Original article

Impact of simulated nitrogen pollution on heathland microfauna, mesofauna and plants

Richard J. Payne [a,b,*], Astrid M. Thompson [c], Valerie Standen [c], Christopher D. Field [a], Simon J.M. Caporn [a]

[a] School of Science and the Environment, Manchester Metropolitan University, Chester Street, Manchester M1 5GD, UK
[b] Geography, School of Environment and Development, University of Manchester, Oxford Road, Manchester M13 9PL, UK
[c] School of Biological and Biomedical Sciences, Durham University, South Road, Durham DH1 3LE, UK

ARTICLE INFO

ABSTRACT

Deposition of reactive nitrogen derived from intensive agriculture and industrial processes is a major threat to biodiversity and ecosystem services around the world; however our knowledge of the impacts of nitrogen is restricted to a very limited range of organisms. Here we examine the response of groups of microfauna (testate amoebae), mesofauna (enchytraeid worms) and plants to ammonium nitrate application in the Ruabon heathland long-term experiment. Plant data showed significant differences between treatments, particularly characterised by a loss of bryophytes in nitrogen-treated plots, by contrast enchytraeids showed a non-significant increase in abundance in response to treatment. Testate amoebae showed no significant changes in abundance or inferred biomass but significant changes in community structure with a reduced abundance of Corythion dubium, interpreted as a response to the loss of bryophytes. Our results suggest that simple indices of plant community may have value for bioindication while the bioindication value of testate amoebae and enchytraeids is not clearly demonstrated.

1. Introduction

Since the first commercial application of the Haber-Bosch process in 1913 human production of reactive nitrogen (N_r) has grown rapidly, with an increase of over 120% since 1970 [1]. N_r deposition in the absence of human activity is generally less than around 0.5 kg N ha^{-1} yr^{-1}, while in the United Kingdom some areas currently receive deposition in excess of 40 kg N ha^{-1} yr^{-1}. These levels of nitrogen deposition are sufficient to lead to a significant reduction in biodiversity [2,3] and damage to ecosystem services. Species-loss from ecosystems is driven by both eutrophication and acidification with the relative contributions of these processes varying by habitat type [5].

Heathlands are a UK Biodiversity Action Plan priority habitat, covering over 2,000,000 ha of upland Britain but in England and Wales their cover declined by an estimated 27% between 1947 and 1980 [6]. A critical load range of 10–20 kg N ha^{-1} yr^{-1} is exceeded in many heathland areas of the British Isles with N deposition

shown to reduce plant biodiversity, particularly marked by a loss of lichens and bryophytes [7]. Large-scale ecological surveillance data shows a reduction in plant species richness along the N deposition gradient even when accounting for other drivers [4]. Impacts of nitrogen on groups of heathland organisms other than plants are however poorly documented. Here we examine the response of plants and major groups of eukaryotic microorganisms and meso-fauna in the same ecological experiment and consider the possible inter-relations between these groups. Our study aims to provide a broader understanding of the ecosystem-wide consequences of nitrogen pollution in heathlands and to identify possible bio-indication approaches.

1.1. The studied groups and their inter-relations

Testate amoebae are a group of eukaryotic microorganisms characterised by a solid shell (test) which can constitute a very large proportion of microbial biomass in organic soils [8] and are likely to have an important role in nutrient cycling [9,10]. Testate amoebae have been shown to respond to soil environmental changes to which other groups are insensitive [11] and have broad feeding preferences making them good synthesisers of overall microbial community change. Previous studies have demonstrated testate

amoeba sensitivity to nutrient enrichment [12–14] and have suggested impacts from NO_2 exposure [15].

The enchytraeidae are a group of detritovorous, bacterivorous and fungivorous annelid worms, typically 3–30 mm in length. Enchytraeids constitute a large proportion of mesofaunal biomass in many temperate soils (c. 75%: [16]) and may fill a keystone role in heathlands [17]. Enchytraeid abundance has been shown to respond to application of nitrogen fertiliser [18]. It seems possible that enchytraeids might predate testate amoebae given their size and observations of predation by other groups of worms [19 cited in 10]. Bacteria feeding on enchytraeid faeces are likely to provide a food supply for some testate amoebae and enchytraeid burrowing may aerate soil, modifying the amoeba's habitat and translocating individuals [cf. 20]. Enchytraeids may compete with testate amoeba species for food, for instance with members of the Centropyxidae for fungi [21–23].

Testate amoeba and enchytraeid communities are both intricately linked to plant communities with plants shaping the organism's physical, chemical and biotic environment. Precise mechanisms are difficult to pin-down but it is probable that for instance amoebae are affected by the chemical quality of plant litter [24], are closely linked to mycorrhizas [25] and are affected by changes in root exudation [e.g. 26]. As decomposers enchytraeids are highly sensitive to the quality of plant litter and experimental removal of different plant species has been shown to differentially modify enchytraeid abundance [27]. Both enchytraeids and testate amoebae are likely to be involved in nutrient mineralisation and thereby influence plant nutrition [28].

2. Site and methods

Experiments were first established on wet upland heath near Ruabon, Clwyd, North Wales (53° 02′N, 3°08′W; 470 m asl) in 1989 and have been extensively discussed in previous publications [29–33]. The climate of the site is cool and oceanic: average annual air temperature is 9.8 °C (2008–9 data), average annual soil temperature 6.9 °C (2008–9 data) and average annual precipitation 1053 mm (2007–2009 data). Vegetation of the site is dominated by Calluna vulgaris with subordinate bryophytes and scattered Vaccinium myrtillus. The site is representative of the Calluna-dominated heaths (NVC type H12: C. vulgaris–V. myrtillus heath [34]) which cover large areas of upland Britain. Soil is silty clay loam with pH around 4.4 and depth of around 50 cm. Ambient nitrogen deposition is around 19.9 kg N ha^{-1} yr^{-1} (UK Air Pollution Information System (APIS) www.apis.ac.uk), at the upper limit of the critical load range (10–20 kg N ha^{-1} yr^{-1}). The original experiments consisted of 1×1 m plots which were established in May 1989, subsequent experiments with 2×2 m plots were established in 1998. Nitrogen as ammonium nitrate is applied ten times a year to plots at concentrations of 0, 40, 80 and 120 kg N_r ha^{-1} yr^{-1} in the 1989-('old') plots and 0, 10, 20, 40 and 120 kg N_r ha^{-1} yr^{-1} in the 1998-('new') plots with four replicates for each concentration. The old plots were burned in 2000 in keeping with normal management practise [35].

For testate amoeba analysis samples were extracted from the control and heaviest treated (120 kg N ha^{-1} yr^{-1}, hereafter termed 120N) of the older (1989) plots in November 2009, more than 20 years after the onset of the treatment. Approximately 5 cm^3 of surface soil with any overlying litter and bryophytes were removed with a knife, sealed in plastic bags and refrigerated. In the laboratory testate amoebae were extracted using a method based on the standard methodology [36]. Sub-sample volume was measured by displacement in deionised water, samples were soaked for c.2 h and stirred to disaggregate. The majority of recent testate amoeba studies have been based on relative abundance data (for ease of

application to the palaeoecological record) however this approach may lead to loss of information [37]. Here we analyse both percentage and concentration data; an exotic Lycopodium clavatum innoculum of counted spores was added to samples to allow calculation of concentrations [38]. Suspensions were sieved at 300 μm but were not back-sieved to avoid loss of small taxa [39]. Samples were mounted in glycerol and a count of 100 individuals aimed for [40]. A variety of taxonomic guides were used [41–43]; the Euglypha rotunda, Centropyxis aerophila (=Centropyxis cassis) and Difflugis pristis types follow [41]. Tests with visible cytoplasm (termed 'live individuals') were recorded separately from empty shells (although it was not possible to distinguish living from simply undecayed individuals). Taxon-specific biovolumes were calculated based on assumed geometric shapes and published biometric data and converted to estimated biomass [8,14].

For enchytraeid analysis soil cores (50 mm diameter, 50 mm depth) were extracted from the 0, 20, 40 and 120 N_r treatments of the newer (1998) plots between May 2002 and September 2003. Three replicate cores were taken from each plot at six intervals over this period (May, July and September in 2002 and 2003) giving a total of 216 samples. Enchytraeids were extracted using the wet funnel technique [44] and identified following Nielsen and Christensen [45].

Changes in plant communities of these plots have been extensively considered over more than 20 years (Table 1). Here we focus solely on vascular plant species with bryophytes and lichens identified to functional types, a simple approach which may have considerable potential as a quick and effective bioindication strategy [cf. 46]. Our analysis updates the previous results of Carroll et al. [30] more than a decade after that study. A 15-point pin quadrat was placed in the centre of each of the old plots (4 replicates of 3 treatments + control) in summer 2005, recording all touches in four categories (C. vulgaris, V. myrtillus, bryophytes and lichens). Lichens were too rare for meaningful data analysis. Calluna canopy height was also measured at each pin point.

2.1. Data analysis

For the testate amoeba data Shannon (H) and Simpson (D) diversity indices, and related equitability measures (E_H, E_D) were calculated. A sequence of nested-ANOVAs were used to identify significant differences between treated and untreated plots for species richness, diversity and equitability, proportion of occupied tests (a measure of general community health) and amoeba concentration and biomass based on both all tests and only live

Table 1
Previous studies of plant response in Ruabon experiments. Showing only properties considered to have value for ecological indication with minimal resources (i.e. excluding properties requiring repeated site visits and chemical and physiological parameters).

Reference	Period	Plots	Response
[29,62]	1992	Old	Increased canopy height.
[63]	1995	Old	Increased canopy height.
			Increased C. vulgaris cover.
			Reduced bryophyte and lichen cover.
[30]	1995–1996	Old	Increased canopy height.
			Increased C. vulgaris cover.
			Reduced bryophyte and lichen cover.
[60]	1998–2002	New	Increased bryophyte cover, non-significant decrease in lichen cover (with 20 kg N ha^{-1} yr^{-1}).
[64]	2005	New	Decreased bryophyte cover. Decreased bryophyte diversity (Shannon (H)).
This study	2005	Old	Decreased bryophyte cover. Increased canopy height.