

Long term atmospheric nitrogen deposition stimulates enzyme activity in montane heathland

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Introduction

Atmospheric deposition of nitrogen (N) is a major component of global environmental change that poses a threat to natural ecosystems. It has been linked with many environmental problems such as soil acidification, eutrophication, changes in species composition and contamination of surface and underground waters (NEG-TAP 2001). These issues are thought to be particularly important in montane heathland ecosystems because they have adapted to low nutrient levels and extreme climatic conditions (Bobbink *et al.* 1998). Furthermore, montane heaths are dominated by slow-growing evergreen shrubs and often have high conservation value.

The maintenance of plant communities in extreme environments such as montane heaths is only possible as a result of the continual turnover of nutrients by soil microorganisms, and yet little is known about how deposition of N may affect the key functions that soil microbes undertake. In other semi-natural ecosystems, there is increasing evidence that deposition of N can affect both the composition of soil microbial populations and the key functions they undertake, especially in carbon and nutrient cycling.

Of particular importance to nutrient cycling is the production of a range of extracellular enzymes by soil microorganisms. These enzymes are common in soil and participate in the breakdown of organic matter providing simple forms of nutrients that plants and microorganisms can assimilate. In this study the activity of three enzymes - phosphomonoesterase (PME), β -glucosidase and β -xylosidase - is measured.

PME is an enzyme responsible for the release of inorganic phosphate from organic compounds produced during the later stages of organic matter breakdown. Its activity is very important for phosphorus (P) mineralization and cycling in soil systems. N deposition is thought to lead to greater P demand since more P is needed in order to keep the N:P ratio constant in both plants and microorganisms under conditions of N enrichment. PME has been used in the past as an indicator to assess increased P demand in soils (Johnson *et al.* 1998) and in bryophytes (Turner *et al.* 2001).

β -Glucosidase and β -xylosidase are two enzymes that catalyse the breakdown of cellulose and hemicellulose, respectively. Their activity, along with that of other enzymes, is important for determining the rate of organic matter decomposition and subsequent release of carbon dioxide as respiration. N enrichment is thought to increase the decomposition rate of labile cellulosic litter by making the C:N ratio more favorable for the decomposing microorganisms. Enhanced activity of cellulolytic enzymes under high N inputs has been observed, supporting this hypothesis (Sinsabaugh *et al.* 2005).

Experimental site and methods

The experimental site is situated on a plateau of the eastern Cairngorm Mountains in North East Scotland, at an altitude of c. 750 m above sea level. The prevailing climate in the area is sub-arctic (Langan & Soulsby 2001) and the average annual temperature c. 5 °C. The soil is a sub-alpine podzol and soil pH ranges from 3.9 to 4.6. A prostrate form of the ericaceous shrub *Calluna vulgaris* is the dominant plant at the site with other less frequent species being present, mainly *Empetrum nigrum* and *Arctostaphylos uva-ursi*, and several lichen species. The National Vegetation Classification (NVC) is H13 *Calluna vulgaris-Cladonia arbuscula* heath (Rodwell, 1992).

An N addition experiment has been running at this site since autumn 1999. The experimental set-up comprises factorial combinations of N addition, burning and grazing, which are applied to 96 1.5 m² plots in three replicate blocks. The plots are contained within metal cages to exclude herbivory. N is added as ammonium nitrate solution in six doses during the growing season (May-October) equivalent to 0, 10, 20 and 50 kg N ha⁻¹ y⁻¹. Also, grazing, burning and climate change simulation treatments are involved at this site but results are not presented here.

Heather litter was collected from within the plots in summer 2005, air-dried and placed in 500 µm nylon mesh litter bags. Litter bags were buried in the plots and collected from the field after a nine-month incubation period. The activity of the three enzymes, PME, β-glucosidase and β-xylosidase was determined.

Soil samples were collected in October 2005, June, August and September 2006 for the determination of PME activity and July 2006 and September 2006 for the determination of β-glucosidase and β-xylosidase activity. In this study the average values from the different time points are presented.

Colorimetric assays with p-nitrophenyl substrates were used. Soil or litter suspension was incubated with the substrate for 60 to 120 min at 5 °C, and then the produced p-nitrophenol was determined by measuring the optical density at 405 nm. The activity is expressed as nmol p-nitrophenol (p-NP) g⁻¹ dwt min⁻¹.

Results and discussion

Soil and litter PME activity increased significantly in response to N addition ($P < 0.001$) (Figure 1), supporting our hypothesis that increased N availability causes an increase in P demand, indicated by increased PME activity. The activity observed in litter samples was much higher, reaching ten times the value for the soil activity (973, 7 and 96.75 nmol p-NP g⁻¹ dwt min⁻¹, respectively). Litter contains greater concentrations of carbon and nutrients than soil, and so is likely to support a more active microbial population, which is reflected in the values obtained for litter and soil PME.

N addition had no effect on the activity of β-glucosidase and β-xylosidase in soil and litter for either of the time points (Figure 2). Nevertheless a trend of increasing enzyme activity with increased N additions is present. However, there is not enough evidence to support our hypothesis that enhanced N availability leads to increased enzyme activity. Further studies are required to investigate whether cellulose decomposition rates (and subsequent up-regulation of β-glucosidase and β-xylosidase) are not influenced by N addition or the enzymes chosen are not good indicators for decomposition rates in this system. The litter and organic matter of the

system is very high in lignin in this community and this also could be limiting the decomposition rates of cellulose.

Figure 1. Average soil and litter PME activity expressed as nmol p-nitrophenol produced per gram soil dry weight per minute. Error bars show the standard error of the mean (n=12). Columns sharing a letter are not significantly different ($P < 0.001$).

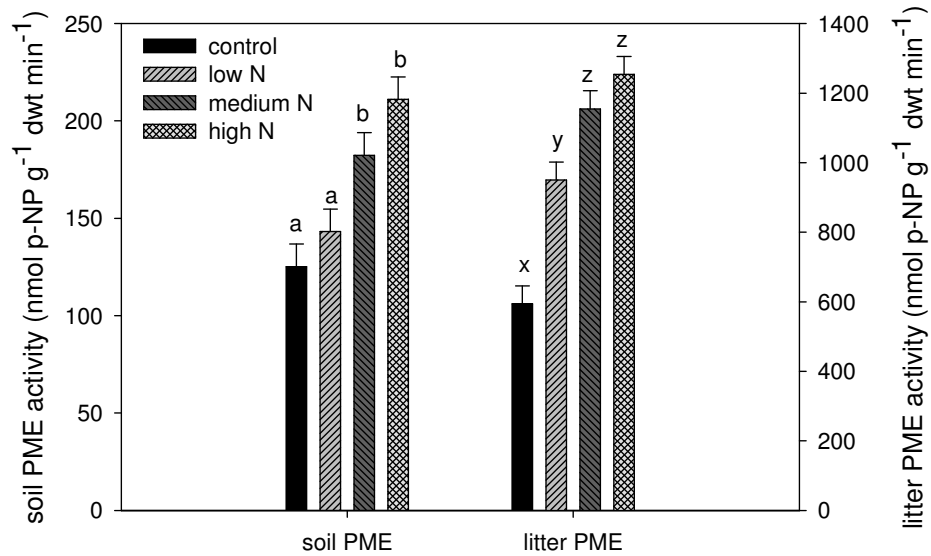
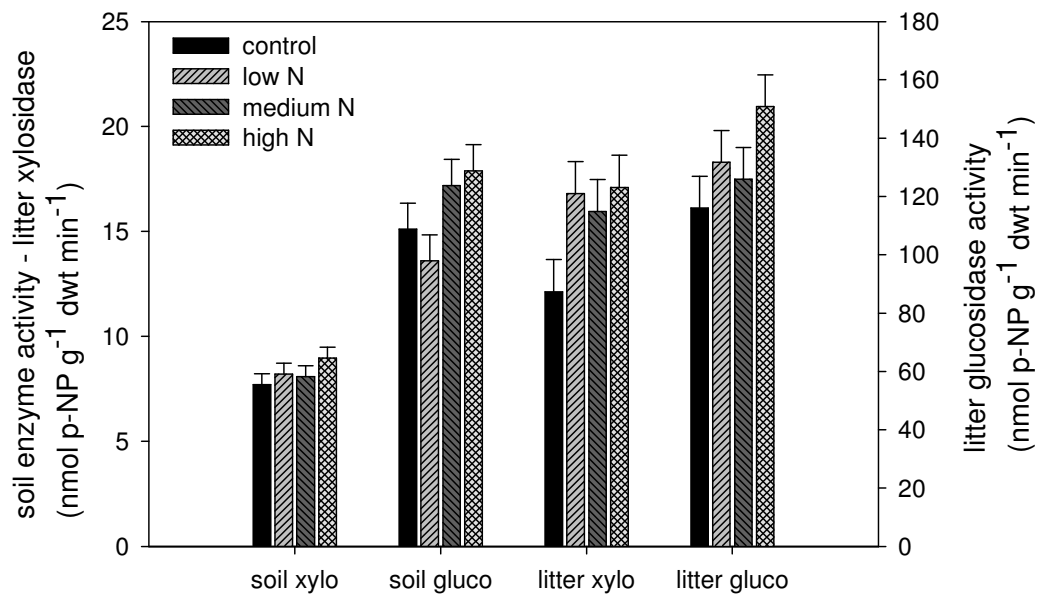


Figure 2. Average soil and litter activity of β -glucosidase and β -xylosidase expressed as nmol p-nitrophenol produced per gram soil dry weight per minute. Error bars show the standard error of the mean (n=12).



Conclusion

Our results suggest that N deposition stimulates the activity of a P mineralizing enzyme indicating a greater P demand and a change in P cycling. Further studies are required to understand the influence of N deposition on carbon cycling and litter decomposition.

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