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Impacts and indicators of nitrogen deposition in moorlands: Results from a national pollution gradient study

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ABSTRACT

Damage to terrestrial ecosystems from long-term atmospheric nitrogen pollution is a key conservation challenge in many industrialized countries. An important research and management priority is the identification of bioindicators to allow pollution exposure and ecological impacts to be determined at an individual site. We evaluate the impacts of nitrogen (N) deposition and identify methods with bioindication potential across a national-scale pollutant deposition gradient for British heather moorlands. Nitrogen deposition is associated with distinct changes in plant community structure, including reduced bryophyte and vascular plant species richness, and changes in the frequency of many species. Notable results include positive correlation with nitrogen for the invasive bryophyte *Campylopus introflexus* and negative correlation for the pollution-sensitive *Hylocomium splendens* and *Pleurozium schreberi*. Higher nitrogen deposition is associated with increased plant foliar N in a dwarf shrub and a bryophyte, increased extractable litter N, and reduced activity of the enzyme phenol oxidase. Although gradient study results cannot prove causation it is clear that Nitrogen deposition exerts a widespread impact on the ecology and biogeochemistry of heather moorlands. Bioindicators can be used to evaluate exposure and impacts, a promising approach could combine plant species richness and litter nitrogen analyses.

1. Introduction

Deposition of reactive forms of nitrogen (N e.g. NH_y, NO_x), primarily derived from intensive agriculture and fossil fuel combustion, is one of the key conservation challenges of the 21st century and affects an increasingly large proportion of global ecosystems (Galloway et al., 1995; Dentener et al., 2006). Nitrogen deposition is implicated in widespread loss of biodiversity and degradation of ecosystem services (Bobbink et al., 1998, 2010; Sala, 2000), which is a particular concern given rapidly increasing rates of N deposition in global biodiversity hotspots and protected areas (Phoenix et al., 2006; Bleeker et al., 2011).

A key semi-natural habitat of conservation concern in oceanic western Europe is heather moorlands. In Britain, these dwarf shrub and bryophyte-dominated ecosystems cover 2000,000 ha, but much of this area is considered threatened and a large proportion receives N deposition in excess of a critical load of 10–20 kg N ha^{-1} yr^{-1} (Thompson et al., 1995; RoTAP, 2012). Evidence for impacts of nitrogen deposition comes from many experimental studies (Cunha et al., 2002; Phoenix et al., 2012) and national ecological surveillance data (Maskell et al., 2010).

Given the threat posed by N deposition, a major topic of research and conservation interest has been the identification of bioindicators which can reveal impacts of, and exposure to, nitrogen pollution over a longer temporal-scale than is possible with individual atmospheric measurements (Sutton et al., 2004) and at a finer spatial scale than is possible with regional and national models of pollutant deposition (e.g. Smith et al., 2000). Bioindication may allow more accurate and precise determination of pollution deposition in complex terrain than deposition models (Sutton et al., 2004), as well as real-time monitoring of developing impacts. Because the UK has a large area of heather moorland and encompasses a wide pollution and climate gradient, it is ideal for identifying potential indicators of pollution.

Three distinct roles of pollutant bioindication are indicating the level of exposure (environmental indicators sensu McGeoch, 1998), the impacts of pollutants on species and ecosystems (ecological indicators sensu McGeoch, 1998) and relating impacts at one trophic level to others (biodiversity indicators sensu McGeoch,

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1998). Here we identify and test environmental and ecological indicators of nitrogen pollution in heather moorlands using a national gradient in nitrogen pollution. By simultaneously investigating a range of potential indicators in a variety of sites, we demonstrate the relative performance of alternative methods.

We consider four types of bioindicators based on plant community composition, plant foliar N, litter N and litter phenol oxidase activity. Several plant community metrics have been investigated in previous studies and shown to correlate with N deposition (Stevens et al., 2009); these methods have the advantage that assessments can be made in the field with minimal equipment. Plant tissue N concentration is the most widely investigated indicator of nitrogen deposition in terrestrial ecosystems (e.g. Innes, 1995; Hicks et al., 2000; Pitcairn et al., 1995, 2002, 2003, 2006; McNeil et al., 2007; Mitchell et al., 2004), with N-addition experiments (e.g. Gundersen et al., 1998; Carroll et al., 1999; Edmondson et al., 2010), gradient studies (e.g. Pitcairn et al., 1995, 1998, 2002; Hicks et al., 2000) and time-series (e.g. Baddeley et al., 1994; Woolgrove and Woodin, 1996) all demonstrating increased tissue N content with increasing N deposition. Similarly it is known that a large proportion of deposited N can be retained in the litter layer in heathlands (Pilkington et al., 2005), and previous studies have shown significant correlations between litter N and N deposition for moorlands in the UK (White et al., 1996; Edmondson et al., 2010). We also investigated phenol oxidase activity – a key enzyme in soil organic matter mineralization – following the finding of correlation with N deposition in a previous study (Edmondson et al., 2010).

2. Methods

2.1. Sites

Twenty two heather moorland sites in north Wales, northern England and eastern Scotland were investigated in June 2006 (Hollis and Perry (2004)Roberts (1986); Supplementary Fig. 1). All sites are classified under the UK National Vegetation Classification (NVC: Rodwell, 1991) as H12 *Calluna vulgaris*–*Vaccinium myrtillus* moorland, mostly in the late building phase (Supplementary Table 1). This community forms a large proportion of the total extent of managed moorlands in the British uplands and is normally maintained by controlled burning together with sheep and deer grazing. Sites were selected in regions of extensive *Calluna* moorland and were stratified to span the modelled nitrogen deposition gradient for the UK (5.9–32.8 kg N ha^{-1} yr^{-1}). While detailed information on long-term land management of the sites is not available the sites form a broadly representative cross-section of British heather moorlands (Supplementary Table 1).

2.2. Plant community

0.5 × 0.5 m quadrats were placed at five randomly-selected locations within each site. All species were recorded as either present or absent in each quadrat, giving each species a score from 0 to 5 for each site. This frequency index provides a measure of species abundance that is coarser than cover estimates, but is efficient and less subject to measurement error. We calculated species richness, Shannon and Simpson diversity and equitability (H', D, E_H, E_D) and unweighted mean Ellenberg *N* (soil fertility) and *R* (acidity) scores using the UK-adjusted values of Hill et al. (1999) for vascular plants and Hill et al. (2007) for bryophytes.

2.3. Plant tissue nitrogen

We consider the foliar N concentration of two species which have shown potential in previous studies and may represent N deposition over differing periods: the dwarf shrub *C. vulgaris* (the keystone species of this habitat), and the most frequently-occurring bryophyte, *Hypnum jutlandicum* (Pitcairn et al., 1995; Hicks et al., 2000; Kirkham, 2001; Sutton et al., 2004, Edmondson et al., 2010). In each vegetation quadrat a sample of *Calluna* shoots (apical 20 mm sections) and *H. jutlandicum* stems (complete length) were collected, stored under cool conditions and returned to the laboratory within two days of collection. Material was air-dried and three replicates of ground 50 mg sub-samples analyzed for total N using a LECO TruSpec CN.

2.4. Litter total nitrogen, ammonium and nitrate

Samples of approximately 25 cm^3 of all standing plant litter (predominantly *C. vulgaris* stems and scale-leaves) were extracted from the centre of each quadrat and analyzed for total nitrogen, KCl-extractable NH_4^+, and KCl-extractable NO_3^-. Total N was analyzed as above. For KCl-extractable NH_4^+ and NO_3^- 2 g of each sample was combined with 20 ml of 6% (0.81 M) KCl and shaken at 200 r.p.m. for 30 min (Allen, 1989). The extracts were filtered using Whatman Number 1 paper and analyzed using an Ion Chromatograph (DIONEX ICS-2000). Where sufficient material remained litter pH was measured in solution in distilled water.

2.5. Phenol oxidase activity

The method used for phenol oxidase analysis was adapted from Pind et al. (1994). 0.5 g of dried, ground litter was suspended in 9 ml distilled water and a 3 ml aliquot of the solution was added to 8.0 ml 10 mM dihydroxy phenylalanine (L-DOPA). The assay was incubated at 18 °C in a shaking water bath for 30 min and subsequently centrifuged for 5 min at 6000 r.p.m. The supernatant fluid was syringe filtered using a Whatman GF/C filter and optical density measured immediately at 460 nm in a spectrophotometer (CECIL CE1020, Cambridge, England). Phenol oxidase activity is expressed as μmol dicq (2,3-dihydroindole-5,6-quinone-2-carboxyate) g dry $weight^{-1}$ min^{-1}.

2.6. Data analysis

Linear regression was used to model the relationships between total inorganic nitrogen deposition and a range of potential indicator variables. We use national-scale modelled nitrogen deposition data on a 5 × 5 km grid from the Centre for Ecology's C-BED model (Smith et al., 2000), following the example of many previous studies (e.g. Stevens et al., 2004). On a national scale, nitrogen deposition is correlated with other environmental variables which may influence moorlands such as climate (N deposition is generally higher in warmer and wetter areas of the UK) and sulphur deposition (areas of high S deposition typically also have high N deposition due to similarity of sources). To account for such multicollinearity we applied stepwise regression which sequentially builds a regression model using those variables that can explain the most additional variability in the dependent variable, taking into account the other independent variables. We assembled data on a large number of additional environmental parameters including climate, air pollution, and local site information from national databases and site observations (Supplementary Table 2) and included all these variables in stepwise regressions. The period of the environmental data varies; we use the available data-set which most closely matches the period in which the sites were sampled. There is typically a high degree of correlation between datasets from different years and as we are interested in national-scale trends rather than absolute values small differences are unlikely to affect our general conclusions.