



# Impacts of nitrogen deposition on herbaceous ground flora and epiphytic foliose lichen species in southern Ontario hardwood forests



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## ABSTRACT

In this study 70 sugar maple (*Acer saccharum* Marsh.) dominated plots in Ontario, Canada were sampled in the spring of 2009 and 2010 and herbaceous plant and epiphytic foliose lichen species data were compared against modeled N and S deposition data, climate parameters and measured soil and plant/lichen S and N concentration. Herbaceous plant species richness was positively correlated with temperature and indices of diversity (Shannon Weiner and Simpson's Index) were positively correlated with soil pH but not N or S deposition or standardized foliar N scores. Herbaceous community composition was strongly controlled by traditional factors, but there was a small and significant influence of atmospheric S and N deposition. Epiphytic lichen species richness exhibited a strong negative relationship with standardized foliar N score and only one lichen species (*Phaeophyscia rubropulchra*) was observed at sites with a standardized foliar N score of 0.76.

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## 1. Introduction

Nitrogen (N) emissions have steadily increased in recent decades and global anthropogenic N deposition now exceeds that of natural N inputs (Galloway et al., 2008, 2004). Numerous experimental and gradient studies have shown that plants and lichens can be adversely impacted by elevated N (Stevens et al., 2004; Diekmann and Falkengren-Grerup, 1998; Glavich and Geiser, 2008), such that there are reductions in species richness, ultimately favouring nitrophilic species under conditions of high N exposure (Diekmann and Falkengren-Grerup, 1998; Tilman, 1987; Ruisi et al., 2005). Because lichens derive their nutrients directly from the atmosphere they have been suggested as perhaps the most sensitive biological organisms to increased atmospheric pollution, making them excellent bio-indicators of air quality (van Dobben and Ter Braak, 1999; Salo et al., 2012). Most studies that have documented adverse impacts of N deposition on herbaceous plant communities have occurred in Europe where deposition may exceed 40 kg N ha<sup>-1</sup> yr<sup>-1</sup> (Stevens et al., 2010; Diekmann and Falkengren-Grerup, 1998). However, studies in western North America have suggested that impacts on lichen communities may

occur above or between 3 and 9 kg N ha<sup>-1</sup> yr<sup>-1</sup> (Geiser et al., 2010; Fenn et al., 2008).

Nitrogen critical loads have been determined for a variety of ecosystems in Europe (Bobbink et al., 2010, 2002) and empirical critical loads for nutrient N are between 10 and 15 kg N ha<sup>-1</sup> yr<sup>-1</sup> for temperate and boreal forest ecosystems (Bobbink et al., 2010). Critical loads for N may be lower: an empirical critical load has been reported for North America by Fenn et al. (2008) as low as 3.1 kg N ha<sup>-1</sup> yr<sup>-1</sup> to protect sensitive lichens in California, USA. In another study in the USA, empirical critical load recommendation of 4 kg N ha<sup>-1</sup> yr<sup>-1</sup> has been suggested for the Rocky Mountains of Colorado, which would reduce the impacts of lake acidification (Williamson and Tonnessen, 2000). In addition, Giordani et al. (2014) reported that the critical load for N (measured in through-fall) for lichens in central Europe is 2.4 kg ha<sup>-1</sup> yr<sup>-1</sup>.

Modelled N deposition values available from Environment Canada indicate that N deposition varies between about 8 and 13 kg N ha<sup>-1</sup> yr<sup>-1</sup> in southern Ontario (Ro and Vet, 2002; Vet and Shaw, 2004), although measured values indicate that N deposition in some areas may approach and possibly exceed 20 kg N ha<sup>-1</sup> yr<sup>-1</sup> (Aherne and Posch, 2013) and are therefore within the range where adverse impacts on forest ecosystems may be expected. Previous gradient studies in grasslands have documented a pronounced decline in species richness with increasing N deposition (Stevens et al., 2004; Dupra et al., 2010). Similar studies

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in European forests have shown that N may also be impacting plant communities but other environmental variables can also have a strong impact on plant community composition (Falkengren-Grerup and Schottelndreier, 2004; van Dobben and de Vries, 2010).

In Canada there are limited, if any, studies, that have attempted to relate plant community composition and species richness to N exposure. Interestingly, recent work in the United States has suggested that over 200 million hectares (90.5% of the area) of eastern forests received N deposition in excess of the critical load when a low value is used ( $5 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ ) with considerable projected loss of herbaceous plant diversity (Clark et al., 2013). However, using a higher critical load value ( $17.5 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ ) the projected impacts of N deposition on eastern forests were minimal (Clark et al., 2013). Clearly, much uncertainty remains regarding the potential impact of N deposition on eastern North American forests. The objective of the present study is to provide further insights regarding the relationships between N deposition and plant and lichen communities in sugar maple (*Acer saccharum* Marsh) dominated forests in southern Ontario, using 70 Ontario Forest Biomonitoring Network (OFBN) plots. We hypothesized that plant herbaceous communities would be most strongly influenced by traditional ecological climate and soil variables, but that S and N deposition will have a detectable impact on plant communities. In contrast, we expected epiphytic lichen communities to be more sensitive to S and N deposition.

## 2. Methods and materials

### 2.1. Study sites

Seventy OFBN sites were selected for this study (Fig. 1). The OFBN sites are part of a large integrated monitoring program developed by the Ontario Ministry of the Environment (M.O.E) in 1985 (McLaughlin et al., 2000). The 70 sites selected for this study encompass a pollution (N and S deposition) and climate gradient, with mean annual temperatures between 3 and 9 °C (30 year mean) and are dominated (>75% basal area) by sugar maple. The 70 sites ranged in elevation between 81 and 507 m above sea level and mean annual precipitation ranged between 820 mm and 1090 mm (30 year mean). Soil textures differ slightly between sites consisting of loams, loamy sands, sands, sandy loams, silt loams and silty sands; clay soils are not usually encountered. The northern study sites overlay the Precambrian Shield and typically have acidic soil with very low base cation weathering rates (Koseva et al., 2010). Sites in the south overlay limestone bedrock and are much less acidic (Miller and Watmough, 2009).

### 2.2. Deposition data

The most recent available (2002) modeled (wet plus dry) N and S deposition, mean temperature (30 year), and mean precipitation (30 year) data were obtained from Environment Canada (Ro and Vet, 2002; Shaw and Vet, 2007) and were interpolated on a  $5 \times 5 \text{ km}$  grid. OFBN sites that were located within a specific modeled  $5 \times 5 \text{ km}$  grid were assigned the corresponding modeled N and S deposition value ( $\text{kg ha}^{-1} \text{ yr}^{-1}$ ). Similar to all other ecological studies that rely on modelled deposition fields, atmospheric contributions from local sources may not be captured with regional scale models, although this is less commonly evaluated. In a previous study (Watmough et al., 2014), we showed that springtime  $\text{NO}_2$  and  $\text{NH}_3$  concentrations of 17 OFBN plots varied considerably, but that concentrations could be well predicted by road density ( $R^2 = 0.79$  ( $\text{NO}_2$ ) and  $R^2 = 0.32$  ( $\text{NH}_3$ )). Furthermore concentrations of N in plant and lichen tissue at the OFBN sites were significantly correlated with modelled  $\text{NO}_2$  and  $\text{NH}_3$  concentrations derived from the road density models. Hence we

believe that the inclusion of plant chemistry as predictor variables is a good indicator of N exposure at the study sites.

### 2.3. Field sampling

Between May 7 – June 20, 2009, and May 1 and June 15, 2010, 30 and 40 OFBN sites respectively, were visited. At each site herbaceous ground vegetation, epiphytic foliose lichens and soil (mineral soil and forest floor) were sampled. Herbaceous ground vegetation in this study is defined as all forbs, ferns, graminoids, vines, and woody plants and woody vines <16 cm in height. Nomenclature follows that of Bradley (2010) for herbaceous plants and Brodo et al. (2001) for epiphytic foliose lichen species.

Herbaceous ground vegetation percent cover was quantified using 16,  $1 \text{ m}^2$  quadrats situated systematically 5 m outside the perimeter of the OFBN sites (four quadrats per site aspect) (Anderson and Eickmeier, 2000; Fraterrigo et al., 2009; Thimonier et al., 2010, 2011). A lichen ladder divided into five  $10 \text{ cm} \times 10 \text{ cm}$  (total  $50 \text{ cm L} \times 10 \text{ cm W}$ ) squares (Asta et al., 2002) was used to quantify the presence and absence of epiphytic foliose lichens (Miller and Watmough, 2009) on five randomly selected sugar maple trees >30 cm diameter at breast height (DBH, 130 cm above the forest floor) and <70 cm DBH outside the perimeter of each OFBN site. The top of the lichen ladder was placed on each aspect of the sugar maple trees 150 cm above the forest floor. Sugar maple trees were only selected if they were free of cankers, rot and extensive moss (Asta et al., 2002). Unknown epiphytic foliose lichens were collected and stored in steel containers for species identification in the laboratory. The boles of sugar maples found growing in this region are dominated by foliose and crustose lichens. Therefore, we focused our sampling efforts on foliose lichens as it is difficult to remove crustose species from their corresponding substrate (Asta et al., 2002). Samples (foliage and thalli) from the two most common lichen species (*Phaeophysica rubropulchra* and *Parmelia sulcata*), and the four most common herbs (*Maianthemum canadense*, *Maianthemum racemosum* ssp. *racemosum*, *Erythronium americanum* and *Trillium grandiflorum*) were sampled from multiple locations at each plot and were stored for a maximum of four days in a cooler, before being transported to the laboratory.

### 2.4. Soil collection

In 2010, one soil pit along each aspect of the OFBN plots (10 m from the edge of the plot) was excavated, for a total of four pits. Soil pits were  $900 \text{ cm}^2$  and 40 cm deep. Soil samples from each face of the soil pit were bulked separately for both the A and B horizons and placed in plastic sealable bags and stored in a cooler for a maximum of four days. Bulk density for both the A and B horizons were collected using cores with a fixed volume from the centre of their respective profiles. Average soil depth (cm) was measured for each of the A and B horizons to estimate total soil mass. The LFH layer was sampled by collecting an area of  $625 \text{ cm}^2$  to the top of the A horizon, and the mean LFH layer depth was recorded. Soil collection of 2009 OFBN sites follow that of Miller and Watmough (2009) and data were available for this study.

### 2.5. Laboratory analysis

#### 2.5.1. Epiphytic foliose lichen thallus and foliar carbon, nitrogen and sulphur analysis

Epiphytic foliose lichens and herbaceous plant foliage were dried for 72 h at 60 °C. Lichens and plant foliage were finely ground using an electric grinder and prepared for C, N and S analysis using an Elementar vario MACRO Analyzer. For every 25 samples analyzed, three reference samples (NIST Standard Reference

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