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# Impact of nitrogen pollution disturbances on forest vegetation and fungi near a fertilizer factory

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## Abstract

The study compared the reaction of different organism groups to nitrogen pollution near a nitrogen factory. Investigations were conducted in an area affected by a nitrogen fertilizer factory (JSC Achema), in central Lithuania. The study was performed in eight permanent plots established for monitoring purposes at different distances (from 2 to 20 km) from the factory, mainly in a prevailing northeast wind direction. The vascular plants, bryophytes and fungi, including lichens, were recorded. The species number of the different organism groups reacted differently to the distance from the factory. The species number of lichens and fungi increased with increasing distance from the nitrogen factory. The number of species of vascular plants was highest near the factory. The number of bryophytes did not correlate with the distance from the factory. Nitrophilic species, *Chelidonium majus*, *Rubus idaeus*, *Sambucus rasemosa*, and *Impatiens parviflora*, were most common, especially near the factory. A majority of the macrolichens, including epixylic species, reacted extremely negatively to NO<sub>2</sub> and NH<sub>3</sub>, and species richness and abundance increased significantly farther from the air pollution source. Fungi did not react significantly to higher NH<sub>3</sub> or NO<sub>2</sub> concentrations in the air, and the distance from the factory was not an important variable.

*Key words:* herbs, mosses, nitrogen deposition, Scots pine, shrubs

## Introduction

Many ecosystems are exposed to extensive human impact which causes various environmental changes: deposition of harmful pollutants (Staszewski et al., 1998, Diekmann and Falkengren-Grerup 2002, Bokwa 2008), species extinction and introduction (Sala et al., 2000), increased soil erosion (Fenn et al., 1988), and changes in plant communities (Chapin et al., 1986).

In previous decades significant changes in forests ecosystems were caused by air pollution, primarily increased ozone (O<sub>3</sub>), sulphur (S), and nitrogen (N) oxides concentration (Bytnerowicz, 1996; Matyssek and Sandermann, 2003; De Vries et al., 2003; Emberson et al., 2007; Rizvi et al., 2012; Augustaitis et al., 2010; Augustaitis 2021).

Different organism groups react differently to air pollution. Lichens and mites are very sensitive to sulphur dioxide (Thormann, 2006; Seniczak et al., 2007), plants react to surface ozone concentrations (Felzer et al., 2008), herbaceous vegetation, dwarf shrubs, and phytophagy insects are impacted by nitrogen (Marozas et al., 2008; Sujatovienė, 2006; Throop and Lerda, 2004). In polluted forest ecosystems, species composition changes and some species become extinct (Lovett et al., 2009). Therefore, invertebrate species are the most abundant organism group in forest ecosystems and respond to various environmental factors (Führer, 1985; Xu et al., 2009; Vasiliu Oromulu et al., 2011). Increased nitrogen can increase the amount of biomass and change the

relationship between plants and insects (Mattson, 1980; Aber et al., 1989; Leith et al., 1999).

Lichens are recognized as being very sensitive to air pollution. Air pollution is one of several factors explaining the distribution of many lichen species. In general, the effect of nitrogen oxides on lichens remains poorly understood (Van Herk, 2001). Some authors reported the decline of epiphytic lichen vegetation in areas characterized by high NO<sub>x</sub> concentrations (Van Dobbenn et al., 2001). On the other hand, they found an increase in nitrophytic species, suggesting that many lichen species readily assimilate to oxidized nitrogen, and it fosters species indicative of eutrophicated areas (Davies et al., 2002).

During the last two decades, there has been a growing interest in air pollution and its effect on vegetation (Bytnerowicz et al., 2006; Danielewska et al., 2013). Within the last three decades, researchers have identified various effects of nitrogen pollution for tree species, especially conifers, and to a lesser extent deciduous forests (Pearson and Stewart, 1993; Fangmeier et al., 1994; Krupa, 2003).

Excess nitrogen may cause various changes in the understory vegetation. For herbaceous plants, such alterations were documented in studies of conifers (Van Dobbenn, 1993; Kellner and Red Bo-Turstensson, 1995; Nordin et al., 1998; Allen et al., 2007) and deciduous forests (Falkengren-Grerup, 1993, 1995, 1998). Increased nitrogen rates have been shown to cause

changes in species composition and decreased species diversity (Bobbink et al., 1998; Sujetoviene, 2006).

Until now, data concerning the effect of nitrogen on various species or organisms have been contradictory. Some studies provide evidence that expressed nitrogen affects different groups, and others do not find any impact.

The acute adverse effects on Lithuanian forests attributable to air pollution were observed near the JSC *Achema* nitrogen fertilizer factory, established in 1965. The effects of its harmful pollutants (NO<sub>x</sub>, NH<sub>3</sub>, SO<sub>2</sub>, and dust) were observed a decade later. In the 1970s and 1980s nitrogen deposition reached 120 kg N ha<sup>-1</sup> yr<sup>-1</sup>. The first dead pine stands were observed in 1972, and by 1976 they covered 6 ha. After 15 years, the zone of damaged coniferous stands had expanded 20–25 km northeast from the factory in the direction of prevailing winds and totalled approximately 500 ha (Armolaitis, 1998). The total area of dead stands (mostly Scots pine and Norway spruce) in 1988 comprised almost 1000 ha. Due to reduced production of the fertilizers since 1990 air pollution has significantly decreased. Technological improvements to the factory in 1997 increased production while significantly reducing pollution, and it is now kept at a stable level.

Researchers conducted various studies in the JSC *Achema* nitrogen factory zone of influence: tree defoliation (Armolaitis and Stakėnas, 2001; Augustaitis et al., 2005); tree radial increment (Juknys et al., 2002, 2003; Stravinskienė, 2004; Stravinskienė et al., 2013, 2014), needles and epiphytic microflora (Kupčinskienė 1999, 2001, 2006; Kupčinskienė et al., 2000), ground vegetation (Armolaitis and Stakėnas, 2001; Sujetoviene, 2006; Marozas et al., 2008), and soil chemical properties (Armolaitis, 1998; Armolaitis and Stakėnas, 2001).

However, a complex evaluation of different species groups (vascular plants, bryophytes, lichens, and fungi) was missing. This study aimed to compare the reaction of different organism groups to the nitrogen pollution near the factory.

## Material and methods

### Site description

Investigations were conducted in the area affected by the nitrogen fertilizer factory (JSC *Achema*) in central Lithuania (55° 05' N, 24° 20' E). A mixed forest of birch and Scots pine covers about 30% of the land in this zone. Between 2000 to 2020 annual total emissions from the factory ranged between 1.9 - 6.9 (10<sup>6</sup> kg yr<sup>-1</sup>): NH<sub>3</sub> – 0.2-0.5 10<sup>6</sup> kg yr<sup>-1</sup>, NO<sub>x</sub> – 0.4-0.7 10<sup>6</sup> kg yr<sup>-1</sup>, and SO<sub>2</sub> – 0.02-0.17 10<sup>6</sup> kg yr<sup>-1</sup> comprised the total emissions (Ministry of Environment of Lithuania). In 1996, nitrogen deposition along a selected transect from the nitrogen fertilizer factory ranged between 48 and 17 kg ha<sup>-1</sup> yr<sup>-1</sup> (Armolaitis, 1998), while recently only small differences in deposition (16-6 kg ha<sup>-1</sup> yr<sup>-1</sup>) were recorded along the transect.

The study was performed in eight permanent plots established for monitoring purposes at different distances (from 2 to 20 km) from the factory, mainly in the prevailing northeast wind direction. The Scots pine (*Pinus sylvestris* L.) stands were 80-100 years old of the *Pinetum vaccinio-myrtillosum* type (Table 1).

At each site, twelve 100 m<sup>2</sup> plots were established. Species composition and projection cover (expressed in %) of herbs and mosses were evaluated (Jongman et al., 1995). Vegetation sampling was conducted from June to August. The overall cover of each herb and moss species was calculated in the separate sites by averaging all subplot data for projection cover, and the total area covered. Nomenclature for vascular plants and mosses follows Jankeviciene (1999).

Macrolichens (both fruticose and foliose) and polypores fungi samples were taken. The nomenclature of polypores fungi followed Niemela (1996) and Motiejūnaitė (2002) was used for macrolichens. Species were studied growing on all substrata types, including the ground. A 4-point scale (1: least abundant, to 4: very abundant) was used to estimate the abundance of lichens and fungi in a plot.

**Table 1.** Characteristics of Scots pine stands along transect from fertilizer factory

Stand Characteristics	Distance from factory (km)							
	3	3	5	5	9	9	20	20
Forest type	<i>Vaccinio-myrtillo-Pinetum</i>	<i>Vaccinio-myrtillo-Pinetum</i>	<i>Vaccinio-myrtillo-Pinetum</i>	<i>Vaccinio-myrtillo-Pinetum</i>	<i>Vaccinio-myrtillo-Pinetum</i>	<i>Vaccinio-myrtillo-Pinetum</i>	<i>Vaccinio-myrtillo-Pinetum</i>	<i>Vaccinio-myrtillo-Pinetum</i>
Site class	Nb	Nb	Nb	Nb	Nb	Nb	Nb	Nb
Stand age	80-100	80-100	80-100	80-100	80-100	80-100	80-100	80-100
Mean 5 years concentrations of NO <sub>2</sub> µg m <sup>-3</sup>	33,9±2,4	33,9±2,4	12,6±1,3	12,6±1,3	8,4±0,2	8,4±0,2	8,2±1,0	8,2±1,0
Mean 5 years concentrations NH <sub>4</sub> µg m <sup>-3</sup>	76,9±51,3	76,9±51,3	33,1±14,1	33,1±14,1	2,1±0,3	2,1±0,3	3,2±1,2	3,2±1,2

Nb – normal humidity, poor fertility forest sites.

Data analyses

We calculated two quantitative species evaluation indices per plot: species richness (mean number of species) and abundance (mean number of specimens of a species found on all substrata units in the plot). To evaluate the species richness of a sample, we used the term “total species richness” and for the abundance of a sample “total abundance” was used.

Data were analysed using a constrained ordination to detect patterns in species assemblages related to environmental variation (Jongman et al., 1995). Initially, Detrended Correspondence Analysis (DCA) was applied on species data to obtain gradient lengths in standard deviation units of species turnover (Ter Braak and Šmilauer, 2004). These results were used to determine whether to use a linear or unimodal model for the data. The results of the DCA indicated that a Redundancy Analysis (RDA) should be used for our data. The environmental data selected for comparison were: distance from pollution sources (Dist), ammonium emissions (NH<sub>3</sub>), nitrogen dioxide emissions (NO<sub>2</sub>), soil pH<sub>KCl</sub> 0-5 cm (5\_pH\_KCl), soil carbon (5\_C), soil nitrogen 0-5 cm (5\_N), soil P<sub>2</sub>O<sub>5</sub> 0-5 cm (5\_P<sub>2</sub>O<sub>5</sub>), soil K<sub>2</sub>O 0-5 cm (5\_K<sub>2</sub>O), cover of first tree layer (Cover1a), cover of spruce second layer (Pic.ab2a), global site factor (GSF), indirect site factor (ISF), direct site factor (DSF). Significance of RDA was tested using the distribution-free Monte Carlo test (999 permutations). The CCA was carried out with CANOCO 4.5 (Ter Braak and Šmilauer, 2004).

Results

The species number of the different organism groups reacted differently to the distance from the factory (Figure 1).

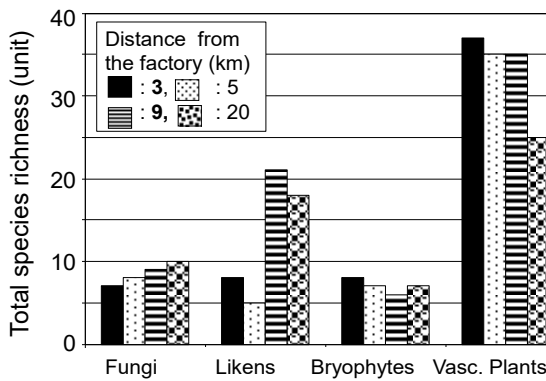


Figure 1. Total species richness on different distance from the “Achema” factory (3, 5, 9, 20 distance from the factory, km)

The species number of lichens and fungi increased with increasing distance from the nitrogen factory (Figure 2). The number of species of vascular plants was highest near the factory (Figure 1) and declined farther from the factory (Figure 3). The number of bryophytes did not correlate with distance from the factory. Slow growing species belonging to *Ericaceae* (*Vaccinium myrtillus*, *V. vitis-idaea*) and ferns were absent near the nitrogen factory. Nitrophilic species, especially

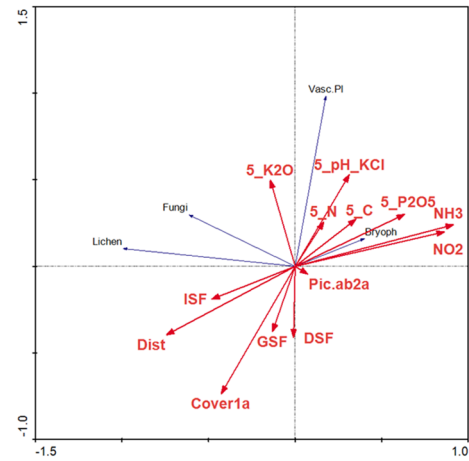


Figure 2. RDA of species number of different organism groups (Bryoh – bryophyte, Vasc.Pl – vascular plants; Dist - distance from pollution sources, NH<sub>3</sub> - ammonium emissions, NO<sub>2</sub> - nitrogen dioxide emissions, 5\_pH\_KCl - soil pH KCl 0-5 cm, 5\_C - soil carbon, 5\_N - soil nitrogen 0-5 cm, 5\_P<sub>2</sub>O<sub>5</sub> - soil P<sub>2</sub>O<sub>5</sub> 0-5 cm, 5\_K<sub>2</sub>O - soil K<sub>2</sub>O 0-5 cm, Cover1a - cover of first tree layer, Pic.ab2a - cover of spruce second layer, GSF - global site factor, ISF - indirect site factor, DSF - direct site factor).

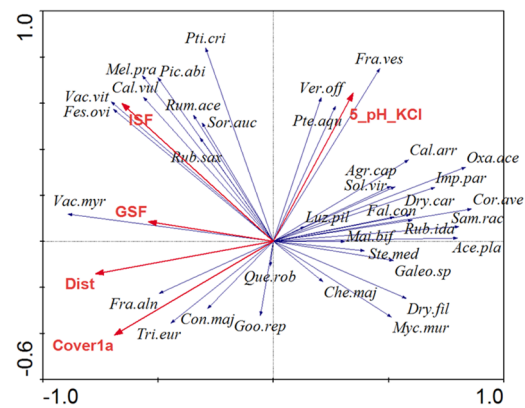


Figure 3. RDA of vascular plants (Dist - distance from pollution sources, 5\_pH\_KCL - soil pHKCl 0-5 cm, Cover1a - cover of first tree layer, GSF - global site factor, ISF - indirect site factor).

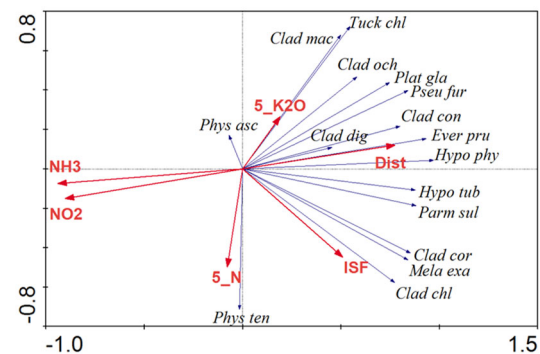
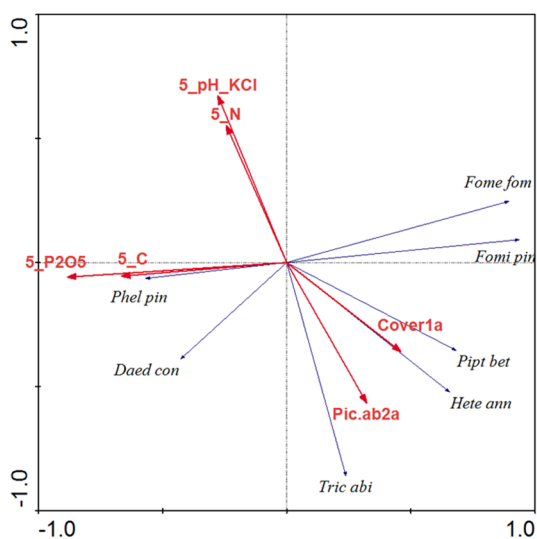


Figure 4. RDA of lichens (Dist - distance from pollution sources, NH<sub>3</sub> - ammonium emissions, NO<sub>2</sub> - nitrogen dioxide emissions, 5\_N - soil nitrogen 0-5 cm, 5\_K<sub>2</sub>O - soil K<sub>2</sub>O 0-5 cm, ISF - indirect site factor).

*Chelidonium majus*, *Rubus idaeus*, *Sambucus rasemosa*, and *Impatiens parviflora*, were most common, near the factory (Fig. 3).

Our studies on lichens showed that a vast majority of macro-lichens, including epixylic species, reacted extremely negatively to  $\text{NO}_2$  and  $\text{NH}_3$  in species richness and in abundance, increasing significantly, farther from the air pollution source (Figure 4). Only two species from the genus *Physcia* (*Ph. tenella* and *Ph. ascendens*) showed no apparent effects from nitrogen compounds of the air pollution.

Fungi did not react significantly to higher  $\text{NH}_3$  or  $\text{NO}_2$  concentrations in the air, and distance from the factory was not a critical variable (Fig. 5). More local factors, such as stand structure, were more important.



**Figure 5.** RDA of fungi (5\_pH\_KCl - soil pHKCl 0-5 cm, 5\_C - soil carbon, 5\_N - soil nitrogen 0-5 cm, 5\_P<sub>2</sub>O<sub>5</sub> - soil P<sub>2</sub>O<sub>5</sub> 0-5 cm, Cover1a - cover of first tree layer, Pic.ab2a - cover of spruce second layer).

## Discussion

Some studies provide evidence that expressed nitrogen effects vegetation, and other data shows that it doesn't. For example, nitrogen concentration in elk sedge (*Carex geyeri* Boott) increased following nitrogen application in a ponderosa pine (*Pinus ponderosa* Dougl.) stand (Wanderschaaf et al., 2003). However, in a loblolly pine plantation the effect of fertilization was not observed. Also, the addition of nitrogen did not affect N concentration of understory shrubs in *Pinus palustris* L. and *Pinus taeda* L. stands of South Carolina, USA (Wood and Tanner, 1985).

Vegetation studies in Great Britain along the transects from livestock buildings showed that species such as *Deschampsia flexuosa* (L.) Trin., *Holcus lanatus* L., *Rubus idaeus* L., and *Urtica dioica* L. were abundant closer to livestock buildings, and their percentage of cover decreased rapidly farther from the ammonia pollution source. More nitrogen sensitive species, such as *Oxalis acetosella* L., *Galium odoratum* (L.) Scop., mosses and ferns, were found some distance downwind from the buildings (Pitcairn et al., 1998, 2001; Skiba et

al., 1999). Similar changes in woodland ground flora have been described for Germany, Sweden, and the Netherlands (Hofmann et al., 1990; Falkengren-Grerup, 1993; Van Dobben, 1993). The greater effects of ammonia pollution for vegetation found in other countries could be explained by the higher (up to 2-3 times) gradients of ammonia concentrations compared to our sites.

In rural areas, the main effects of nitrogen deposition on lichens are changes in the communities (Van Dobben and De Bakker, 1996), a greater occurrence of nitrophytic species (De Bakker, 1989), often associated with a rise of bark pH (De Bakker 1989; Van Dobben and De Bakker 1996; Van Herk, 2001), a decrease in the biodiversity (Cepeda and Garcia Rowe, 1998; Van Dobben and Ter Braak, 1998), and increasing uptake of nitrogen near sources of pollution (Søchting, 1995).

Van Dobben and ter Braak (1998) found that  $\text{NO}_2$ , predominantly released by vehicles, was the second-most important factor explaining the distribution of lichen species, apart from  $\text{SO}_2$ . However, Cape et al. (2004) found that  $\text{NO}_2$  contributes to only 10% of the excess nitrogen and acidity deposition compared to  $\text{NH}_3$  at equal air concentrations.  $\text{NO}_2$  could only acidify phorophytes when present in very high concentrations (Krupa 2003). The possibility that increased nitrogen content, caused by  $\text{NO}_2$  and  $\text{NH}_3$ , influences lichen vegetation was already demonstrated by van Dobben and ter Braak (1998).

There is a strong relationship between ammonia air concentration and nitrophytic lichens. For the first time, it was observed that a decrease in nitrophytic lichens was related to a fall in the ammonia air concentration. Neutrophytes, i.e., many large macrolichens, have an optimum relationship with ammonia on acid-barked trees.

## Conclusions

The species number of the different organism groups reacted differently to the distance from the factory. The species number of lichens and fungi increased with increasing distance from the nitrogen factory, but the number of species of vascular plants was highest near it. The number of bryophytes did not correlate with the distance from the factory. Nitrophilic species were the most common, especially *Chelidonium majus*, *Rubus idaeus*, *Sambucus rasemosa*, and *Impatiens parviflora* near the factory.

The majority of macrolichens, including epixylic species, reacted extremely negatively to  $\text{NO}_2$  and  $\text{NH}_3$  in both species richness and abundance increasing significantly farther from the air pollution source. Higher  $\text{NH}_3$  and  $\text{NO}_2$  concentrations in the air did not significantly impact fungi, and the distance from the factory was not an important variable.

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