N availability, and that they can sometimes have drastic effects on communities (Flückiger & Braun, 1998; Strengbom et al., 2002; Thrall et al., 2007). The lack of interest might be due to the view that lichens have extremely good defenses and are rarely parasitised (Lawrey, 1995; Richardson, 1999). However, at least two species, Athelia arachnoidea and Nectria parmeliae, are known to kill or cause serious damage to lichens in polluted areas (Arvidsson, 1978; Richardson, 1999) and fungal parasites were suspected of causing lichen mass-deaths after heavy rains in Norway (Gauslaa, 2002). Most studies on lichen parasites, or lichenicolous fungi (fungi growing on lichens), have been purely taxonomical or related to chemical host-parasite interactions (Lawrey, 1995). Hedenäs et al. (2006) is an exception which deals with disease incidence of the fungus Abrothallus suecicus on the lichen Ramalina sinensis in different forest successional stages.

The mechanisms by which increased N could make lichens susceptible to parasites are not well studied, but are probably related to those N-related factors connected to the decline of species (substrate pH, shifted biont ratio, etc.; see above). Cytotoxic effects can weaken the lichen's structural and chemical defenses (Duffy et al., 2003, Munzi et al., 2009), and shifts in the biont ratio or other physiological problems could give opportunities for parasites to appropriate sugar and nutrients. N absorbed by lichens is invested in growth of either the photobiont or the mycobiont, or in an increased metabolism. Some lichens have the ability to reject excessive N (Dahlman et al., 2002). Increased N might also affect the production of secondary carbon-based compounds (“lichen acids”). The secondary compounds are often toxic and can be used for defense or against competition from other lichens and plants (Land
& Lundström, 1998). The relative investment in these compounds differs between species and might represent different strategies for dealing with parasites. A recent study involving A. sarmentosa among other lichens showed no significant effects on most species' defensive compound levels after N-fertilisation, except in the pollution-tolerant Platismatia glauca, where they were significantly lower (Nybakken et al., 2009). P. glauca instead invested the extra N into photobiont and thallus growth (although this was a short study that could not detect long term changes, also there could be qualitative differences that are important).

The ability to feed on lichen tissue has evolved mainly among ascomycetes and deuteromycetes, but also basidiomycetes. These fungi are on a scale from mutualistic to parasitic; most are believed to be commensalistic (Richardson, 1999). There are examples of both generalist and species-specific lichen parasites (Lawrey, 1993). Most of the selective pressure leading to species-specificity is probably related to the defensive compounds produced by lichens. Parasitic fungi have their own array of toxins used to penetrate hosts' defenses or against competing parasites (Duffy et al., 2003). Strong co-evolution between lichen and parasite is indicated by the fact that non-lichenicolous relatives of parasites are often not affected by lichen defensive compounds, but they also lack the enzymes necessary to feed on lichen tissue (Lawrey, 1995). There are examples of a parasite actually being benefited by defensive compounds (Lawrey, 1995). The complexity of these interactions is exemplified in the infection of the lichen Punctelia rudecta by Nectria parmeliae, where N. parmeliae depends on a second fungus, Fusarium, to disable a certain defensive compound in the lichen host (Lawrey, 2000). Parasites can also themselves be parasitised (Richardson, 1999).

The purpose of this study is to test the prediction that increased N deposition increases the abundance of parasitic fungi on lichens. This could indicate whether parasitic fungi are responsible for the universal pattern of lichen diversity decline following N pollution. The experiment was made in a remote boreal forest with a rich flora of Alectoria sarmentosa, a pollution-sensitive epiphytic lichen. The occurrence of two unidentified (and probably undescribed) hyphomycetes was surveyed on thalli of A. sarmentosa, growing on trees irrigated with fertiliser of different concentrations. In addition possible vertical gradients will be discussed. Parasitic fungi usually prefer shade and moisture which is found in the lower parts of trees (Duffy et al., 2003).

Materials and methods

Study site
The site is located in an old growth spruce forest at Kulbäcksliden (64°12’N, 19°33’E) in Vindeln, Sweden and is part of The Unit for Field-based Forest Research, The Swedish University of Agricultural Sciences. The trees are 10–15 m high, 50-100 cm Ø in breast height and stand relatively widely spaced. Background deposition of biologically reactive N is low (c. 2 kg N ha⁻¹ year⁻¹) in this part of Sweden (Forsum et al., 2006).
Fertilisation experiment

Trees that are conspicuously rich in lichens, taller than 8 meters and have no large neighbouring trees closer than 2-3 m have been selected. Around these trees wooden towers have been built, with an irrigation system spraying a solution of NH$_4$NO$_3$ onto the trees each morning for a period of 4 months each year since 2006. Five levels of fertiliser have been used, equaling depositions of 0.6, 6*, 12.5*, 25 and 50 kg N ha$^{-1}$ during each 4-month period, where 0.6 kg represents the N-concentration of rainwater at the study site and 50 kg represents levels actually measured in polluted areas. For further details see Johansson, et al., 2010.

* Not included in this study.

Sampling

12 trees from 3 treatment levels (0.6, 25 and 50) were sampled, including 3 control trees without irrigation. Trees were divided into two height levels (1-4 m and 4-6 m respectively). 20 thalli of Alectoria sarmentosa were sampled at each sampling position (in total 40 per tree) and larger thalli were favored. Two of the control trees had to be climbed by ladder which put their upper sampling position 1-2 meters lower than the rest.

Two hyphomycete species, as of yet unidentified, were observed on the lichens and distinguished according to the following description by Lars Ericson (pers. com.):

- *Species 1* (Fig. 1ab). Attacked parts of the host are colored brown by the numerous conidia. Destroys the cortex of the host exposing the medulla. Severe attacks will result in destruction/fragmentation of the host.

- *Species 2* (Fig. 1c). Is characterised by its dark-brown hyphae which through a hand lens appears like a tiny species of Bryoria. Attacked parts of the host do not show any visible damages.

Their occurrence on thalli was surveyed using a 10x hand lens. In addition instances of algal colonies on thalli were recorded. These were clearly visible as green spots around 0.3 mm. Sampling was carried out between August 20 – September 5, 2009.

Statistical analysis

Responses to treatment and vertical position were tested with hierarchial ANOVA using tree as a block factor. To account for the vertical difference in nitrogen uptake by thalli, additional analyses were made substituting treatment for thallus N concentrations (using data from 2008). Also, a chi-square test was made to test for possible interactions between the two species on thallus level. Analyses were made using the statistical package R (http://www.r-project.org/).
Results

Nitrogen
Species 1 and the number of algal colonies on thalli increased with higher N treatments (P = 0.009 and P = 0.002), while Species 2 showed no statistically significant response (fig. 2, table 1). Both species appeared to be present on Alectoria sarmentosa throughout the forest; Species 1 was fairly abundant while Species 2 was quite rare. Algal colonies were completely absent from all but the two highest treatments.

Spatial pattern
Species 2 was significantly more abundant at the lower than at the higher sampling positions (P = 0.042). No other significant vertical patterns were found (fig. 2, table 1). I tested whether a vertical pattern in N-uptake could influence the distribution of Species 1, but substituting treatment for thallus N content (data from 2008) in the analysis did not significantly alter the results.

Only 4 thalli of the total 470 were infected by both species simultaneously. A chi-square test confirmed that coexistence of the two parasitic fungi was significantly less common than what should be expected based on their abundance (χ² = 5.24, d.f. = 1, P = 0.022).
Fig. 2. Occurrence rates of two unidentified hyphomycetes (Species 1 & 2) and algal colonies on Alectoria sarmentosa under 3 different nitrogen treatments (units: kg N ha⁻¹). Species 1 and algae responded positively to nitrogen treatments, while Species 2 showed a significant vertical pattern (more abundant on lower branches). L/U = lower/upper sampling position. Error bars: ± 1 S.E..

Table 1. Hierarchical ANOVAs on responses to nitrogen treatment and height level. Significant P-values in bold (P<0.05).

<table>
<thead>
<tr>
<th></th>
<th>Species 1</th>
<th></th>
<th>Species 2</th>
<th></th>
<th>Algae</th>
<th></th>
</tr>
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<tr>
<td></td>
<td>d.f.</td>
<td>F</td>
<td>P</td>
<td>d.f.</td>
<td>F</td>
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<td>3,8</td>
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<td>0.009</td>
<td>3,8</td>
<td>0.287</td>
<td>0.833</td>
</tr>
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<td>0.752</td>
<td>1,8</td>
<td>5.825</td>
<td>0.042</td>
</tr>
<tr>
<td>Treatment:Height</td>
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<td>0.248</td>
<td>0.860</td>
<td>3,8</td>
<td>0.561</td>
<td>0.656</td>
</tr>
</tbody>
</table>

Discussion

Lichens infected by a tissue damaging parasitic fungus ("Species 1") increased significantly with higher N load, indicating that lichens can suffer from increased parasite attacks under higher nitrogen deposition. Parasitic fungi could thus be an important factor behind the decrease of lichen diversity in polluted areas. The high abundance of the tissue damaging
parasite supports previous suggestions that parasitic fungi can be important for the population dynamics of lichens and their responses to extreme climate events, such as the mass-deaths of lichens after heavy rains in Norway (Gauslaa, 2002). Increased precipitation leads to higher background N-deposition, suggesting that increased parasite attacks could occur also in otherwise unpolluted areas, where more rain is expected in the future (Hole & Engardt, 2008). However, the lowest N deposition treatment (0.6 kg/ha), that should be fairly similar to rainwater, had no detectable effect in this experiment. A long-term fertilisation experiment was conducted in a close-by forest stand already in the 1920's by Hesselman (1937). 8-9 years into the experiment he noted that the rich growth of *A. sarmentosa* had fallen off the trees. He attributed this to faster shedding of the bark under the improved growth conditions, which seems likely considering the fertiliser was applied at ground level rather than sprayed onto the trees, thereby not making it directly available to epiphytes. Still, it remains to be seen if the same thing will happen if the present experiment is allowed to continue.

Because Species 1 was recorded by its effects (i.e. tissue damage) rather than observations of the actual fungus, it was probably overlooked where it did not cause noticeable damage. I believe that most lichen thalli were infected to some extent, even in the forest outside the experiment area. The high infection rates indicate an equilibrium, i.e. the lichen is able to maintain a net growth, at least large enough to replace killed parts of the population. This balance could be delicate, and if shifted it could potentially lead to local extinction of the lichen. Mutualisms or commensalisms can shift into parasitism when environmental quality increases (Thrall et al., 2007). It is possible that otherwise harmless lichenicolous fungi will become virulent with increased N, perhaps because of improved growth conditions, weakened host defenses or other changes in the lichen's physiology. *A. sarmentosa* was shown to maintain a solid chemical defense throughout the N treatments (Nybakken et al., 2009), which points towards other causes for the increased parasite attacks in this study.

The physiological responses of lichens to N are species-specific (Dahlman et al., 2002). This, together with independently evolved chemical defenses and parasite host-specificity, suggests highly species-specific effects of parasites. In many cases parasites are probably of small or no importance. A similar study using multiple lichen species would be able to address these differences between species, and also host preferences of parasites. The role of generalist/opportunist fungi such as *Athelia arachnoidea* is perhaps more difficult to study, since they are often only visible through their effects (Arvidsson, 1978), and their distribution is probably patchy.

Parasitic fungi on plants have been used to monitor air pollution. For example, the fungus *Rhytisma acerinum*, or tar spot disease, was used to estimate atmospheric sulphur dioxide, by counting the number of tar spots on leaves (Bevan & Greenhalgh, 1976). Using lichen parasites as air quality indicators has to my knowledge never been tested, but it could potentially be a more efficient and more accurate alternative to "normal" lichen monitoring. It could also provide early warning signs that an environment is degrading to a point where the lichen community is threatened. This study indicates that parasitic fungi increase faster than lichen communities respond to environmental changes. For example, Species 1 shows a clear response to increased N deposition after only 4 years, while it would probably take longer for effects to show in the lichen community. Calibrating the number and severity of
parasite infections against e.g. a N gradient could perhaps be a means to detect smaller variations than is possible using only lichen species occurrences. Using a parasite that exists in polluted areas would be more useful for this purpose, rather than Species 1 in this study (assuming Species 1 only uses A. sarmentosa as its host - the lichen is completely absent from polluted areas).

Green algal colonies were observed growing on the surface of several lichens. If “surplus” nitrogen taken up by the lichen is invested into growth of photosynthetic tissue, the photobiont might be able to outgrow and escape the lichen, for example through cracks in the thallus (Richardson, 1999). This appears to have happened primarily on the upper branches under the highest treatments. Independent green algal colonies are on the other hand common throughout the forest and the environmental conditions presumably leading to a shift in the lichen biont ratio (high N, high temperature/light, high humidity) coincide with the optimal conditions for green algae (Bråkenhielm & Qinghong, 1995), making the origin of the green spots uncertain.

The distance between the upper and lower sampling levels was only between 2-4 meters, still Species 2 showed a significant vertical pattern. This is probably due to microclimate preferences. Parasitic fungi are usually favored by moisture and disfavored by direct sunlight (Duffy et al., 2003), and can thus be expected to be more abundant in the shady microclimate under the lowest branches near the ground. A vertical pattern in the species distribution of alectoroid lichens has been observed in several studies, where Alectoria and Usnea are more common in the lower canopy while Bryoria is more common in the upper canopy (Coxson & Coyle, 2003; Gauslaa et al., 2008). Coxson & Coyle (2003) tested this pattern against some physiological and microclimate factors, and concluded that most, but not all, of the variation could be explained by differences in pigmentation and microclimate. Considering the vertical pattern of Species 2 and the differences in usnic acid content between the mentioned genera (Gauslaa et al., 2008), suggesting different levels of chemical defense, it could be interesting to include parasitic fungi in the model.

In this study I have shown that the lichen A. sarmentosa suffers from increased attacks by an unidentified parasitic fungus under higher N. This could partly explain why the lichen is sensitive to air pollution, but more importantly it shows that parasites need to be acknowledged as a factor responsible for the disappearance of species from areas with increased N deposition. I conclude that increased parasite attacks are widespread among communities affected by higher N, but with large variations between species.

Further studies using multiple lichen species are needed to:
1) identify parasites and their host preferences,
2) investigate the effects of these parasites on various lichens under different N availability,
3) understand the importance of parasites on the community level.
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