Slow recovery of High Arctic heath communities from nitrogen enrichment

Lorna E. Street[1], Nancy R. Burns[2], and Sarah J. Woodin[1]

1. I.B.E.S, University of Aberdeen, St Machar Drive, Aberdeen, AB24 3UU, UK
2. School of Geosciences, University of Edinburgh, Edinburgh, EH9 3JN

Author for correspondence: Sarah Woodin, Tel 01224 272688, s.woodin@abdn.ac.uk

Word counts:

- Main body of text: 7078
- Introduction: 1249
- Materials and Methods: 1563
- Results: 1849
- Discussion: 2292
- Acknowledgements: 125

Number of Figures: 7

- Colour figures: Figure 1, Figure 3

Number of Tables: 3

Summary

- Arctic ecosystems are strongly nutrient limited and exhibit dramatic responses to nitrogen (N) enrichment, the reversibility of which is unknown. This study uniquely assesses the potential for tundra heath to recover from N deposition and the influence of phosphorus (P) availability on recovery.
- We re-visited an experiment in Svalbard, established in 1991, in which N was applied at rates representing atmospheric N deposition in Europe (10 and 50 kg N ha^{-1} yr^{-1}; "low" and "high") for 3-8 years. We investigated whether significant effects on vegetation composition and ecosystem nutrient status persisted up to 18 years post-treatment.
- Although the tundra heath is no longer N saturated, N treatments effects persist and are strongly P-dependent. Vegetation was more resilient to N where no P was added, although shrub cover is still reduced in low N plots. Where P was also added (5 kg P ha^{-1} yr^{-1}), there are still effects of low N on community composition and nutrient dynamics. High N, with and without P, has many lasting impacts. Importantly, N+P caused dramatically increased moss abundance, which influences nutrient dynamics.
- Our key finding is that Arctic ecosystems are slow to recover from even small N inputs, particularly where P is not limiting.

200 words

Key words: bryophytes, critical load, nitrogen deposition, phosphorus, recovery, tundra, winter injury

Introduction

This study investigates the potential for high Arctic tundra to recover following reduction in atmospheric nitrogen (N) deposition. The potential for recovery, defined here as the reversal of physiological or ecological impacts, is a key component of our understanding of how ecosystems respond to external pressures. In the case of N deposition, this includes understanding the potential trajectories of change in response to changing trends in N deposition, and the ability to robustly quantify the degree of protection required to prevent significant, lasting effects.

Arctic ecosystems are typically strongly nutrient limited, and fertilisation experiments have shown that vegetation composition and function respond dramatically to increases in nutrient availability (Dormann & Woodin, 2002). High sensitivity to atmospheric N deposition would thus be expected, indicating a low "critical load" of N for Arctic habitats - the amount below which no significant harmful effects occur, according to current understanding. However, Arctic fertilisation experiments typically applied N in combination with phosphorus (P) or used high application rates (e.g. Robinson *et al.,* 1998; Bret-Harte *et al.,* 2008), and so did not provide a basis for quantifying the critical load. To address this, we established an experiment on Svalbard in 1991 with more realistic N treatments than used before on tundra: 10 kg N ha^{-1} y^{-1} representing the highest deposition rates known in the Arctic and 50 kg N ha^{-1} y^{-1} approximating the highest rates of deposition to analogous alpine heath in Europe (Gordon *et al.*, 2001). This study provided the basis for the empirical critical load of N for tundra to be set at 5-10 kg N ha^{-1} y^{-1} (Achermann & Bobbink, 2003); a subsequent experiment in Greenland (Arens *et al.*, 2008) led to revision to 3-5 kg N ha^{-1} y^{-1} (Bobbink & Hettelingh, 2010).

The quantification and mapping of empirical critical loads of N for European natural and semi-natural ecosystems provided the basis for international pollution control (UNECE, 1999; EU, 2001) as a result of which European emissions of nitrogen oxides and ammonia decreased by 44% and 25% respectively during 1990-2011 (www.eea.europa.eu). While the ecological effects of N deposition have been well documented (e.g. Bobbink *et al.* 2010; Phoenix *et al.* 2012), there have been few studies of the potential for recovery once N deposition is reduced. Of the studies undertaken, many have used experiments in which the initial N additions were at very high rates (e.g. Strengbom *et al.*, 2001; Nordin *et al.*, 2005; Strengbom & Nordin, 2008) and often in combination with other nutrients (e.g. Klaudisová *et*

al., 2009; Królovec *et al.*, 2009; Pavlů *et al.*, 2012). An additional constraint on most recovery studies is that the period of recovery is no longer than the period of N addition; notable exceptions are re-visitations, after decades, of fertilisation trials in boreal forest (Strengbom *et al.*, 2001; Nordin *et al.*, 2005; Strengbom & Nordin, 2008) and sub-alpine grassland (Spiegelberger *et al.*, 2006; Klaudisová *et al.*, 2009).

Notwithstanding these limitations, patterns of vegetation response to the cessation or reduction of N inputs have been demonstrated. Tissue N concentration in bryophytes has been shown to recover over as little as 1-2 years (Arróniz-Crespo *et al.*, 2008; Limpens & Heijmans, 2008; Mitchell *et al.*, 2004; Armitage *et al.*, 2011). After cessation of high rates of boreal forest fertilisation, amino acid N in feather moss was still elevated at 9 years, but recovered by c. 50 years (Nordin *et al.*, 2005). Vascular plant tissue chemistry may respond more slowly than bryophytes: tissue N recovery has been shown after 12-15 years in temperate grasslands (Clark *et al.*, 2009; Stevens *et al.*, 2012) but increased tissue N persisted after 22 years in boreal forest (*Picea, Vaccinium, Deschampsia*), by which time moss tissue N had recovered (Strengbom & Nordin, 2008).

The impacts of N addition on plant species composition or diversity can also be long-lived. Effects were still seen after recovery periods of c. 15-20 years in temperate grasslands (Królovec *et al.*, 2009; Stevens *et al.*, 2012; Isbell *et al.*, 2013) and boreal forest (Strengbom & Nordin, 2008). Findings from upland/alpine grasslands are, however, contradictory, with both the persistence of marginal influence of small N(PK) inputs after 70 years (Spiegelberger *et al.*, 2006) and rapid reversal of effects of large N(PK) inputs within 8 years (Pavlů *et al.*, 2012). Non-vascular plants are particularly sensitive to N deposition (Gordon *et al.*, 2001; Phoenix *et al.*, 2012) and are affected both directly, through physiological effects of increased tissue N, and indirectly, through increased shading by the vascular plant canopy (e.g. van der Wal et al. 2005). Given their slow growth rate, recovery of non-vascular plant abundance might be expected to be slow, and will depend on both the persistence of N recycling within the system and the legacy effects of N on the vascular plant community. For example, boreal forest moss and lichen species' abundance showed no recovery after 22 years (Strengbom & Nordin, 2008) and was still affected after c. 50 years, by which time the vascular plant community had recovered (Strengbom *et al.*, 2001).

Clearly the effects of N deposition may persist after reduction of N inputs and, just as initial impacts of N deposition differ between plant functional groups and between systems of different nutrient status, so will the rate of recovery. Whilst N is regarded as the most widely limiting nutrient, some nutrient-poor ecosystems - including tundra heath - are co-limited by P (e.g. Gordon et al. 2001). Indeed P limitation can be induced by N deposition (e.g. Britton & Fisher 2007), and the interaction between N and P availability determines the response to N. In tundra heath, P limitation constrained the effects of N on vegetation composition, which much were greater where P was also added (Gordon *et al.*, 2001; Madan *et al.*, 2007; Arens *et al*., 2008) and it has been suggested that P status should be taken into account when assigning a specific N critical load value to a particular site (Achermann & Bobbink, 2003). Given the strength of influence of P availability on the impacts of N deposition, we would also expect it to influence the potential for recovery. Yet, to our knowledge, only our experiment and one other, which thus far has only reported short term (22 month) recovery in temperate grassland (Arróniz-Crespo *et al.*, 2008), enable investigation of this.

To investigate the potential for Arctic tundra to recover from N deposition, we revisited our Svalbard experiment, established in 1991, in which two heath communities received N treatments in factorial combination with P, for 3 and 8 years. Here we document the recovery over almost two decades of plant species composition, the nutrient status and dynamics of the moss layer, and ecosystem N saturation. We assess the influence of P availability on the magnitude and persistence of the effects of deposited N, and explore the mechanisms by which historic N inputs may still be influencing the system. Due to the conservative nature of nutrient cycling in high Arctic tundra, we expect that the effects of N enrichment at rates well above the critical load will still be apparent on a time scale of decades, but that at application rates closer to the critical load there will be a greater degree of recovery. We also predict that non-vascular plant communities will recover more slowly than vascular plants, due to a combination of higher sensitivity to N and slower growth rates. Finally, we consider the implications of our findings for critical loads and for expectations of ecosystem responses to decreasing emissions of nitrogenous pollutants.

Materials and Methods

Site description

We utilised our previous nutrient addition experiment, established in 1991, approximately 1.5 km east of Ny-Ålesund, Svalbard (78° 54' 56" N 11° 58' 22" E) in two dwarf shrub heath types; one dominated by *Cassiope tetragona (*L.) D. Don., the other by *Dryas octopetala* L. Treatment plots (1.5 x 1.5 m) were selected to be representative, to contain the dominant shrub and >25 % vegetation cover, and treatments were allocated randomly. *Cassiope* plots were treated from 1991–1993 (with 60% treatment in 2000) and *Dryas* plots from 1991–1998; no further treatments have been applied since. Full details of the experiment are in Baddeley *et al.* (1994) and Gordon *et al*. (2001) and Table 1 provides a summary of the treatments applied, recovery periods and timing of measurements.

During the original experiment, nitrogen was applied as NH_4NH_3 in solution at application rates of 0, 10 (low N, LN) and 50 kg N ha^{-1} yr^{-1} (high N, HN), in factorial combination with P at 0 and 5 kg P ha^{-1} yr^{-1} as KH_2PO_4; thus there were six treatments and each was replicated five times on each heath type. Nutrient solutions were applied with a watering can, five times at two-weekly intervals, over the growing season. Background N deposition in precipitation is c. 0.74 kg N ha^{-1} yr^{-1}, with a small additional input (<10 %) from dry deposition, and has not shown any trend over time since this experiment was established (Kühnel *et al.*, 2011; Björkman *et al.*, 2013).

We re-visited the site during the growing season of 2011, 13 and 18 years after the initial treatment periods on the *Dryas* and *Cassiope* heaths respectively. The treatment plots were relocated using original plot maps and marker posts, most of which were still in place. To assess recovery, vegetation in plots which previously received fertiliser was compared with that in control plots. We consider the vegetation to have recovered when significant treatment effects on a parameter (e.g. plant tissue nutrient content) which were apparent during or shortly after treatment, are no longer detectable in 2011. For many parameters, qualitative comparison was also made with data from the end of the treatment period. Due to the different histories of the experiment on the two heath types (Table 1) no direct, formal comparison was made between them.

Community composition

In 2011 we used point intercept sampling to characterise community composition using a 10 cm grid point quadrat (196 points per plot). All plots were sampled during 9-21st July. A 'top hit' was recorded for the vascular canopy if present, then a 'bottom hit' for the ground layer (moss, lichen, bare ground etc.) We also present species cover data from previous years, which were obtained using a variety of methods, summarised in Table 2.

Plant nutrient content

On 9th July 2011 we sampled moss and vascular leaf tissue for nutrient analysis. We sampled *C. tetragona* and *Salix polaris Walenb.* leaves from *Cassiope* heath plots, *D. octopetala* leaves from *Dryas* heath plots, and the moss *Dicranum spadiceum* (J.E. Zetterst.) from all plots. We separated the top 5 mm of green moss tissue in the laboratory for analysis. Tissues were dried (65°C for 3 days) then milled using a ball-mill (Retch MM100). Sub-samples (100 mg) were digested using sulphuric acid/ hydrogen peroxide digestion (Allen, 1989) and analysed colorimetrically for total N and P using flow injection analysis (Foss Tecator FIAstar 5000).

We also present previously published tissue nutrient data from 1993 (Baddeley *et al.*, 1994) and 1998 (Gordon *et al.*, 2001) and unpublished data from 1996 and 2000, which were all obtained following the same methods. Samples were collected at a similar time in each year (between second week of July and first week of August).

Nitrate reductase activity

We measured moss nitrate reductase activity (NRA) as an indicator of N demand. On 9th July 2011 we collected samples of *Dicranum spadiceum* tissue for NRA assay following the methods of Gordon *et al.* (2001). We also present previously unpublished data for *D. spadiceum* from the *Cassiope* plots, which were sampled in 2000, 7 years post treatment (before application of the partial treatment in that year), and from the *Dryas* plots in their final year of treatment, 1998 (previously published in Gordon *et al.* 2001). Briefly, green tips of the moss shoots were fully hydrated and placed in a cool greenhouse or growth cabinet (depending on year), both with 24 h light, for at least 24 h prior to assay. Several shoots were used in each assay (c. 50-100 mg dry wt); these were vacuum infiltrated in 5 ml of 75 mM KNO_3 in 100 mM phosphate buffer (pH 7.5) with 0.75 % propan-1-ol, and incubated in the dark at 25 °C for 1 h. Nitrite concentration of the incubation medium was then determined

colorimetrically. For determination of inducible NRA the moss was sprayed, 4.5 h before assay, with 1 mM KNO_3.

^{15}N labelling

To quantify ecosystem N retention in 2011 we used ^{15}N stable isotope labelling, following a protocol used in earlier years of the experiment in which the ^{15}N was added as one of the series of treatment applications. *Cassiope* (all treatments) and *Dryas* (control (C), high N, high N+P) plots were labelled with $^{15}NH_4{}^{15}NO_3$. We randomly selected one 0.2 x 0.2 m area in each plot for labelling, avoiding previous sampling areas. In the *Cassiope* control plots we randomly selected two areas, one for a low N control (CL) and one for a high N control (CH). We took one 4.2 cm diameter core from each plot before treatments were applied to quantify background ^{15}N. Normal nutrient treatments were then applied on 8th July, i.e. one fifth of the annual treatment in 80 ml solution applied with a hand-held sprayer to each 0.2 x 0.2 m area. This was followed on 15th July by a second treatment containing 10 atom% excess ^{15}N. On 21st July a further normal treatment was applied so that any remaining ^{15}N would be rinsed from the leaves such that subsequent recovery would be through N uptake rather than adsorption. Further 4.2 cm diameter cores were taken on 27th July to quantify ^{15}N retention.

Sampled cores were separated into four pools: aboveground vascular plant material, moss and litter, organic soil, and mineral soil. All were dried at 65°C for 3 days. Leaf material was milled using a ball mill (Retsch MM100). Moss/litter and organic soils samples were pre-ground using a laboratory blender, then sub-sampled for ball-milling. Mineral soil samples were ground to a fine powder using a larger ball-mill (Retsch MM200).

We also report previously unpublished data from ^{15}N retention studies carried out by the same methods on the *Dryas* heath in 1996 (6th year of treatment; total retention only) and on on *Cassiope* heath in 2000 (7 years post treatment; retention in the four pools in control, HN, HN+P treatments only).

Statistics

To assess recovery, we test for statistically significant differences between plots which received nutrient treatment in the past, and control plots (which never received nutrient treatment). All analyses were carried out in R 3.0.0. To examine treatment effects on plant community composition in 2011, we performed a nonmetric multidimensional scaling

(nMDS) analysis of species composition (excluding non-living categories: bare ground, rock, litter) using the metaMDS function within the 'Vegan' package. Dissimilarities were calculated using the Bray-Curtis method, and vectors representing N and P addition rate were overlain onto the ordination. Significance values for the treatment vectors are based on 999 random permutations of the data (Oksanen *et al.*, 2012).

To test for treatment effects on % cover of individual plant functional groups (shrubs, lichens and bryophytes), bryophyte and shrub tissue nutrient concentrations and bryophyte tissue nitrate reductase activity we performed separate two-way ANOVAs for each time point. The decision to use separate two-way ANOVAs, rather than repeated measures (with two time-points), was based on the consideration that 1) measurements of plant abundance varied between years both in method and in sampling intensity (Table 1) making repeated measures analysis inappropriate, and 2) the effect of interest is the treatment effect in relation to the controls, not changes through time. It should be noted that technically the chances of type 1 error (i.e. detecting a significant difference where there is none) is increased by carrying out two separate analyses. *C. tetragona* cover in 2011 was negligible in HN+P, and zero in the HN treatments (see Fig. 3 later in the article) so we excluded the high N treatment level from the ANOVA models for 2011 to avoid violating assumptions due to zero-inflation. We instead used one-way ANOVA to test for differences in cover and tissue nutrient concentrations between treatments. Due to unbalanced design we analysed total recovery of ^{15}N using separate one way ANOVAs for each year/N application rate combination, and analysed the effect of nutrient treatment on the distribution of ^{15}N between pools (i.e. vascular plant, moss/litter, organic soil, mineral soil) using two-way ANOVA, again with a separate analysis for each year/N application rate. Tukey HSD tests were used for post-hoc pair wise means comparisons. Data were transformed (square root or reciprocal square root) where necessary to meet assumptions of normality and homogeneity of variance.

To allow comparison of all the measured variables on the same scale, we calculate the effect size (R) for each variable, at each time point as:

$$R = \frac{\text{treatment mean}}{\text{control mean}} \qquad (1)$$

Results

Community composition

In both *Cassiope* heath, 18 years after nutrient applications, and *Dryas* heath, 13 years post-treatment, plant community composition differed between treatments with a clear clustering of ordination scores by treatment in the nMDS analysis (Fig. 1). The effect of N was clearest in the *Cassiope* vegetation, but there were significant relationships ($P < 0.01$) between ordination score and N treatment rate for both heath types. Phosphorus treatment also influenced both *Dryas* and *Cassiope* heath composition (both $P < 0.01$). Differences in the direction of the N and P treatment vectors, particularly for *Cassiope* heath where the vectors were almost orthogonal, indicate differing effects of N and P on community composition.

The predominance of bryophyte species towards the nutrient enriched side of ordination space indicates that in general bryophytes have responded positively to nutrient addition, though some species responded most strongly to N (e.g. *Polytrichastrum alpinum* (Hedw.) G.L. Sm.), some to P (e.g. *Hylocomium splendens* (Hedw.) Schimp.) and some to both (e.g. *D. spadiceum)* (Fig. 1). These positive effects of nutrient addition on bryophyte cover have increased since the end of the treatments (Fig. 2a,b, Table 3). In 1998 bryophyte abundance in *Dryas* plots receiving both nutrients was approaching double that in control plots, but by 2011 the difference was threefold. The only vascular plant species to respond positively to nutrient addition was *Salix polaris* (Fig. 1), with c. 60 % increase in cover in *Cassiope* heath plots receiving high N (two-way ANOVA, $F_{2,24} = 5.5$, $P < 0.05$). Cover also increased in response to P and additive effects of high N+P resulted in a doubling in *S. polaris* cover, compared to control, in *Cassiope* heath in 2011 (Tukey's HSD, $P < 0.01$); responses in *Dryas* heath were similar but less pronounced (data not shown).

Most vascular plant species responded negatively to N addition, most notably the dominant shrubs. The high N treatment resulted in complete mortality of *C. tetragona*, whether or not P had also been added, and the low N treatment also resulted in a strong reduction in cover compared to the control (Fig. 3a). In high N plots there has been no re-establishment of *C. tetragona*. At the low N addition rate (with and without P) there is some indication of increased cover between 1995 and 2011, but it was still significantly lower than in control plots in 2011 (Tukey HSD, $P < 0.01$) (Table 3). There was also a reduction in cover, though not complete mortality, of *D. octopetala* in treated plots compared to controls during treatment particularly in high N+P treated plots (Fig. 3b). Whilst *D. octopetala* showed fairly

311 rapid recovery in subsequent years, there were significant effects of N on abundance in 2011
312 (two-way ANOVA, p = 0.019) and cover in the plots which had received high N+P was 41 %
313 of that in plots receiving no N (Fig. 3b, Table 3). Treatment effects were also still apparent
314 for *Saxifraga oppositifolia* L. on *Cassiope* heath (this species was infrequent on *Dryas* heath)
315 in 2011 (Fig. 1); in comparison to controls, cover was reduced by 80 % by high N treatment
316 (Tukey HSD, $P < 0.05$), and by 50 % by P (Tukey HSD, $P < 0.05$) with no NxP interaction
317 (two-way ANOVA, $F_{2,24} = 0.63$, p = 0.54) and there was no evidence of any recovery since
318 2000 (7 years post treatment; data not shown). The only species group which has recovered
319 from negative effects of N is the lichens, which are a component of *Dryas* heath. Total lichen
320 abundance did not differ between treatments in 2011 (Fig. 2d), although at the end of
321 treatment in 1998, lichen abundance had been significantly reduced by application of high N
322 and, most notably, the combination of low or high N and P (Gordon *et al.*, 2001, Fig. 2c,
323 Table 3).
324
325 *Plant nutrient content*
326 MOSS TISSUE N AND P
327 Past nutrient additions significantly influenced tissue nutrient concentrations in *D. spadiceum*
328 in 2011, with similar effects on both heaths (Fig. 4); however, the magnitude of effect was
329 less than it had been immediately after treatment (Table 3). At the end of 8 years of treatment
330 of the *Dryas* heath (1998), tissue N concentration of *D. spadiceum* reflected N inputs and in
331 the high N treatments (with and without P) was more than double that in the control (Fig. 4a).
332 After 13 years' recovery (2011), moss tissue N concentration in the high N treatment plots
333 was c. 25 % greater than the control (Fig. 4b). In the *Cassiope* heath, 7 years after 3 years of
334 treatment, moss which had received high N (with and without P) had tissue N concentration
335 c. 55 % greater than in control plots (Fig 4c); 18 years post-treatment the difference in high N
336 plots was c. 35 % (Fig. 4d). Thus, although diminishing, the effect of added N on moss tissue
337 N persists almost two decades after treatment. It is notable that in 2011, whilst tissue N
338 concentration of moss which received N alone still increased with N treatment rate, there was
339 now a strong response to P treatment, such that the overall treatment effect is driven by the
340 NxP interaction. The tissue N concentration of moss which had received P but not N was now
341 greatly increased and equal to that in the moss which had received high N only. Conversely,
342 tissue N in moss which had received high N+P was reduced to the concentration in control
343 moss (Fig. 4b,d).
344

Immediately after 8 years of treatment (1998), *Dryas* heath moss tissue P concentration was elevated over fourfold by P addition, and c. sixfold by the addition of N with P, compared to control moss (Fig. 4e). On *Cassiope* heath, 7 years after three years of treatment (2000), tissue P concentration was doubled in moss which had received P, compared to controls, but not influenced by N treatment (Fig. 4g). In 2011, on both heath types, tissue P concentration was still significantly increased, by c. twofold overall, in moss which had received P, but was now less elevated where N had been applied in combination with P (Fig. 4f,h). Thus the influence of P addition on tissue P appears to have stabilised in the early years after treatment and persists, whilst the influence of N addition on moss which had received both nutrients has switched over time from increasing tissue P to decreasing it.

At the end of treatment of the *Dryas* heath, moss which had not received P had a tissue N:P ratio around 8, whilst moss which had received P had a N:P ratio of c. 2 (Fig. 4c). After recovery on both heaths (*Dryas* 2011, *Cassiope* 2000, 2011) the N:P ratio of moss which had received N only increased with N treatment, ranging from 8 to 14, whilst the N:P ratio of the moss which had received P had stabilised close to 5, irrespective of N treatment (Fig. 4j-l).

SHRUB TISSUE N

At the end of treatment, the leaf tissue N concentrations of *C. tetragona, D. octopetela* and *S. polaris* were all significantly increased in response to N addition. However, we found no such significant effects of treatment on leaf N concentrations in 2011 (Fig. 5, Table 3).

Nitrate reductase activity in moss tissue

Nitrate reductase activity (NRA) was measured in *Dicranum spadiceum* from both heaths; from *Dryas* heath in the final year of treatment (1998) and 13 years later (2011) (Fig. 6) and from *Cassiope* heath 7 and 18 years post treatment (2000, 2011). Whilst there was some variation in absolute NRA rates between years, presumably due to differences in moss or assay conditions, clear patterns of treatment effect are apparent and these patterns were very similar on both heaths (hence data are shown for the *Dryas* heath only). Throughout the years, high N treatment resulted in decreased NRA activity, and this was still apparent after 13 years for inducible activity on the *Dryas* heath (Fig. 6) and 18 years for constitutive activity on the *Cassiope* heath ($P < 0.01$, $F = 8.25$) (Table 3). Phosphorus treatment stimulated constitutive NRA at the end of treatment (Fig. 6) and 7 years post treatment ($P < 0.001$, $F = 17.45$).

^{15}N recovery

In 2011 total ^{15}N recovery was close to 100 % for all treatments on both heath types (Fig. 7a). In contrast, 7 years post-treatment on the *Cassiope* heath (2000), total ^{15}N recovery in the high N treated plots was significantly lower than in both the control (Tukey HSD, $P < 0.05$) and the high N+P treatments (Tukey HSD, $P < 0.01$) (Fig 7a). In the sixth year of treatment on the *Dryas* heath (1996) there was a similar pattern of effect of high N treatments, with greater recovery in high N+P plots than in high N alone (Tukey HSD, $P < 0.05$) but no significant differences between the low N treatments (Fig. 7a). This suggests that the addition of high N in both heath types without concomitant addition of P caused N saturation, resulting in N leakage from the system, but that this effect has not persisted. The much lower total ^{15}N recovery in *Dryas* plots in 1996, in all treatments including controls, may have been due at least in part to unusually low temperatures limiting biological activity (Ny-Ålesund (1.5 km from site) average temperature between ^{15}N application and harvest, 3.37 °C in 1996, 6.47 °C in 2011; data from eKlima, http://sharki.oslo.dnmi.no/).

Vascular plants always retained < 10 % of the ^{15}N applied, reflecting their low biomass (Fig.7 b-e). In 2011, the largest single fraction of ^{15}N was recovered in the moss and litter layer (comprising mainly moss) in both vegetation types and all treatments (Fig. 7 c-e). In 2000, the distribution of ^{15}N between fractions was more even, with the majority being recovered from the organic and mineral soil layers (Fig. 7b). This may have resulted from

much higher precipitation in 2000, prior to and during the experiment, enabling greater downward mobility of ^{15}N (June+July 2000, 58.5 mm; 2011, 27.8 mm; data from eKlima, http://sharki.oslo.dnmi.no/). Beyond these inter-annual differences we found clear patterns of treatment effect on ^{15}N distribution (Table 3). Seven years post-treatment on the *Cassiope* heath (2000), less ^{15}N was recovered from the moss and litter layer in high N plots than in either control or high N+P plots (Tukey HSD, $P < 0.05$, and $P < 0.001$ respectively, Fig. 7b) This pattern had changed by 2011, when much more ^{15}N was recovered from moss and litter in plots which had received both N (low or high) and P, than in controls and plots which had received N only (Fig. 7 c,d,e); recovery of ^{15}N in moss and litter was inversely related to moss tissue N:P (^{15}N % recovery = - 4.7 * N:P + 113.0, $R^2 = 0.41$, $P < 0.0001$). More ^{15}N appeared to be recovered from mineral soil in high N than in control and high N+P plots in 2011 (though only differences between HN and HN+P in *Cassiope* plots were statisticially significant; Tukey HSD, $P < 0.05$, Fig.7e), a pattern which had already begun to emerge in 2000 (Fig. 7b).

Discussion

Our experiment has provided a unique insight into the potential for tundra heath vegetation to recover from nitrogen enrichment. Whilst N deposition rate across the Arctic is generally < 2 kg N ha^{-1} y^{-1} (Vet *et al.*, 2014), our low treatment of 10 kg N ha^{-1} y^{-1} represented the highest local deposition rates known in the Arctic in the early 1990s (Woodin, 1997) and our high treatment of 50 kg N ha^{-1} y^{-1} approximated to the highest rates of deposition to analogous alpine tundra heath in Europe at that time (> 30 kg N ha^{-1} y^{-1}, e.g. INDITE, 1994; http://emep.int/mscw/). Treatments applications were of very short duration compared to background atmospheric N deposition. Two decades post-treatment there are some signs of recovery, but many effects of the N treatments persist, some as a legacy of a past impact and others via currently active mechanisms.

Community composition

Previous nutrient treatments still influence vegetation species composition, via different mechanisms. Nitrogen treatment has resulted in dramatically reduced cover of the dominant shrubs, *C. tetragona* and *D. octopetala,* and of *Saxifraga oppositifolia*. This is most likely due to the death of shoots following an unusually mild, wet winter in 1993/94 which resulted in ice encasement of vegetation. Nitrogen treatment delayed hardening and resulted in increased winter injury, as observed on nearby polar heath (Robinson *et al.*, 1998). In contrast to these species, *Salix polaris* responded positively to nutrient addition, presumably escaping winter damage because it is deciduous and has overwintering buds protected in the moss layer. Similar positive responses to fertilisation have been reported for *Salix polaris* (Robinson *et al.*, 1998; Madan *et al.*, 2007) and *Salix arctica* (Arens *et al*., 2008).

Mortality of N treated plants was greatest for *C. tetragona* and, although partially recovered in plots which had received low N, this species was almost eradicated and is still virtually absent in high N plots, irrespective of P status. Abundance of *S. oppositifolia* in high N treated plots is also still reduced by 80 %, with no evidence of recovery over 11 years, and although there was some initial recovery of *D. octopetala,* cover is still only c. 40 % of that in control plots. Slow recovery may reflect the very low rate of seed germination in tundra heath (Cooper *et al.*, 2004; Müller *et al.*, 2011); it is possible that if seedlings established now they would be able to survive as the recovery of tissue nutrient status in remaining shrubs suggests that the treatments are no longer affecting them. Thus this dramatic effect of N may be a legacy of a past climatic event (i.e. an unusually mild winter) from which the vegetation will,

449 in time, recover; but indications are that recovery will be slow and depend on suitable
450 summer conditions for recruitment, which may occur infrequently.
451
452 In Arctic vegetation, fertilisation has been shown to cause decline in non-vascular plants
453 through increased competition from vascular plants; however, in open vegetation such as
454 tundra heath there is insufficient canopy for this to occur and fertilisation affects lichens and
455 moss directly (Madan *et al.*, 2007). Lichen cover on the *Dryas* heath initially decreased in
456 response to N, exacerbated by P treatment (Gordon *et al.*, 2001). However, contrary to our
457 expectations, in one of the clearest examples of recovery in this experiment, total lichen cover
458 no longer differs between treatments. Direct negative effects of N have been previously
459 reported for arctic and alpine lichens (e.g. Robinson et al. 1998; Fremstad et al. 2005; Britton
460 & Fisher 2010) but we do not know of any other studies of recovery in directly comparable
461 systems. In boreal forest, recovery of ground living lichens was slower, with abundance still
462 decreased 22 years after the second of two applications of 150 kg N ha^{-1} (Strengbom &
463 Nordin, 2008), but this may be an effect of the increased density of the ground layer vascular
464 plant canopy which also persisted. We did not examine shifts in lichen community
465 composition but it is possible that, despite the observed recovery in total abundance, there is
466 reduced diversity with decreased presence of those species more sensitive to nutrient rich
467 conditions (e.g. Klanderud, 2008).
468
469 In contrast to the lichens, nutrient addition increased moss abundance and this response has
470 continued over time. Similar positive responses of bryophytes to N plus P have been
471 observed on polar semi-desert in Svalbard (Robinson *et al.*, 1998; Madan *et al.*, 2007), on
472 dwarf shrub heath in Greenland (Arens *et al.*, 2008) and *Calluna* heath in the UK (Pilkington
473 *et al.*, 2007). Whereas the current effects of N on *D. octopetala, C. tetragona* and *S.*
474 *oppositifolia* appear to be the result of past physiological damage, the mechanisms of effect
475 of N on mosses are suggested by physiological parameters (discussed below) to still be
476 active.
477
478 It should be noted that the experimental site is grazed by reindeer, at low intensity. It is
479 possible that there may have been differential grazing between treatments, but we have no
480 way of quantifying this. However, there is no evidence of differential grazing effects on
481 lichens, which have recovered from initial treatment effects, or on *Cassiope* and *Dryas*,

which in the high N treatments are frequently still present, but dead. Grazing is unlikely to have confounded the treatment effects on mosses, as reindeer do not select them.

Moss nutrient status

Although somewhat diminished over time, effects of N addition on moss tissue N concentration remain. This is in contrast to other studies which have shown a rapid reduction of moss tissue N to control levels following the cessation of nutrient treatment (see Introduction), and must be due to efficient recycling of N, probably through internal translocation (Eckstein & Karlsson, 1999).

From ^{15}N recovery, the moss in the high N plots initially appeared to be N saturated, suggesting decreased ability to retain newly available N (Curtis et al. 2005). Decreased nitrate reductase activity (NRA) in moss under high N treatment in both heaths supported this, indicating feedback inhibition by accumulated products of N assimilation (Woodin & Lee, 1987). We found no evidence of persistent N saturation in 2011. However, treatment effects were still evident in the influence of moss tissue N:P on ^{15}N retention in the moss layer, and the effects of N were still apparent on NRA. This sensitive indicator suggests that the N metabolism of the moss continues to be influenced by the historic N treatments, with the amount of N available within the moss tissue still being greater than can be used for growth where P is limiting. The persistence of this effect is notable, and suggests that *D. spadiceum* has physiological mechanisms which allow for "luxury" translocation of nutrients beyond current demands for growth. In contrast, recovery of NRA was observed in less than 2 years in *Rhytidiadelphus squarrosus* in temperate acid grassland (Arróniz-Crespo *et al.*, 2008).

The effects of N treatment on moss physiology are strongly dependent on P availability. In the early years after treatment, moss tissue N reflected N treatment irrespective of P availability. In 2011 this remained the case for moss which had not received P. However, tissue N in moss which received high N+P (and low N+P on *Dryas* heath) had recovered to control values. There was a similar decline in tissue P concentration, indicating growth dilution through increased productivity in mosses for which both N and P limitations have been alleviated. By 2011 moss cover in high N+P plots had increased to 3 x that of controls and as biomass is closely related to cover (subset of plots: moss biomass g m^{-2} = 9.055 x

%cover – 59.92, R^2 = 61.4%, p<0.001) so, theoretically, growth dilution could account for all of the observed reduction in tissue N concentration.

Interestingly, in 2011 tissue N concentrations in moss treated with P alone were as high as those in moss which had received high N alone. Phosphorus addition had clearly caused strong N limitation, with a tissue N:P ratio of c. 2 at the end of treatment. In addition to stimulation of moss NRA in initial years, we suggest that the low N:P ratio may have stimulated N_2 fixation by moss-associated cyanobacteria (DeLuca et al. 2007; Bay *et al.*, 2013). Furthermore, in moss which received P, the N:P ratio has levelled to *c.* 5 irrespective of N treatment; it appears that where P limitation has been removed, the moss N:P ratio may be tightly regulated via the mechanisms above. Similar patterns of moss N:P equilibration in *Pseudoscleropodium purum* (at N:P *c.* 6) were seen at the end of fertilisation treatments in an acid grassland in northern England (Arróniz-Crespo *et al.*, 2008), so perhaps a tissue N:P of around 5 is indicative of co-limitation of moss by N and P in these systems.

Ecosystem N saturation

The ^{15}N studies on *Dryas* heath in the final year of treatment, and *Cassiope* heath 7 years post-treatment, suggested that the high N-only plots were N-saturated; less added ^{15}N was recovered than from control and high N+P plots, with the remainder presumably lost to leaching or denitrification. Similar decreases in ^{15}N retention have been observed in several ecosystems subject to fertilisation or N deposition (Templer *et al.*, 2012). In contrast, both the control and high N+P treated systems tightly conserved added N, suggesting N limitation due to low N availability and low N:P ratio respectively. By 2011 there was no indication of N saturation of the whole system, but partitioning of ^{15}N between components was still influenced by the original nutrient treatments. Recovery of ^{15}N from mineral soil was increased in high N plots, probably as a result of the markedly thinner moss and organic soil layers in these plots (Street et al, pers. obs.). Recovery of ^{15}N from the moss layer was greatest in plots receiving both N (low and high) and P, again reflecting the high abundance of mosses. These results highlight the key role of moss in influencing ecosystem responses to N inputs (Curtis *et al.* 2005). Persistent differences in the initial fate of deposited N in the system may influence its further cycling; N moving straight to the mineral soil may be more readily lost via leaching during periods when there is greater water movement (e.g. spring thaw). N assimilated by moss is more likely to be retained and recycled or taken up by plant roots and fungal hyphae, which preferentially colonise decomposing moss.

549 *Statistical considerations*

550 In this study we chose to use separate ANOVA analysis at two time points to assess the
551 statistical significance of treatment effects. Our definition of "recovery" is based on
552 comparison to the control plots; where previously significant effects of treatment were
553 undetectable in 2011, we consider the vegetation to have recovered. It should be noted that,
554 even if the effect size after recovery is statistically significant, if it is small compared to the
555 effect size immediately after treatment, this could also be interpreted as recovery. However,
556 Table 3 shows that where we detect statistically significant treatment effects, often the size of
557 these effects is of similar magnitude to, or even larger than, those immediately after
558 treatment.

559

560 *Recovery and critical loads*

561 Almost two decades after just a few years' treatment of tundra heath with relatively low
562 inputs of N, the system no longer appears to be N saturated and some elements of species
563 composition have recovered. Leaf tissue N concentrations in shrubs have returned to control
564 levels, and tissue N in N-treated moss is less elevated than it was at the end of treatment.
565 Thus some components of the system are recovering.

566

567 On the other hand, many effects of N deposition are still apparent. Overall vegetation
568 composition differs between N treatments and abundance of *D. octopetala, C. tetragona* and
569 *S. oppositifolia* in plots which received 50 kg N ha^{-1} y^{-1} is still severely compromised, with
570 no evidence of recovery since previous measurements. In contrast, moss abundance is now
571 dramatically increased by both N treatments where P was also added, the treatment effect
572 having increased over time. Both ^{15}N and NRA data demonstrate that the moss is still
573 responding physiologically to the added nutrients, although the magnitude of the NRA
574 response has diminished over time. In all these respects, the tundra heath is showing only
575 very slow signs, if any, of recovery. Added nutrients may be so efficiently recycled, within
576 the moss layer in particular, that effects will continue in the much longer term. Moss
577 functions as an ecosystem engineer, influencing the soil environment, decomposition and
578 vascular plant growth (Gornall *et al.*, 2007, 2011; Turetsky *et al.*, 2012). The increase in
579 abundance of moss in plots which received N+P will have changed soil conditions, and may
580 therefore result in alternate stable states of the vegetation (van der Wal, 2006).

581

582 Whilst many of the long-lasting effects of N are in response to addition of 50 kg N ha^{-1} y^{-1},
583 there are still clear responses to deposition of just 10 kg N ha^{-1} y^{-1} for 3 years, supporting the
584 critical load, which is set lower than this at 3-5 kg N ha^{-1} y^{-1}. The system is less sensitive to N
585 if P is limiting; the only persistent effect of 10 kg N ha^{-1} y^{-1} without P addition that we
586 detected was reduced cover of *C. tetragona.* Where P is not limiting, low N inputs have
587 greater influence on community composition and on the productivity and nutrient dynamics
588 of moss. The persistence of all these impacts further argues for a low critical load, and such
589 evidence of potential for recovery - or lack of it - has begun to be considered in their revision
590 (Bobbink & Hettelingh, 2010). Although legislation based on the critical loads approach has
591 resulted in major reductions in emissions of nitrogenous pollutants in Europe, it may be that
592 recovery of the most sensitive ecosystems will be slow to follow.
593

Acknowledgements

We are indebted to Ian Alexander who initiated the fertilisation experiment with SJW; to successive researchers involved in the experiment, John Baddeley, Nanette Madan, Lars Hogbom, Bernard Moyersen, Carmen Gordon; and to field assistants, Alison Horsburgh, Andrew Coughlan, Jo Wynn, Lora Crabtree. We thank Hans Kruijer and Michael Stech for assistance with bryophyte species identification in 2011. Funding for the initial experiment was provided by the NERC Arctic Terrestrial Ecology Special Topic Programme (GR3/9424, GR9/3433) with additional support from the CEC TMR Programme, Ny-Ålesund LSF and the British Ecological Society. This recovery study was funded by NERC (NE/I016899/1). The research was made possible by use of NERC facilities at Harland Huset; special thanks to Nick Cox and colleagues for their unfailing hospitality and support.

608 *References*

609 Achermann B, Bobbink R. 2003. Empirical Critical Loads for Nitrogen. In: *Environmental*
610 *Documentation No.164 Air*, pp. 43–170. Swiss Agency for Environment, Forest and
611 Landscape SAEFL, Berne.

612 Allen SE. 1989. *Chemical analysis of ecological materials*, 2nd edn. Oxford, UK: Blackwell
613 Scientific Publications.

614 Arens SJT, Sullivan PF, Welker, JM. 2008. Nonlinear responses to nitrogen and strong
615 interactions with nitrogen and phosphorus additions drastically alter the structure and
616 function of a high Arctic ecosystem. *Journal of Geophysical Research* **113**: G03S09.

617 Armitage HF, Britton AJ, Woodin SJ, van der Wal R. 2011. Assessing the recovery potential
618 of alpine moss-sedge heath: reciprocal transplants along a nitrogen deposition gradient.
619 *Environmental Pollution* **159**: 140–147.

620 Arróniz-Crespo M, Leake JR, Horton P, Phoenix GK. 2008. Bryophyte physiological
621 responses to, and recovery from, long-term nitrogen deposition and phosphorus
622 fertilisation in acidic grassland. *New Phytologist* **180**: 864–874.

623 Baddeley JA, Woodin SJ, Alexander IJ. 1994. Effects of increased nitrogen and phosphorus
624 availability on the photosynthesis and nutrient relations of three Arctic dwarf shrubs
625 from Svalbard. *Functional Ecology* **8**: 676–685.

626 Bay G, Nahar N, Oubre M, Whitehouse MJ, Wardle DA, Zackrisson O, Nilsson M-C,
627 Rasmussen U. 2013. Boreal feather mosses secrete chemical signals to gain nitrogen.
628 *New Phytologist* **200**: 54–60.

629 Björkman MP, Kühnel R, Partridge DG, Roberts TJ, Aas W, Mazzola M, Viola A, Hodson
630 A, Ström J, Isaksson E. 2013. Nitrate dry deposition in Svalbard. *Tellus B* **65**: 19071.

631 Bobbink R, Hettelingh J-P. 2010. Review and revision of empirical critical loads-response
632 relationships. In: *Proceedings of an expert workshop, Noordwijkerhout, 23-25 June*
633 *2010*, p. 246.

634 Bobbink R, Hicks K, Galloway J, Spranger T, Alkemade R, Ashmore M, Bustamante M,
635 Cinderby S, Davidson E, Dentener F *et al*. 2010 Global assessment of nitrogen
636 deposition effects on terrestrial plant diversity: a synthesis. *Ecological Applications* **20**:
637 30–59.

638 Bret-Harte MS, Mack MC, Goldsmith GR, Sloan DB, DeMarco J, Shaver GR, Ray PM,
639 Biesinger Z, Chapin FS. 2008. Plant functional types do not predict biomass responses
640 to removal and fertilization in Alaskan tussock tundra. *Journal of Ecology* **96**: 713–726.

641 Britton A, Fisher J. 2007. NP stoichiometry of low-alpine heathland: Usefulness for bio-
642 monitoring and prediction of pollution impacts. *Biological Conservation* **138**: 100–108.
643 Britton AJ, Fisher JM. 2010. Terricolous alpine lichens are sensitive to both load and
644 concentration of applied nitrogen and have potential as bioindicators of nitrogen
645 deposition. *Environmental Pollution* **158**: 1296–1302.
646 Clark CM, Hobbie SE, Venterea R, Tilman D. 2009. Long-lasting effects on nitrogen cycling
647 12 years after treatments cease despite minimal long-term nitrogen retention. *Global*
648 *Change Biology* **15**: 1755–1766.
649 Cooper EJ, Alsos IG, Hagen D, Smith FM, Coulson SJ, Hodkinson ID. 2004. Plant
650 recruitment in the High Arctic: Seed bank and seedling emergence on Svalbard. *Journal*
651 *of Vegetation Science* **15**: 115–124.
652 Curtis CJ, Emmett BA, Grant H, Kernan M, Reynolds B, Shilland E. 2005. Nitrogen
653 saturation in UK moorlands: the critical role of bryophytes and lichens in determining
654 retention of atmospheric N deposition. *Journal of Applied Ecology* **42**: 507–517.
655 DeLuca TH, Zackrisson O, Gentili F, Sellstedt A, Nilsson M-C. 2007. Ecosystem controls on
656 nitrogen fixation in boreal feather moss communities. *Oecologia* **152**: 121–30.
657 Dormann CF, Woodin SJ. 2002. Climate change in the Arctic: using plant functional types in
658 a meta-analysis of field experiments. *Functional Ecology* **16**: 4–17.
659 Eckstein RL, Karlsson PS. 1999. Recycling of nitrogen among segments of Hylocomium
660 splendens as compared with Polytrichum commune: implications for clonal integration
661 in an ectohydric bryophyte. *Oikos* **86**: 87–96.
662 EU. 2001. Directive 2001/81/EC of the European Parliament and of the Council on national
663 emission ceilings for certain atmospheric pollutants.
664 *http://ec.europa.eu/environment/air/pdf/nec_eu_27.pdf*.
665 Fremstad E, Paal J, Mols T. 2005. Impacts of increased nitrogen supply on Norwegian lichen-
666 rich alpine communities: a 10-year experiment. *Journal of Ecology* **93**: 471–481.
667 Gordon C, Wynn JM, Woodin SJ. 2001. Impacts of increased nitrogen supply on high Arctic
668 heath: the importance of bryophytes and phosphorus availability. *New Phytologist* **149**:
669 461–471.
670 Gornall JL, Jónsdóttir IS, Woodin SJ, Van der Wal R. 2007. Arctic mosses govern below-
671 ground environment and ecosystem processes. *Oecologia* **153**: 931–41.
672 Gornall JL, Woodin SJ, Jónsdóttir IS, van der Wal R. 2011. Balancing positive and negative
673 plant interactions: how mosses structure vascular plant communities. *Oecologia* **166**:
674 769–782.

675 INDITE 1994. Impacts of Nitrogen Deposition in Terrestrial Ecosystems. U.K. Review
676 Group on Impacts of Atmospheric Nitrogen Deposition on Terrestrial Ecosystems.
677 London, UK: Department of the Environment.
678 Isbell F, Tilman D, Polasky S, Binder S, Hawthorne P. 2013. Low biodiversity state persists
679 two decades after cessation of nutrient enrichment. *Ecology Letters* **16**: 454–60.
680 Klanderud K. 2008. Species-specific responses of an alpine plant community under simulated
681 environmental change. *Journal of Vegetation Science* **19**: 363-372.
682 Klaudisová M, Hejcman M, Pavlů V. 2009. Long-term residual effect of short-term fertilizer
683 application on Ca, N and P concentrations in grasses *Nardus stricta L.* and *Avenella*
684 *flexuosa L.*. *Nutrient Cycling in Agroecosystems* **85**: 187–193.
685 Královec J, Pocová L, Jonášová M, Macek P, Prach K. 2009. Spontaneous recovery of an
686 intensively used grassland after cessation of fertilizing. *Applied Vegetation Science* **12**:
687 391–397.
688 Kühnel R, Roberts TJ, Björkman MP, Isaksson E, Aas W, Holmén K, Ström J. 2011. 20-Year
689 Climatology of NO_3^- and NH_4^+ Wet Deposition at Ny-Ålesund, Svalbard. *Advances in*
690 *Meteorology* **2011**: 1–10.
691 Limpens J, Heijmans MMPD. 2008. Swift recovery of *Sphagnum* nutrient concentrations
692 after excess supply. *Oecologia* **157**: 153–161.
693 Madan NJ, Deacon LJ, Robinson CH. 2007. Greater nitrogen and/or phosphorus availability
694 increase plant species' cover and diversity at a High Arctic polar semidesert. *Polar*
695 *Biology* **30**: 559–570.
696 Mitchell RJ, Sutton MA, Truscott AM, Leith ID, Cape JN, Pitcairn CER, Van Dijk N. 2004.
697 Growth and tissue nitrogen of epiphytic Atlantic bryophytes: Effects of increased and
698 decreased atmospheric N deposition. *Functional Ecology* **18**: 329.
699 Müller E, Cooper EJ, Alsos IG. 2011. Germinability of Arctic plants is high in perceived
700 optimal conditions but low in the field. *Botany* **89**: 337–348.
701 Nordin A, Strengbom J, Witzell J, Näsholm T, Ericson L. 2005. Nitrogen deposition and the
702 biodiversity of boreal forests: implications for the nitrogen critical load. *Ambio* **34**: 20-
703 24.
704 Oksanen J, Blanchet FG, Kindt R, Legendre P, Minchin PR, O'Hara RB, Simpson GL,
705 Solymos P, Stevens MHH, Wagner H. 2012. vegan: Community Ecology Package. *R*
706 *package version*, **1**, R package version 2.0–4.

Pavlů V, Gaisler J, Pavlů L, Hejcman M, Ludvíková V. 2012. Effect of fertiliser application and abandonment on plant species composition of *Festuca rubra* grassland. *Acta Oecologica* **45**: 42–49.

Phoenix GK, Emmett BA, Britton AJ, Caporn SJ, Dise NB, Helliwell R, Jones L, Leake JR, Leith ID, Sheppard LJ *et al*. 2012. Impacts of atmospheric nitrogen deposition: responses of multiple plant and soil parameters across contrasting ecosystems in long-term field experiments. *Global Change Biology* **18**: 1197–1215.

Pilkington MG, Caporn SJM, Carroll JA, Cresswell N, Lee JA, Emmett BA, Bagchi R. 2007. Phosphorus supply influences heathland responses to atmospheric nitrogen deposition. *Environmental Pollution* **148**: 191–200.

Robinson CH, Wookey PA, Lee JA, Callaghan TV, Press MC. 1998. Plant community responses to simulated environmental change at a high Arctic polar semi-desert. *Ecology* **79**: 856–866.

Spiegelberger T, Hegg O, Matthies D, Hedlund K, Schaffner U. 2006. Long-term effects of short-term perturbation in a subalpine grassland. *Ecology* **87**: 1939–1944.

Stevens CJ, Mountford JO, Gowing DJG, Bardgett RD. 2012. Differences in yield, Ellenberg N value, tissue chemistry and soil chemistry 15 years after the cessation of nitrogen addition. *Plant and Soil* **357**: 309–319.

Strengbom J, Nordin A. 2008. Commercial forest fertilization causes long-term residual effects in ground vegetation of boreal forests. *Forest Ecology and Management* **256**: 2175–2181.

Strengbom J, Nordin A, Näsholm T, Ericson L. 2001. Slow recovery of boreal forest ecosystem following decreased nitrogen input. *Functional Ecology* **15**: 451–457.

Templer PH, Mack MC, Chapin FS, Christensen LM, Compton JE, Crook HD, Currie WS, Curtis CJ, Dail DB, D;Antonio CM *et al*. (2012) Sinks for nitrogen inputs in terrestrial ecosystems: a meta-analysis of ^{15}N tracer field studies. *Ecology*, **93**, 1816–29.

Turetsky MR, Bond-Lamberty B, Euskirchen E, Talbot J, Frolking S, McGuire a D, Tuittila E-S. 2012. The resilience and functional role of moss in boreal and arctic ecosystems. *New Phytologist* **196**: 49–67.

UNECE. 1999. Gothenburg Protocol to Abate Acidification, Eutrophication and Ground-level Ozone. *http://www.unece.org/env/lrtap/multi_h1.html*.

Van der Wal R. 2006. Do herbivores cause habitat degradation or vegetation state transition? Evidence from the tundra. *Oikos* **114**: 177–186.

740 Van der Wal R, Pearce ISK, Brooker RW. 2005. Mosses and the struggle for light in a
741 nitrogen-polluted world. *Oecologia* **142**: 159–68.
742 Vet RRS, Artz SC, Shaw M, Ro C-U, Aas W, Baker A, Bowersox C, Dentener F, Galy-Lacaux
743 C, Hou A, Pienaar JJ, Gillett R, Forti CM, Gromov S, Hara H, Khodzher T, Mahowald NM,
744 Nickovic S, Rao PSP, Reid NW. 2014. A global assessment of precipitation chemistry and
745 deposition of sulfur, nitrogen, sea salt, base cations, organic acids, acidity and pH, and
746 phosphorus. *Atmospheric Environment* **93**: 3-100.
747 Woodin SJ 1997. Effects of acid deposition on arctic vegetation. In: Woodin SJ, Marquiss M,
748 *eds. Ecology of Arctic Environments*. Oxford, UK: Blackwell Science, 219-239.Woodin
749 SJ, Lee JA. 1987. The fate of some components of acidic deposition in ombrotrophic
750 mires. *Environmental Pollution* **45**: 61–72.
751

Tables

Table 1 *Cassiope* and *Dryas* heath: Comparison of treatment and recovery durations, treatment and ambient N inputs and parameters measured prior to 2011.

	***Cassiope* heath**	***Dryas* heath**
Dates of treatment	1991-1993 (+ 60 % treatment in 2000)	1991-1998
Duration of treatment	3 years (+ 0.6 year)	8 years
Duration of recovery to 2011	18 years	13 years
Total cumulative N treatment, kg ha^{-1}	low N = 36; high N = 180	low N = 80; high N = 400
Total cumulative ambient N deposition during recovery , kg ha^{-1}	*c*. 14.5	*c*. 10.5
Dates of parameter measurements *prior to 2011* (number of years' recovery in parentheses):		
Moss and lichen cover	-	1998 (0)
Dryas cover	-	1991, 1993-6, 1998 (all 0)
Cassiope abundance	1991 (0) , 1993 (0), 1995 (2), 2000 (7)	-
Moss tissue nutrients	2000 (7)	1998 (0)
Shrub tissue nitrogen	1993 (0)	1996 (0)
Nitrate reductase activity	2000 (7)	1998 (0)
^{15}N recovery	2000 (7)	1996 (0)

Table 2. Summary of historical plant abundance data and their collection methods on *Dryas* and *Cassiope* heath at intervals during 1991-2011.

	year	species	parameter	collection method
Dryas heath	1991	*Dryas*	cover	score from 0-4 in each of 225 (10 x 10 cm) squares
	1993-6	*Dryas*	cover	score from 0-4 in each of 225 (10 x 10 cm) squares
	1998	All spp.	cover	point intercept sampling on 20 x 20 cm grid (n = 49)
	2011	All spp.	cover	point intercept sampling on 10 x 10 cm grid (n = 196)
Cassiope heath	1991	*Cassiope*	cover	score from 0-4 in each of 225 (10 x 10 cm) squares
	1993	*Cassiope*	leaf biomass	dry mass of leaves per unit ground area
	1995	*Cassiope*	number of live shoots	Number of live shoots in central 0.8 x 0.8 m of plot
	2000	8 spp.	frequency	presence/absence of each in 225 (10 x 10 cm) squares
	2011	All spp.	cover	point intercept sampling on 10 x 10 cm grid (n = 196)

Table 3. Treatment effect sizes (effect size = treatment mean/control mean) for **a)** *Cassiope* heath and **b)** *Dryas* heath for all variables where there is a significant effect of treatment (ANOVA main effect or interaction). Treatments: LN = low N, HN = high N, LNP = low N+P, HNP = high N+P. n.s. = no significant effect of treatment. Cells are shaded grey where no data is available. * indicates the variable was measured in 1996.

***a) Cassiope* heath**	**Post treatment (1993)**				**7 years recovery (2000)**				**18 years recovery (2011)**			
	LN	HN	LNP	HNP	LN	HN	LNP	HNP	LN	HN	LNP	HNP
Plant physiology												
Dicranum tissue N					1.21	1.56	1.18	1.53	1.08	1.36	1.19	0.87
Dicranum P					1.07	0.91	2.27	1.94	0.89	1.01	1.94	1.56
Dicranum tissue N:P					1.21	1.69	0.51	0.80	1.21	1.49	0.62	0.56
Cassiope tissue N	1.06	1.24	1.04	1.28					*n.s*	*n.s*	*n.s*	*n.s*
Salix tissue N	1.11	1.14	1.06	1.26					*n.s*	*n.s*	*n.s*	*n.s*
Dicranum constitutive NRA					0.88	0.80	1.08	1.41	0.87	0.89	0.79	0.73
Dicranum inducible NRA					0.87	0.79	1.06	1.50	*n.s*	*n.s*	*n.s*	*n.s*
Community composition												
Cassiope abundance	*n.s.*	*n.s.*	*n.s.*	*n.s.*					0.41	0.00	0.68	0.02
^{15}Nitrogen retention												
Total ^{15}N recovery						0.75		1.09	*n.s*	*n.s*	*n.s*	*n.s*
^{15}N recovery moss & litter						0.42		1.29	1.04	0.95	1.57	1.91
^{15}N recovery vascular plants						0.69		1.57	3.14	0.84	1.31	0.36
^{15}N recovery organic soil						0.76		0.94	0.55	1.75	0.33	0.51
^{15}N recovery mineral soil						1.47		1.02	0.63	2.27	0.35	0.53

***b) Dryas* heath**	**Post treatment (1998)**				**13 years recovery (2011)**			
	LN	HN	LNP	HNP	LN	HN	LNP	HNP
Plant physiology								
Dicranum tissue N	1.61	2.14	1.47	2.55	1.21	1.26	0.94	0.99
Dicranum P	1.88	2.07	5.44	6.13	1.10	0.99	1.74	1.64
Dicranum tissue N:P	0.73	0.72	0.19	0.29	1.13	1.29	0.54	0.60
Dryas tissue N *	1.25	1.39	1.19	1.41	*n.s.*	*n.s.*	*n.s.*	*n.s.*
Dicranum constitutive NRA	1.04	0.32	2.45	1.31	*n.s.*	*n.s.*	*n.s.*	*n.s.*
Dicranum inducible NRA	0.50	0.17	2.70	-0.21	0.77	0.61	0.83	0.75
Community composition								
Dryas abundance	0.78	0.61	0.65	0.42	0.98	0.69	0.74	0.41
Moss % cover	1.17	0.97	1.85	1.78	1.06	1.36	2.98	3.31
Lichen % cover	1.18	0.64	0.30	0.41	*n.s.*	*n.s.*	*n.s.*	*n.s.*
^{15}Nitrogen retention								
Total ^{15}N recovery	*n.s*	0.50	*n.s*	1.37		*n.s.*		*n.s.*
^{15}N recovery in moss & litter						0.97		1.82
^{15}N recovery in vascular plants						0.75		0.50
^{15}N recovery in organic soil						0.69		0.25
^{15}N recovery in mineral soil						1.49		0.65

Figure legends

Figure 1. Non-metric multidimensional scaling (NMDS) analysis of the community composition of **a)** *Cassiope* and **b)** *Dryas* heath vegetation in 2011, following previous treatment with N and P. Species for which occurrence was recorded < 5 times (total number of hits < 5 out of a possible 196) are not shown, but are included in the analysis. *Cassiope* heath stress = 0.14, *Dryas* heath stress = 0.13. Arrows indicate fitted vectors for the N and P treatment rates, $P < 0.01$ for all treatment vectors. Treatments: C = control, LN = low N, HN = high N, P = P only, LNP = low N+P, HNP = high N+P. Bryophyte species names are shown in bold. Ellipses are drawn using the standard error of the (weighted) average of scores, the (weighted) correlation defines the axis of the ellipse. Species names are abbreviated as: A.tur = *Aulacomnium turgidum*, B.viv = *Bistorta viviparum*, C.rup = *Carex rupestris*, C.tet = *Cassiope tetragona*, Dic spp = *Dicranum* species, Ditrich. = Ditrichaceae, D.oct = *Dryas octopetala*, H.spl = *Hylocomium splendens* , O.dig = *Oxyria digyna* , L.arc = *Luzula arctuata*, L.con = *Luzula confusa* , O.wah = *Oncophorus wahlenbergii* , P.alp = *Polytrichastrum alpinum* , P.cil = *Ptilidium ciliare* , Ped.spp = *Pedicularis species*, Phil.spp = *Philonotis* species, S.auc = *Silene acaulis* , S.opp = *Saxifraga oppositifolia*, S.pol = *Salix polaris* , S.unc = *Sanionia uncinata* , R.lan = *Racomitrium lanuginosum.*

Figure 2. Effects of previous nitrogen and phosphorus treatment of *Dryas* heath on total cover of **a)** bryophytes at the end of treatment (1998), **b)** bryophytes 13 years post-treatment (2011) **c)** lichens at the end of treatment (1998) and **d)** lichens 13 years post-treatment (2011). Data expressed as % of control; mean ± 1 S.E, n = 5. Treatments: C = control, LN = low N, HN = high N, P = P only, LNP = low N+P, HNP = high N+P. Significance of factors in two-way ANOVA are indicated by the number of symbols: NS non-significant, ^ $P < 0.1$, * $P \leq 0.05$, ** $P \leq 0.01$, ***$P \leq 0.001$

Figure 3. Shrub abundance since the beginning of nitrogen and phosphorus treatments, for **a)** *Cassiope tetragona*, expressed as % of control (cover in control plots was 29 % in 1991 decreasing to 11 % by 2011) and **b)** *Dryas octopetala*, expressed as % ground cover. Mean ± 1 S.E, n = 5. Treatments: C = control, LN = low N, HN = high N, P = P only, LNP = low N+P, HNP = high N+P.. Significance of factors in two-way ANOVA are indicated by the number of symbols: NS non-significant, ^ $P < 0.1$, * $P \leq 0.05$, ** $P \leq 0.01$, ***$P \leq 0.001$

Figure 4. a-d) Tissue N and **e-h)** tissue P concentrations (% dry weight), and **i-l)** N:P ratio (g g^{-1}) in *Dicranum spadiceum* previously treated with nitrogen and phosphorus, sampled from *Dryas* heath

in 1998 and 2011 and from *Cassiope* heath in 2007 and 2011. The number of years post-treatment are indicated in parentheses. Treatments: C = control, LN = low N, HN = high N, P = P only, LNP = low N+P, HNP = high N+P.. Mean ± 1 S.E, n = 5. Significance of factors in two-way ANOVA are indicated by the number of symbols: NS non-significant, ^ $P < 0.1$, * $P \leq 0.05$, ** $P \leq 0.01$, ***$P \leq 0.001$

Figure 5. Leaf tissue N concentrations (% dry weight) at the end of nitrogen and phosphorus treatments and after recovery: for **a,b)** *Dryas octopetala*, **c,d)** *Cassiope tetragona* and **e,f)** *Salix polaris**. Mean ± 1 S.E, n=5 (except for HNP *C. tetragona* 2011, where n = 1). Number of years post-treatment indicated in parentheses in Figure. Treatments: C = control, LN = low N, HN = high N, P = P only, LNP = low N+P, HNP = high N+P. Significance of factors in two-way ANOVA are indicated by the number of symbols: NS non-significant, ^ $P < 0.1$, * $P \leq 0.05$, ** $P \leq 0.01$, ***$P \leq 0.001$

N.B. data for *S. polaris* in 1993 are from a separate set of treatment plots in nearby *Salix* dominated heath, many of which were damaged by snow scooter tracks between 1998 and 2011 and were therefore not re-sampled.

Figure 6. Effects of previous nitrogen and phosphorus treatments on **a,b)** constitutive and **c,d)** inducible nitrate reductase activity (μmol NO_2 g^{-1} dry wt h^{-1}) in *Dicranum spadiceum* on *Dryas* heath. Mean ± 1 S.E, n=5. Number of years post-treatment are indicated in parentheses. Significance of factors in two-way ANOVA are indicated by the number of symbols: NS non-significant, ^ $P < 0.1$, * $P \leq 0.05$, ** $P \leq 0.01$, ***$P \leq 0.001$

Figure 7: ^{15}N recovery (as % of that added) from dwarf shrub heath previously treated with nitrogen and phosphorus **a)** total recovery from *Dryas* heath in 1996 and 2011 and *Cassiope* heath in 2000 and 2011. ^{15}N recovery in vascular plants, moss and litter, organic soil and mineral soil from **b)** *Cassiope* heath in 2000, **c)** *Dryas* heath in 2011, **d)** *Cassiope* heath in 2011, low N treatments, **e)** *Cassiope* heath in 2011, high N treatments. Mean ± 1 S.E, n = 5. Number of years post-treatment are indicated in parentheses in Figure. Significant differences (Tukey HSD) within year and N addition rate (in **(a)**) and within fraction (in (**b) – (d)**) are indicated by shared symbols above bars, the number of symbols indicating the level of significance: ^ $P < 0.1$, * $P \leq 0.05$, ** $P \leq 0.01$, ***$P \leq 0.001$. Treatments: C = control, LN = low N, HN = high N, P = P only, LNP = low N+P, HNP = high N+P.