

1 Slow recovery of High Arctic heath communities from nitrogen enrichment

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24 **Summary**

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

42
43 *200 words*

44
45 Key words: bryophytes, critical load, nitrogen deposition, phosphorus, recovery, tundra,
46 winter injury

47

48 **Introduction**

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

56
57 Arctic ecosystems are typically strongly nutrient limited, and fertilisation experiments have
58 shown that vegetation composition and function respond dramatically to increases in nutrient
59 availability (Dormann & Woodin, 2002). High sensitivity to atmospheric N deposition would
60 thus be expected, indicating a low “critical load” of N for Arctic habitats - the amount below
61 which no significant harmful effects occur, according to current understanding. However,
62 Arctic fertilisation experiments typically applied N in combination with phosphorus (P) or
63 used high application rates (e.g. Robinson *et al.*, 1998; Bret-Harte *et al.*, 2008), and so did not
64 provide a basis for quantifying the critical load. To address this, we established an experiment
65 on Svalbard in 1991 with more realistic N treatments than used before on tundra: 10 kg N ha⁻¹
66 y⁻¹ representing the highest deposition rates known in the Arctic and 50 kg N ha⁻¹ y⁻¹
67 approximating the highest rates of deposition to analogous alpine heath in Europe (Gordon *et al.*,
68 2001). This study provided the basis for the empirical critical load of N for tundra to be
69 set at 5-10 kg N ha⁻¹ y⁻¹ (Achermann & Bobbink, 2003); a subsequent experiment in
70 Greenland (Arens *et al.*, 2008) led to revision to 3-5 kg N ha⁻¹ y⁻¹ (Bobbink & Hettelingh,
71 2010).

72
73 The quantification and mapping of empirical critical loads of N for European natural and
74 semi-natural ecosystems provided the basis for international pollution control (UNECE,
75 1999; EU, 2001) as a result of which European emissions of nitrogen oxides and ammonia
76 decreased by 44% and 25% respectively during 1990-2011 (www.eea.europa.eu). While the
77 ecological effects of N deposition have been well documented (e.g. Bobbink *et al.* 2010;
78 Phoenix *et al.* 2012), there have been few studies of the potential for recovery once N
79 deposition is reduced. Of the studies undertaken, many have used experiments in which the
80 initial N additions were at very high rates (e.g. Strengbom *et al.*, 2001; Nordin *et al.*, 2005;
81 Strengbom & Nordin, 2008) and often in combination with other nutrients (e.g. Klaudivová *et*

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

87

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

98

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

114

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

129

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

144

145 **Materials and Methods**

146 *Site description*

147 We utilised our previous nutrient addition experiment, established in 1991, approximately 1.5
148 km east of Ny-Ålesund, Svalbard (78° 54' 56" N 11° 58' 22" E) in two dwarf shrub heath
149 types; one dominated by *Cassiope tetragona* (L.) D. Don., the other by *Dryas octopetala* L.
150 Treatment plots (1.5 x 1.5 m) were selected to be representative, to contain the dominant
151 shrub and >25 % vegetation cover, and treatments were allocated randomly. *Cassiope* plots
152 were treated from 1991–1993 (with 60% treatment in 2000) and *Dryas* plots from 1991–
153 1998; no further treatments have been applied since. Full details of the experiment are in
154 Baddeley *et al.* (1994) and Gordon *et al.* (2001) and Table 1 provides a summary of the
155 treatments applied, recovery periods and timing of measurements.

156

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

165

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

176

177 *Community composition*

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

183

184 *Plant nutrient content*

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

193

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

198

199 *Nitrate reductase activity*

200 We measured moss nitrate reductase activity (NRA) as an indicator of N demand. On 9th July
201 2011 we collected samples of *Dicranum spadicum* tissue for NRA assay following the
202 methods of Gordon *et al.* (2001). We also present previously unpublished data for *D.*
203 *spadicum* from the *Cassiope* plots, which were sampled in 2000, 7 years post treatment
204 (before application of the partial treatment in that year), and from the *Dryas* plots in their
205 final year of treatment, 1998 (previously published in Gordon *et al.* 2001). Briefly, green tips
206 of the moss shoots were fully hydrated and placed in a cool greenhouse or growth cabinet
207 (depending on year), both with 24 h light, for at least 24 h prior to assay. Several shoots were
208 used in each assay (c. 50-100 mg dry wt); these were vacuum infiltrated in 5 ml of 75 mM
209 KNO₃ in 100 mM phosphate buffer (pH 7.5) with 0.75 % propan-1-ol, and incubated in the
210 dark at 25 °C for 1 h. Nitrite concentration of the incubation medium was then determined

211 colorimetrically. For determination of inducible NRA the moss was sprayed, 4.5 h before
212 assay, with 1 mM KNO₃.

213

214 ¹⁵N labelling

215 To quantify ecosystem N retention in 2011 we used ¹⁵N stable isotope labelling, following a
216 protocol used in earlier years of the experiment in which the ¹⁵N was added as one of the
217 series of treatment applications. *Cassiope* (all treatments) and *Dryas* (control (C), high N,
218 high N+P) plots were labelled with ¹⁵NH₄¹⁵NO₃. We randomly selected one 0.2 x 0.2 m area
219 in each plot for labelling, avoiding previous sampling areas. In the *Cassiope* control plots we
220 randomly selected two areas, one for a low N control (CL) and one for a high N control (CH).
221 We took one 4.2 cm diameter core from each plot before treatments were applied to quantify
222 background ¹⁵N. Normal nutrient treatments were then applied on 8th July, i.e. one fifth of
223 the annual treatment in 80 ml solution applied with a hand-held sprayer to each 0.2 x 0.2 m
224 area. This was followed on 15th July by a second treatment containing 10 atom% excess ¹⁵N.
225 On 21st July a further normal treatment was applied so that any remaining ¹⁵N would be
226 rinsed from the leaves such that subsequent recovery would be through N uptake rather than
227 adsorption. Further 4.2 cm diameter cores were taken on 27th July to quantify ¹⁵N retention.

228

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

234

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

239

240 *Statistics*

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

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

250

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

271

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

274

$$275 \quad R = \frac{\text{treatment mean}}{\text{control mean}} \quad (1)$$

276

277 **Results**

278 *Community composition*

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

287

288 The predominance of bryophyte species towards the nutrient enriched side of ordination
289 space indicates that in general bryophytes have responded positively to nutrient addition,
290 though some species responded most strongly to N (e.g. *Polytrichastrum alpinum* (Hedw.)
291 G.L. Sm.), some to P (e.g. *Hylocomium splendens* (Hedw.) Schimp.) and some to both (e.g.
292 *D. spadiceum*) (Fig. 1). These positive effects of nutrient addition on bryophyte cover have
293 increased since the end of the treatments (Fig. 2a,b, Table 3). In 1998 bryophyte abundance
294 in *Dryas* plots receiving both nutrients was approaching double that in control plots, but by
295 2011 the difference was threefold. The only vascular plant species to respond positively to
296 nutrient addition was *Salix polaris* (Fig. 1), with c. 60 % increase in cover in *Cassiope* heath
297 plots receiving high N (two-way ANOVA, $F_{2,24} = 5.5$, $P < 0.05$). Cover also increased in
298 response to P and additive effects of high N+P resulted in a doubling in *S. polaris* cover,
299 compared to control, in *Cassiope* heath in 2011 (Tukey's HSD, $P < 0.01$); responses in *Dryas*
300 heath were similar but less pronounced (data not shown).

301

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

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

355

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

361

362 SHRUB TISSUE N

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

366

367 *Nitrate reductase activity in moss tissue*

368 Nitrate reductase activity (NRA) was measured in *Dicranum spadiceum* from both heaths;
369 from *Dryas* heath in the final year of treatment (1998) and 13 years later (2011) (Fig. 6) and
370 from *Cassiope* heath 7 and 18 years post treatment (2000, 2011). Whilst there was some
371 variation in absolute NRA rates between years, presumably due to differences in moss or
372 assay conditions, clear patterns of treatment effect are apparent and these patterns were very
373 similar on both heaths (hence data are shown for the *Dryas* heath only). Throughout the
374 years, high N treatment resulted in decreased NRA activity, and this was still apparent after
375 13 years for inducible activity on the *Dryas* heath (Fig. 6) and 18 years for constitutive
376 activity on the *Cassiope* heath ($P < 0.01$, $F = 8.25$) (Table 3). Phosphorus treatment
377 stimulated constitutive NRA at the end of treatment (Fig. 6) and 7 years post treatment ($P <$
378 0.001 , $F = 17.45$).

379

380 *¹⁵N recovery*

381 In 2011 total ¹⁵N recovery was close to 100 % for all treatments on both heath types (Fig. 7a).
382 In contrast, 7 years post-treatment on the *Cassiope* heath (2000), total ¹⁵N recovery in the
383 high N treated plots was significantly lower than in both the control (Tukey HSD, $P < 0.05$)
384 and the high N+P treatments (Tukey HSD, $P < 0.01$) (Fig 7a). In the sixth year of treatment
385 on the *Dryas* heath (1996) there was a similar pattern of effect of high N treatments, with
386 greater recovery in high N+P plots than in high N alone (Tukey HSD, $P < 0.05$) but no
387 significant differences between the low N treatments (Fig. 7a). This suggests that the addition
388 of high N in both heath types without concomitant addition of P caused N saturation,
389 resulting in N leakage from the system, but that this effect has not persisted. The much lower
390 total ¹⁵N recovery in *Dryas* plots in 1996, in all treatments including controls, may have been
391 due at least in part to unusually low temperatures limiting biological activity (Ny-Ålesund
392 (1.5 km from site) average temperature between ¹⁵N application and harvest, 3.37 °C in 1996,
393 6.47 °C in 2011; data from eKlima, <http://sharki.oslo.dnmi.no/>).

394

395 Vascular plants always retained < 10 % of the ¹⁵N applied, reflecting their low biomass
396 (Fig.7 b-e). In 2011, the largest single fraction of ¹⁵N was recovered in the moss and litter
397 layer (comprising mainly moss) in both vegetation types and all treatments (Fig. 7 c-e). In
398 2000, the distribution of ¹⁵N between fractions was more even, with the majority being
399 recovered from the organic and mineral soil layers (Fig. 7b). This may have resulted from

400 much higher precipitation in 2000, prior to and during the experiment, enabling greater
401 downward mobility of ^{15}N (June+July 2000, 58.5 mm; 2011, 27.8 mm; data from eKlima,
402 <http://sharki.oslo.dnmi.no/>). Beyond these inter-annual differences we found clear patterns of
403 treatment effect on ^{15}N distribution (Table 3). Seven years post-treatment on the *Cassiope*
404 heath (2000), less ^{15}N was recovered from the moss and litter layer in high N plots than in
405 either control or high N+P plots (Tukey HSD, $P < 0.05$, and $P < 0.001$ respectively, Fig.
406 7b) This pattern had changed by 2011, when much more ^{15}N was recovered from moss and
407 litter in plots which had received both N (low or high) and P, than in controls and plots which
408 had received N only (Fig. 7 c,d,e); recovery of ^{15}N in moss and litter was inversely related to
409 moss tissue N:P (^{15}N % recovery = $- 4.7 * \text{N:P} + 113.0$, $R^2 = 0.41$, $P < 0.0001$). More ^{15}N
410 appeared to be recovered from mineral soil in high N than in control and high N+P plots in
411 2011 (though only differences between HN and HN+P in *Cassiope* plots were statistically
412 significant; Tukey HSD, $P < 0.05$, Fig.7e), a pattern which had already begun to emerge in
413 2000 (Fig. 7b).

414

415 Discussion

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

426

427 *Community composition*

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

438

439 Mortality of N treated plants was greatest for *C. tetragona* and, although partially recovered
440 in plots which had received low N, this species was almost eradicated and is still virtually
441 absent in high N plots, irrespective of P status. Abundance of *S. oppositifolia* in high N
442 treated plots is also still reduced by 80 %, with no evidence of recovery over 11 years, and
443 although there was some initial recovery of *D. octopetala*, cover is still only c. 40 % of that in
444 control plots. Slow recovery may reflect the very low rate of seed germination in tundra heath
445 (Cooper *et al.*, 2004; Müller *et al.*, 2011); it is possible that if seedlings established now they
446 would be able to survive as the recovery of tissue nutrient status in remaining shrubs suggests
447 that the treatments are no longer affecting them. Thus this dramatic effect of N may be a
448 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*,

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

484

485 *Moss nutrient status*

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

491

492 From ^{15}N recovery, the moss in the high N plots initially appeared to be N saturated,
493 suggesting decreased ability to retain newly available N (Curtis et al. 2005). Decreased
494 nitrate reductase activity (NRA) in moss under high N treatment in both heaths supported
495 this, indicating feedback inhibition by accumulated products of N assimilation (Woodin &
496 Lee, 1987). We found no evidence of persistent N saturation in 2011. However, treatment
497 effects were still evident in the influence of moss tissue N:P on ^{15}N retention in the moss
498 layer, and the effects of N were still apparent on NRA. This sensitive indicator suggests that
499 the N metabolism of the moss continues to be influenced by the historic N treatments, with
500 the amount of N available within the moss tissue still being greater than can be used for
501 growth where P is limiting. The persistence of this effect is notable, and suggests that *D.*
502 *spadiceum* has physiological mechanisms which allow for “luxury” translocation of nutrients
503 beyond current demands for growth. In contrast, recovery of NRA was observed in less than
504 2 years in *Rhytidiadelphus squarrosus* in temperate acid grassland (Arróniz-Crespo et al.,
505 2008).

506

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

515 %cover – 59.92, $R^2 = 61.4\%$, $p < 0.001$) so, theoretically, growth dilution could account for all
516 of the observed reduction in tissue N concentration.

517

518 Interestingly, in 2011 tissue N concentrations in moss treated with P alone were as high as
519 those in moss which had received high N alone. Phosphorus addition had clearly caused
520 strong N limitation, with a tissue N:P ratio of c. 2 at the end of treatment. In addition to
521 stimulation of moss NRA in initial years, we suggest that the low N:P ratio may have
522 stimulated N_2 fixation by moss-associated cyanobacteria (DeLuca et al. 2007; Bay *et al.*,
523 2013). Furthermore, in moss which received P, the N:P ratio has levelled to c. 5 irrespective
524 of N treatment; it appears that where P limitation has been removed, the moss N:P ratio may
525 be tightly regulated via the mechanisms above. Similar patterns of moss N:P equilibration in
526 *Pseudoscleropodium purum* (at N:P c. 6) were seen at the end of fertilisation treatments in an
527 acid grassland in northern England (Arróniz-Crespo *et al.*, 2008), so perhaps a tissue N:P of
528 around 5 is indicative of co-limitation of moss by N and P in these systems.

529

530 *Ecosystem N saturation*

531 The ^{15}N studies on *Dryas* heath in the final year of treatment, and *Cassiope* heath 7 years
532 post-treatment, suggested that the high N-only plots were N-saturated; less added ^{15}N was
533 recovered than from control and high N+P plots, with the remainder presumably lost to
534 leaching or denitrification. Similar decreases in ^{15}N retention have been observed in several
535 ecosystems subject to fertilisation or N deposition (Templer *et al.*, 2012). In contrast, both the
536 control and high N+P treated systems tightly conserved added N, suggesting N limitation due
537 to low N availability and low N:P ratio respectively. By 2011 there was no indication of N
538 saturation of the whole system, but partitioning of ^{15}N between components was still
539 influenced by the original nutrient treatments. Recovery of ^{15}N from mineral soil was
540 increased in high N plots, probably as a result of the markedly thinner moss and organic soil
541 layers in these plots (Street et al, pers. obs.). Recovery of ^{15}N from the moss layer was
542 greatest in plots receiving both N (low and high) and P, again reflecting the high abundance
543 of mosses. These results highlight the key role of moss in influencing ecosystem responses to
544 N inputs (Curtis *et al.* 2005). Persistent differences in the initial fate of deposited N in the
545 system may influence its further cycling; N moving straight to the mineral soil may be more
546 readily lost via leaching during periods when there is greater water movement (e.g. spring
547 thaw). N assimilated by moss is more likely to be retained and recycled or taken up by plant
548 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 $50 \text{ kg N ha}^{-1} \text{ y}^{-1}$ 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 $50 \text{ kg N ha}^{-1} \text{ y}^{-1}$,
583 there are still clear responses to deposition of just $10 \text{ kg N ha}^{-1} \text{ y}^{-1}$ for 3 years, supporting the
584 critical load, which is set lower than this at $3\text{-}5 \text{ kg N ha}^{-1} \text{ y}^{-1}$. The system is less sensitive to N
585 if P is limiting; the only persistent effect of $10 \text{ kg N ha}^{-1} \text{ y}^{-1}$ 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

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595

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606

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4
5

Tables

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

	<i>Cassiope</i> heath	<i>Dryas</i> 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)
<i>Dryas</i> cover	-	1991, 1993-6, 1998 (all 0)
<i>Cassiope</i> 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)

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

2

	year	species	parameter	collection method
Dryas heath	1991	<i>Dryas</i>	cover	score from 0-4 in each of 225 (10 x 10 cm) squares
	1993-6	<i>Dryas</i>	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	<i>Cassiope</i>	cover	score from 0-4 in each of 225 (10 x 10 cm) squares
	1993	<i>Cassiope</i>	leaf biomass	dry mass of leaves per unit ground area
	1995	<i>Cassiope</i>	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)

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1 **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
 2 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
 3 of treatment. Cells are shaded grey where no data is available. * indicates the variable was measured in 1996.

<i>a) Cassiope</i> heath	Post treatment (1993)				7 years recovery (2000)				18 years recovery (2011) ⁴			
	LN	HN	LNP	HNP	LN	HN	LNP	HNP	LN	HN	LNP	HNP
<i>Plant physiology</i>												
<i>Dicranum</i> tissue N					1.21	1.56	1.18	1.53	1.08	1.36	1.19	0.87
<i>Dicranum</i> P					1.07	0.91	2.27	1.94	0.89	1.01	1.94	1.56
<i>Dicranum</i> tissue N:P					1.21	1.69	0.51	0.80	1.21	1.49	0.62	0.56
<i>Cassiope</i> tissue N	1.06	1.24	1.04	1.28					n.s.	n.s.	n.s.	n.s.
<i>Salix</i> tissue N	1.11	1.14	1.06	1.26					n.s.	n.s.	n.s.	n.s.
<i>Dicranum</i> constitutive NRA					0.88	0.80	1.08	1.41	0.87	0.89	0.79	0.73
<i>Dicranum</i> inducible NRA					0.87	0.79	1.06	1.50	n.s.	n.s.	n.s.	n.s.
<i>Community composition</i>												
<i>Cassiope</i> abundance	n.s.	n.s.	n.s.	n.s.					0.41	0.00	0.68	0.02
¹⁵Nitrogen 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

<i>b) Dryas heath</i>	Post treatment (1998)				13 years recovery (2011)			
	LN	HN	LNP	HNP	LN	HN	LNP	HNP
<i>Plant physiology</i>								
<i>Dicranum</i> tissue N	1.61	2.14	1.47	2.55	1.21	1.26	0.94	0.99
<i>Dicranum</i> P	1.88	2.07	5.44	6.13	1.10	0.99	1.74	1.64
<i>Dicranum</i> tissue N:P	0.73	0.72	0.19	0.29	1.13	1.29	0.54	0.60
<i>Dryas</i> tissue N *	1.25	1.39	1.19	1.41	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>
<i>Dicranum</i> constitutive NRA	1.04	0.32	2.45	1.31	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>
<i>Dicranum</i> inducible NRA	0.50	0.17	2.70	-0.21	0.77	0.61	0.83	0.75
<i>Community composition</i>								
<i>Dryas</i> 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	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>
¹⁵Nitrogen retention								
Total ¹⁵ N recovery	<i>n.s.</i>	0.50	<i>n.s.</i>	1.37		<i>n.s.</i>		<i>n.s.</i>
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

1

1 *Figure legends*

2

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

18

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

25

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

32

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

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

6
7 **Figure 5.** Leaf tissue N concentrations (% dry weight) at the end of nitrogen and phosphorus
8 treatments and after recovery: for **a,b)** *Dryas octopetala*, **c,d)** *Cassiope tetragona* and **e,f)** *Salix*
9 *polaris**. Mean \pm 1 S.E, n=5 (except for HNP *C. tetragona* 2011, where n = 1). Number of years
10 post-treatment indicated in parentheses in Figure. Treatments: C = control, LN = low N, HN = high
11 N, P = P only, LNP = low N+P, HNP = high N+P. Significance of factors in two-way ANOVA are
12 indicated by the number of symbols: NS non-significant, $^{\wedge} P < 0.1$, $* P \leq 0.05$, $** P \leq 0.01$, $*** P$
13 ≤ 0.001
14 N.B. data for *S. polaris* in 1993 are from a separate set of treatment plots in nearby *Salix* dominated
15 heath, many of which were damaged by snow scooter tracks between 1998 and 2011 and were
16 therefore not re-sampled.

17
18 **Figure 6.** Effects of previous nitrogen and phosphorus treatments on **a,b)** constitutive and **c,d)**
19 inducible nitrate reductase activity ($\mu\text{mol NO}_2 \text{ g}^{-1} \text{ dry wt h}^{-1}$) in *Dicranum spadicum* on *Dryas*
20 heath. Mean \pm 1 S.E, n=5. Number of years post-treatment are indicated in parentheses.
21 Significance of factors in two-way ANOVA are indicated by the number of symbols: NS non-
22 significant, $^{\wedge} P < 0.1$, $* P \leq 0.05$, $** P \leq 0.01$, $*** P \leq 0.001$

23
24 **Figure 7:** ^{15}N recovery (as % of that added) from dwarf shrub heath previously treated with
25 nitrogen and phosphorus **a)** total recovery from *Dryas* heath in 1996 and 2011 and *Cassiope* heath
26 in 2000 and 2011. ^{15}N recovery in vascular plants, moss and litter, organic soil and mineral soil
27 from **b)** *Cassiope* heath in 2000, **c)** *Dryas* heath in 2011, **d)** *Cassiope* heath in 2011, low N
28 treatments, **e)** *Cassiope* heath in 2011, high N treatments. Mean \pm 1 S.E, n = 5. Number of years
29 post-treatment are indicated in parentheses in Figure. Significant differences (Tukey HSD) within
30 year and N addition rate (in **a)**) and within fraction (in **b)** – **d)**) are indicated by shared symbols
31 above bars, the number of symbols indicating the level of significance: $^{\wedge} P < 0.1$, $* P \leq 0.05$, $** P \leq$
32 0.01 , $*** P \leq 0.001$. Treatments: C = control, LN = low N, HN = high N, P = P only, LNP = low
33 N+P, HNP = high N+P.