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Short communication

Interpreting nitrogen pollution thresholds for sensitive habitats: The importance of concentration versus dose

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Nitrate and ammonium concentration in wet deposition detrimentally impacted a sensitive pollution indicator species irrespective of the nitrogen dose.

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1. Introduction

Atmospheric nitrogen (N) pollution has caused severe alteration and loss of habitats across the globe (Jefferies and Maron, 1997; Vitousek et al., 1997). The most damaging effects occur in typically nutrient-poor ecosystems where the additional N can dramatically alter species composition, either directly through toxic effects and competition for N, or indirectly through enhanced levels of shading, herbivory and disease (Van der Eerden et al., 1991; Jonasson, 1992; Press et al., 1998; Strengbom et al., 2002; Pearce et al., 2003; Van der Wal et al., 2003, 2005). N pollution can similarly impact ecosystem function through influencing below-ground processes such as nutrient cycling, and lead to the loss of carbon from deeper soil layers (Zogg et al., 2000; Mack et al., 2004). Impacts from unremittingly elevated N pollution rates can extend beyond the directly affected plant-soil systems, and impinge on ecosystem services such as provision of clean drinking water, through enhanced concentrations of dissolved organic N and carbon in drainage waters from habitats saturated by atmospheric N inputs (Curtis et al., 2005). It is therefore vital that effective methods are in place to monitor and analyse detrimental impacts from atmospheric N pollution.

Many networks have been established across Europe to monitor N emission and deposition rates (for example, European Monitoring and Evaluation Programme). To link N deposition with its impact on habitats the concept of 'pollutant critical loads' has been developed to highlight areas where current N enrichment is likely to cause unacceptable habitat damage (Bobbink et al., 2002; Hall et al., 2003). Critical loads of N can be defined as the estimate of exposure to N deposition below which changes in ecosystem structure and function are limited (Hornung et al., 1995). The total N loading is typically expressed in kg N ha^{-1} yr^{-1} and also referred to as N-dose. As well as models of long-term soil changes, critical loads are also based on observed changes in the structure and function of ecosystems subject to a range of empirical loads of the pollutant, and are thus critically dependent upon experimental trials.

Studies manipulating N loads on semi-natural habitats have investigated atmospheric deposition impacts by applying inorganic N solutions specifically relevant to wet N deposition, which can form the dominant contribution to total anthropogenic N enrichment in a wide range of N-sensitive habitats (NEGTAP, 2001). To set habitat-specific thresholds for wet N deposition, the initial focus has been on N dose to express critical pollutant loads (Green and Ashmore, 1998; NEGTAP, 2001). However, there is growing awareness that unacceptable habitat change may not only be due to critical load exceedence, but can also derive from exposure to high N concentrations. Widely used annual maps of deposition dose and

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concentration fail to identify the frequency distribution of concentrations to which the vegetation is exposed and do not accommodate the typically episodic character of N deposition leading to the occurrence of high N concentration spikes (Fowler et al., 2004). The concentration of a pollutant in precipitation is largely determined by nucleation scavenging in which aerosols act as the condensation nucleus on which cloud droplets form and can range by a factor of 200 for ammonium and nitrate (NEGTAP, 2001). Concentration of N in the first rainfall after a prolonged period of low precipitation can therefore be several orders of magnitude higher than average, whilst the total N dose remains unaffected (Woodin, 1986; Pearce et al., 2003). Exposure to high N concentration pollution events can lead to a build-up of ammonium ions in plant tissue, potentially increasing acidity to toxic levels (Limpens and Berendse, 2003; Paulissen et al., 2004; van den Berg et al., 2005).

To date, the majority of investigations have reported on the effects of N dose rather than concentration, (for example, Chapin and Shaver, 1996; Carroll et al., 2000; Pearce and Van der Wal, 2002; Carroll et al., 2003; Fremstad et al., 2005) whilst using N concentrations that are well above the natural deposition range (from <0.01 mmol–2.0 mmol N in a severe pollution event, Fowler et al., 2004). The handful of studies that do consider the role played by realistic N concentrations do not compensate for the different N doses received (Van der Eerden et al., 1991; Paulissen et al., 2005; van den Berg et al., 2005). Although solution concentration alters with dose and vice versa, the studies do not separate out dose versus concentration effects, nor is this issue considered when data are interpreted. Therefore now, as habitat critical loads are progressed, there is an urgent need to better understand the relative importance N concentration and N dose play in driving detrimental change.

We report, for the first time, how both N dose and N concentration impact on a biological indicator species of N pollution. The moss *Racomitrium lanuginosum* was chosen as both glasshouse and field N addition studies have demonstrated it to be extremely sensitive to N loading (Soares and Pearson, 1995; Jones et al., 2002; Pearce and Van der Wal, 2002). Detrimental physiological effects such as decreased shoot growth, membrane damage and even shoot death have been observed at deposition rates as low as 20 kg N ha^{-1} yr^{-1} (Pearce et al., 2003). To determine the importance of N concentration relative to N dose, we determined the growth response of *Racomitrium* subject to a low background and an elevated dose of wet deposited N (10 and 20 kg N ha^{-1} yr^{-1}) applied as N solutions ranging from relatively low naturally occurring to high experimentally used concentrations (0.1–8 mmol N).

2. Methodology

Shoots of *Racomitrium lanuginosum* were collected from summit vegetation at two sites in the Central Highlands of Scotland: Glas Maol (1068 m a.s.l) and Morven (871 m a.s.l) for which total wet N deposition is estimated as 18 kg N ha^{-1} yr^{-1} and 12 kg N ha^{-1} yr^{-1} respectively. The shoots were packed at a natural density into 4 cm diameter pots, with granite chips in the base. Ten shoots were removed from each pot, cut to 4 cm lengths, loosely tied together with thread, and replaced into the centre of the pot. Pots were placed into a glasshouse maintained at 12 °C, kept moist by an automatic fogging facility (capture rate for pots equivalent to 5.17 ml s^{-1} m^{2}), and lit by halogen lamps for 12 h day^{-1}. A sheet of transparent spectral neutral filter (130 Clear; Lee filters, Andover) protected the moss from desiccation. Pots ($n = 154$) were divided over 7 replicate blocks, with 11 pots (one per treatment) from each site per block. Over a period of 12 weeks, all pots were automatically misted every 2 h with distilled water containing N at a concentration of 0.1 mmol and applied as NH_4NO_3. Pots from both sites were subjected to additional hand-held misting treatments with NH_4NO_3 solution at one of six N concentrations (0.1, 0.4, 1.0, 2.0, 4.0 and 8.0 mmol N), calculated together with the background misting to give two total doses equivalent to 10 and 20 kg N ha^{-1} yr^{-1}. For practical reasons, the 0.1 mmol N treatment could only be applied as 10 kg N ha^{-1} yr^{-1}. The number of hand-held applications varied (from 58 to 2) according to the concentration and dose required. The frequency of the background misting ensured the moss was constantly well hydrated, so the small amount of extra water received during hand-held applications was unlikely to impact significantly on the total water received. At the end of the study, the tagged shoots were re-measured to give a mean shoot extension for each treatment. Data were analysed using the REML mixed procedure in SAS version 8.2, with site, N dose and concentration as fixed effects. N concentration was log transformed to meet the assumptions of the model.

3. Results and discussion

Increasing N concentration negatively affected *Racomitrium* performance (Fig. 1), although the relationship was not linear, and the severe reduction in *Racomitrium* growth between 0.1 and 2.0 mmol N suggests that many pollution events are damaging to moss-dominated habitats. The failure of dose to significantly affect *Racomitrium* growth within the timescale of this study indicates that detrimental effects on N-sensitive species may not always be due to the N solution dose but its concentration. Indeed, a dose of 20 kg N ha^{-1} yr^{-1} resulted in marginally greater shoot extension than 10 kg N ha^{-1} yr^{-1} when applied at the same concentration, whilst at the highest

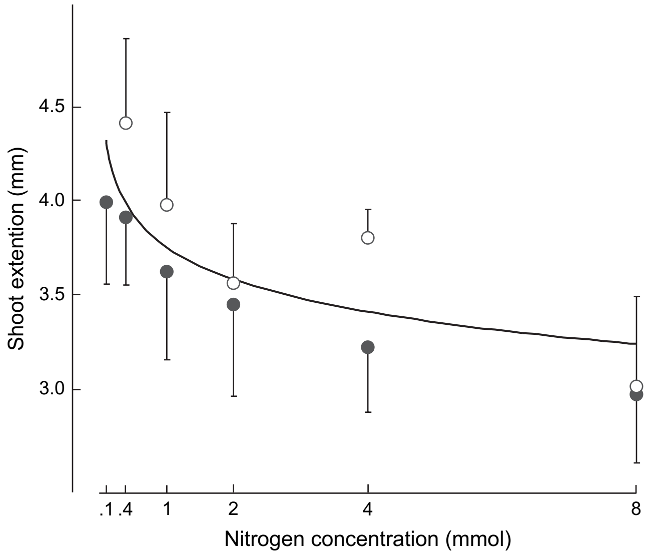

Fig. 1. Mean shoot extension for *Racomitrium lanuginosum* subjected to NH_4NO_3 solution at different concentrations, and receiving a total dose of either 10 kg N ha^{-1} yr^{-1} (closed circles) or 20 kg N ha^{-1} yr^{-1} (open circles) over 2 months. REML: dose ($F_{1,147} = 2.52$, $p = 0.11$), concentration ($F_{1,147} = 8.99$, $p = 0.003$), site ($F_{1,147} = 1.87$, $p = 0.23$). Relationship between concentration and growth: $y = -0.2368\,\mathrm{Ln}(x) + 3.752$, $R^2 = 0.53$.

N concentration of 8 mmol, growth was similarly compromised in both dose treatments. This investigation suggests that the concentrations applied in many N-addition studies may have confounded their results, and so an important aspect of pollution impacts often not considered.

It is possible that in some N-dose studies substantial changes in bryophyte cover occurred across all treatments due to the high N concentrations applied, thus limiting the potential for further impacts. In this trial N concentrations above 4 mmol had little additional negative impact on *Racomitrium* growth (Fig. 1). This suggests that where long-term N-addition studies applied concentrations above 4mmol (for example, Pearce and Van der Wal, 2002; Carroll et al., 2000) but still found increasing detrimental effects on bryophyte survival with increasing N addition, impacts may be due to the particularly high N doses applied as well as solution concentrations. However, where species are exposed to naturally occurring N-pollution spikes, or short-term experimentally raised N supply, solution concentration appears to be more important for species performance than dose, as evidenced by the severe reduction in *Racomitrium* growth subject to N concentrations up to 2 mmol.

The period of exposure to a pollutant is vitally important when evaluating critical levels. For example, the detrimental impact of increasing ammonium concentrations (ranging from 0.01 to 1.0 mmol) on the survival of acid-sensitive heathland plants increased with increasing exposure time (van den Berg et al., 2005). This negative impact over a realistic N-concentration range concurs with the sensitivity of *Racomitrium* found in this study. Therefore, although background N deposition in the UK is on average below 0.1 mmol (NEGTAP, 2001), the frequency of high N concentrations and pollution events in many areas is a cause for concern. Additionally, interaction with environmental stress factors such as herbivory (Van der Wal et al., 2003) and desiccation (Jones et al., 2002) can amplify the impact of N deposition. Although in this study the moss was kept constantly hydrated, naturally occurring periods of dry conditions allow vegetation surfaces to dry and may influence the fate of deposited N and its subsequent effects. Within and between year variation of stress factors such as periods of low rainfall volume or drought, that also lead to raised N concentration in wet deposition, are likely to increase the importance of deposition concentration over dose, especially for species that may be reliant on rainfall to ‘flush out’ protons in order to regulate cell acidity.

The majority of bryophytes are typically adapted to absorb atmospheric nutrients directly through leaf tissue and the lack of regulatory mechanisms leaves them vulnerable to high levels of tissue N accumulation. This makes bryophytes highly sensitive to atmospheric pollution (Lee et al., 1988), and suitable bioindicators of pollution loading (Pearce et al., 2003; Mitchell et al., 2005). In contrast to the direct toxic effects of N solutions on lower plants, vascular species are largely influenced by N additions through impacts on the soil environment. This is reflected in the typically more gradual response of vascular plants compared to bryophytes in N-addition studies (for example Lee and Caporn, 1998; Carroll et al., 2003). In this case N accumulation, and thus dose, rather than direct toxicity is likely to have the greatest influence on vascular species response. However, relative impacts will be species specific, with increasing ammonia concentration and a low pH more likely to be detrimental to acid-sensitive species (van den Berg et al., 2005). Therefore, plant functional type and the different regulatory mechanisms involved must be taken into account when considering the impacts of N dose relative to concentration.

This study demonstrates that the concentration of wet atmospheric N deposition plays a vital role in the detrimental impact on sensitive species and habitats, irrespective of the N dose. Our findings are in line with the suggestion that bryophyte tissue N is more sensitive to N concentration rather than deposition dose (Pitcairn et al., 2006), with tissue pH regulation potentially a key factor in this response. Taking into consideration the concentration of solutions applied in previous dose manipulation studies, along with the natural occurrence of high concentrations in pollution spikes, will have an important influence on our understanding and prediction of N impacts. It is therefore vital that we gain a better understanding of the role concentration and dose play in threatening the survival of sensitive species and their habitats, and that for effective pollution management, the concept of critical N loads is broadened to critical N thresholds in order to encompass the impacts of N concentration.

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