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Detecting the nitrogen critical loads on European forests by means of epiphytic lichens. A signal-to-noise evaluation

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ABSTRACT

Lichens are considered to be among the most sensitive organisms for several types of pollutants. In this work, we analyzed a dataset of 286 epiphytic lichen species observed on 1155 trees at 83 ForestBIOTA plots, which is a subsample of approx. 500 plots of the European ICP Forests Level II network. We aimed at examining the amount of nitrogen deposition for which a significant variation of the relative diversity of morpho-functional groups of epiphytic lichens in the sampled plots is expected. Moreover, the study aimed at determining how much variance of these diversity variables could be explained by nitrogen depositions only. We used correlation and multiple regression models as well as hierarchical partitioning to evaluate the relative importance of environmental predictors in explaining variation in lichen diversity descriptors. The analysis splits the variation explained by each variable into a joint effect together with the other explanatory variables, and into an independent effect not shared with any other variable. The percentage of macrolichens in the plots was shown to be the most important indicator, since 56.7% of its variation could be explained by deposition, particularly by nitrogen compounds. It was shown that approx. 75% of the ForestBIOTA plots are affected by an unsustainably high throughfall nitrogen deposition. Based on these outcomes, it was possible to determine a nitrogen critical load of 2.4 kg ha^{-1} yr^{-1}.

1. Introduction

Reactive nitrogen (N_r, i.e., all N forms apart from N_2) is a valuable resource in nature which, in many ecosystems, still limits plant productivity. Plants are adapted to consume and re-use it effectively. In the 20th century, the discovery of the Haber–Bosch process allowed the fixation of atmospheric N to produce synthetic fertilizers. This has substantially increased crop productivity thus sustaining population growth. On the other hand, this process, in combination with the release of large amounts of N into the atmosphere from fossil fuel combustion, has produced adverse collateral effects both on human health and in the environment (Erisman et al., 2011). N_r causes eutrophication and acidification in fresh waters and soils with consequences for respective biota. Atmospheric N_r deposition is regarded as a major driver for the loss of biodiversity in Europe (Dise et al., 2011). Species and communities adapted to low nutrient levels or from habitats poorly buffered against acidification are the most vulnerable. Changes in the flora usually include the invasion of nitrophytic species and a decline of sensitive native species (Bobbink et al, 2010). As an example, heathlands dominated by the common heather (*Calluna vulgaris*), when exposed to high N deposition, are invaded by acid grasslands species (Bobbink et al., 1998).

Unlike vascular plants that take nutrients mainly from the soil through the roots, lichens, and in particular epiphytic lichens, take up water, solutes and gases over the entire thallus, thus depending on the atmosphere for nutrition. For this reason, they respond directly to atmospheric pollution and they have been widely used for monitoring air pollution (Nimis et al., 2002). There are many studies in which lichens have been used to map the effect of sulphur dioxide deposition in and around industrial areas and cities (e.g. Nimis et al., 1990). With the observed decline of SO_2 deposition in the last two decades, they are re-colonizing urban environments (e.g. Hawksworth and McManus, 1989). Lichens are as well very sensitive to changes in N deposition and to gaseous NH_3 concentrations in the atmosphere (Sheppard et al., 2009), but responses are different depending on the species. Some species (termed 'oligotrophic') are very sensitive to eutrophication (e.g., Frati et al., 2007; Pinho et al., 2008; Geiser et al., 2010) therefore disappearing from N-rich habitats, while others (termed 'nitrophytic') are favored by high N levels. Changes in lichen communities

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(i.e., a reduction in oligotrophic lichens and an increase in nitrophytic species), in lichen cover or the loss of species diversity are commonly reported as one of the first changes observed in different types of ecosystems due to increasing N deposition (e.g., Dise et al., 2011). The increase in nitrophytic lichen species, paralleled by a decrease in oligotrophic ones observed in some areas, has been attributed to an increase in atmospheric NH_3 (e.g., Pinho et al., 2009). An increase of the strictly nitrophytic lichen species is also observed around NH_3 point sources such as farms (Frati et al., 2007). However, an important challenge when conducting studies using lichens as ecological indicators is to be able to separate the responses caused by pollutants, such as N inputs, from other environmental factors associated to substrate, forest stands or others. Climatic variables (specifically average yearly temperature and rainfall), anthropogenic pressures (like harvesting), bark pH and texture and forest structure are particularly relevant for the diversity of lichens (Giordani, 2006, 2012; Giordani and Incerti, 2008).

In order to protect ecosystems, components and services from the effects of pollutants, Critical Loads (CLOs) and Critical Levels (CLEs) have been defined. The CLO is defined as "a quantitative estimate of deposition of one or more pollutants below which significant harmful effects on specified elements of the environment do not occur according to present knowledge" (Posthumus, 1988). On the other hand, the CLEs refer to the concentration of pollutants in the atmosphere above which adverse effects occur (Cape et al., 2009). While the main goal of the CLEs is the protection of sensitive plant species, that of the CLOs is protecting proper functioning of ecosystems (Cape et al., 2009; Bobbink et al., 2011). Basically, CLEs are used to established limits for individual pollutants, but in the absence of detailed information on the exact N species, the CLO is used to integrate all N species. The CLOs for N deposition have been recently revised for Europe. For the majority of the forest ecosystems they range between 10 and 20 kg ha^{-1} yr^{-1} (Bobbink et al., 2011). De Vries et al. (2007) suggest somewhat lower CLOs for European boreal forests (5–10 kg ha^{-1} y^{-1}), based on community studies of lichens, bryophytes, and vascular plants in Scandinavia. For North American forest ecosystems CLOs between 1 and 39 kg ha^{-1} yr^{-1} are given (Pardo et al., 2011). Lichens are mentioned as the ecosystem component that determines the CLO in the most sensitive forest ecosystems. At sites with higher precipitation CLOs are mostly higher, because precipitation presumably dilutes the effects of N concentrations to which lichens are exposed (Geiser et al., 2010). For long-term (annual) CLEs, a reduction from previously 8 $\mu g\, m^{-3}$ to 1 $\mu g\, m^{-3}$ was proposed for ecosystems in which lichens and bryophytes are important (Cape et al., 2009). This again underlines the higher sensitivity of these organisms. Therefore, recent revisions of the CLOs and CLEs (e.g., Bobbink et al., 2011; Cape et al., 2009; Fenn et al., 2008) for a large number of ecosystems are based on lichens. This is for example the case of semi-natural Mediterranean evergreen woodlands, for which CLOs of nitrogen deposition and CLEs of atmospheric ammonia have been defined using epiphytic lichens (Pinho et al., 2011, 2012).

Europe is a major source region for N_r production (Erisman et al., 2011), and part of this N is finally deposited in natural environments such as forest. Bulk and throughfall deposition are regularly measured in European forests at sites of the Intensive Monitoring Plots of ICP Forests (International Co-operative Programme on Assessment and Monitoring of Air Pollution Effects on Forests). Throughfall deposition is more relevant for lichens than bulk open field deposition as it is measured inside the forest. In European forests, yearly throughfall N–NO_3 deposition ranged from 0.2 to 16.9 kg ha^{-1} yr^{-1} (mean 2000–2005). Plots with the highest throughfall values (6.3–16.9 kg ha^{-1} yr^{-1}) are largely situated in areas with high vehicle exhaust (i.e., central Germany, Belgium and northern Italy), while plots with lowest throughfall (0.2–1.8 kg ha^{-1} yr^{-1}) are located mainly in northern Europe and in the Alps (Lorenz and Granke, 2009). Throughfall N—NH_4 deposition shows a similar spatial pattern, with some plots receiving inputs of up to 23.8 kg ha^{-1} yr^{-1}. Therefore, comparing these values with the above mentioned CLOs, changes in lichen communities may be expected.

In the present paper, a dataset of lichen samplings from ICP Forests intensive monitoring (Level II) plots from 10 countries, in combination with measurements of N deposition, has been analyzed with the following main objectives:

(1) To quantify the independent effects of nitrogen pollutants on selected lichen functional groups against other environmental predictors.
(2) To establish nitrogen critical loads for European forests, based on the relative diversity of functional groups of epiphytic lichens.

2. Methods

2.1. Sampling design, plot allocation

The plots were selected by the participating countries of the ForestBIOTA project (Forest Biodiversity Test Phase Assessments). The set-up of the ForestBIOTA plots was based on existing intensive monitoring (Level II) plots of ICP Forests (cf. de Vries et al., 2003). Eleven countries assessed epiphytic lichens on 83 plots in 10 European countries (see Fig. 1). More general information on the study area and the overall sampling design are described by Fischer et al. (2009) and follow Stofer et al. (2012).

Epiphytic lichen assessments were carried out at living trees with a minimum circumference at breast height of 50 cm within the 0.25 ha ForestBIOTA plots. Sample trees were pre-stratified by means of existing ICP Forests data into four groups according to the acidity of the bark (acidic bark/more or less neutral bark) and stem diameter (DBH $\leqslant$ 36 cm/DBH > 36 cm). Then, twelve trees were randomly selected considering the proportion of the four groups on the plot. If groups were represented by less than three trees, additional trees were randomly selected within these groups in order to get a more robust estimation of the epiphytic lichen diversity until at least three trees per group had been chosen. A detailed description of the pre-stratification is included in the ForestBIOTA methodology of lichen assessment (Stofer et al., 2003).

2.2. Lichen sampling

At each of the selected trees four frequency ladders – each of them with a total sampling area of 10 $\times$ 50 cm – were set up at the four directions of the compass. All lichenized fungi, except parasites, with a minimum size of 5 mm occurring inside the ladders were listed and their frequency (number of 10 $\times$ 10 cm units in which they occurred) was recorded. Branches growing inside the ladder were not considered. However, if stems were typically densely branched in the parts where the grid should be placed (e.g. on spruce) also the lichens growing on branches were considered. In such cases, lichens growing on branches were projected onto the surface of the frequency ladder.

In the case of unclear species identifications in the field, specimens for further identification in the laboratory (microscopy and thin-layer chromatography) were collected. Fieldwork and lichen determination was carried out by lichenologists of the participating countries. Specimens of the genus *Bacidina*, *Megalaria*, *Melaspilea*, *Mycomicrothelia* and *Thelopsis* could only be determined at the genus level.