

1 **Title: Long-term nitrogen deposition increases heathland carbon sequestration.**

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12

13 **Abstract**

14 The large increases in reactive nitrogen (N) deposition in developed countries since the Industrial

15 Revolution have had a marked impact on ecosystem functioning, including declining species

16 richness, shifts in species composition, and increased N leaching. A potential mitigation of these

17 harmful effects is the action of N as a fertiliser, which, through increasing primary productivity

18 (and subsequently, organic matter production), has the potential to increase ecosystem carbon

19 (C) storage. Here we report the response of an upland heath to 10 years of experimental N

20 addition. We find large increases in plant and soil C and N pools, with N-driven C sequestration

21 rates in the range of 13-138 kg C kg N⁻¹. These rates are higher than those previously found in

22 forest and lowland heath, mainly due to higher C sequestration in the litter layer. C sequestration

23 is highest at lower N treatments (10, 20, and 40 kg N ha⁻¹ yr⁻¹ above ambient), with evidence of

24 saturation at the highest N treatment, reflecting a physiologically aged *Calluna vulgaris*
25 (*Calluna*) canopy. To maintain these rates of sequestration, the *Calluna* canopy should be
26 managed to maximise its time in the building phase. Scaling our results across UK heathlands,
27 this equates to an additional 0.77 Mt CO₂e per annum extra C sequestered into plant litter and
28 the top 15 cm of heathland soil as a result of N deposition. The bulk of this is found in the litter
29 and organic soil horizons that hold an average of 23% and 54% of soil C, respectively. This
30 additional C represents around 0.44% of UK annual anthropogenic GHG emissions. When
31 considered in the context of falling biodiversity and altered species composition in heathland,
32 policy focus should remain on reducing N emissions.

33 **1. Introduction**

34 Since the Industrial Revolution and throughout most of the 20th century the level of reactive
35 nitrogen (N) in the atmosphere (primarily NH₃, NH₄⁺, NO_x, NO₃⁻, and organic N) has increased
36 due to fossil fuel burning and agricultural intensification (Galloway et al., 2004). Between 1860
37 and 1990, there was a 10-fold increase in reactive N, with a further doubling predicted by 2050
38 (Galloway et al., 2004). This greatly enhanced atmospheric N deposition has had profound effects
39 on ecosystem functioning, including reduced terrestrial plant species diversity, altered species
40 composition, and leaching of N to freshwater habitats following N saturation (Stevens et al. 2004;
41 Clark and Tilman, 2008; Dise et al. 2011; Phoenix et al. 2012; Field et al. 2014).

42
43 Potentially counteracting these negative effects of elevated N deposition is increased carbon (C)
44 sequestration into ecosystems through enhanced plant growth (Yue et al. 2016) and, in some cases,
45 a retardation of long-term decomposition rates (Berg and Laskowski 2006), thereby mitigating
46 rising atmospheric CO₂. In forests, both regional-scale N-gradient studies and N-addition

47 experiments have demonstrated N-driven increases in ecosystem C storage ranging from 12-36 kg
48 C kg N⁻¹ ha⁻¹ (De Vries et al. 2006; Hyvönen et al. 2007; Pregitzer et al. 2007). However, most of
49 the additional C stored in forests in response to N deposition is in new tree biomass rather than
50 soil (Nadelhoffer et al. 1999), with high rates of soil C turnover further suggesting that Forests
51 represent poor long-term soil C stores (Tipping et al. 2010; Mills et al. 2014). In contrast,
52 ecosystems such as bogs and heathlands, that primarily sequester new C in soil can be significant
53 C sinks for hundreds or thousands of years (Dise, 2009). This is due to high moisture levels and
54 vegetation rich in recalcitrant compounds (e.g. *Sphagnum* mosses and ericaceous plants such as
55 *Calluna*) limit decomposition rates, causing a build-up of soil organic matter (Anderson and
56 Hetherington, 1999; Berg and Laskowski, 2006).

57
58 Heathland ecosystems occur throughout much of the UK and north-western Europe, with closely
59 related ecosystems in Western Australia (for example *E. impressa* heathland) and New Zealand,
60 the oak-heathlands of eastern America, and arctic dwarf-shrub tundra. All of these habitats are
61 characterised by vegetation in the Ericaceae family and nutrient-poor, acidic soils. As such,
62 heathlands represent potentially important long-term soil C stores: in the UK alone, they sequester
63 around 120 Mt C in topsoil (0-15 cm) (Ostle et al. 2009); with some soil and ecological overlap
64 existing between bog and heathland. Overall, UK soil C storage is around 10,000 Mt (all depths)
65 and 1600 Mt (top 15 cm) (Emmett et al. 2010), almost half of which is in the organic rich soils of
66 bogs and heaths (Milne and Brown, 1997).

67
68 However, direct experimental evidence of changes in C accumulation in response to N deposition
69 in heathland is limited. N has been shown to increase plant growth and litter production of the key

70 heathland species *Calluna* (Caporn et al. 1995; Power et al. 1995) and significant increases in
71 heathland soil and plant N pools in response to N addition have been observed (Pilkington et al.
72 2005a). Earlier work on smaller plots suggested that N addition enhanced soil C sequestration at
73 the upland heath (Ruabon Moor-the subject of this study), largely through the increasing dry
74 weight of the organic soil horizon and maintenance of C/N ratios (Evans et al. 2006). This work
75 suggested a soil C increase of between 20 and 34 kg C kg N⁻¹ addition, but assumed a fixed C%
76 for peat and mineral soil of 39.3 and 3.9, bulked soil samples, and less real-world realistic N
77 additions of 40, 80 and 120 kg N. A study in a lowland heath in north-west England estimated a
78 slightly lower sequestration rate of 20 kg C kg N⁻¹ due to lower N retention in the more sandy soil
79 (de Vries et al. 2009; Evans et al. 2006; Pilkington et al. 2005a). In south-east England, C
80 sequestration estimates based upon N pools in soil and vegetation were approximately 33 kg C kg
81 N⁻¹ (de Vries et al. 2009). However, neither of these estimates are based on direct measurement
82 of C, instead they use measurements or model simulations of N pools and stoichiometric
83 relationships to convert N to C.

84

85 Here we report the first detailed analysis of organic and mineral soil C content in response to
86 experimental N addition on an upland heath ecosystem. We also upscale the data to estimate the
87 magnitude of N induced C sequestration at a landscape scale. We hypothesise that 1) N addition
88 increases the rate of sequestration of C in the organic and mineral soil horizons, 2) that C/N
89 stoichiometry is not fixed and therefore the rate of C sequestration will vary in response to N
90 addition, and 3) N-induced sequestered C in heathland is a potentially significant sink in relation
91 to the CO₂ equivalents (CO₂ eq) emitted in the UK through human activities.

92

93 2. Methods

94

95 2.1. Study site

96

97 Ruabon Moor is an upland heath situated at an altitude of 480 m, approximately 6 km north of
98 Llangollen in North Wales, UK (Figure 1, UK Grid Reference SJ224491). Annual precipitation is
99 approximately 1000 mm, and total inorganic N deposition in 2008 was estimated as 23.1 kg N ha⁻¹
100 yr⁻¹ from APIS (Air Pollution Information System) data, which uses the CBED model (APIS,
101 2008). The canopy is dominated by *Calluna*, although where burning has taken place or a gap in
102 the canopy occurs naturally, *Vaccinium myrtillus* grows well before it is shaded out by *Calluna*
103 regrowth. Understory vegetation consists mainly of the moss *Hypnum jutlandicum*; this
104 combination of vegetation gives the site a British NVC classification of H12 *Calluna – Vaccinium*
105 *myrtillus* heath (Rodwell, 1991) or a European EUNIS classification of F4.2. Soil is an iron pan
106 stagnopodzol (F.A.O. Placic Podzol) (Evans et al. 2006). N additions (of 0, 40, 80 and 120 kg N
107 ha⁻¹yr⁻¹) to 20 plots at the site began in 1989 (Caporn et al. 1995) and these ‘old’ plots (1 × 1 m)
108 were used by de Vries et al. (2009) in their study of N-induced C sequestration. In 1998, 36
109 rectangular (2 × 2 m) ‘new’ plots were established. N as NH₄NO₃ solution is mixed with rainwater
110 collected at the site and applied monthly to these new plots using a watering can at more realistic
111 N additions of 0, 10, 20, 40 kg N ha⁻¹yr⁻¹ (‘+0, +10N, +20N, and +40N’, respectively). A higher
112 120 kg N ha⁻¹yr⁻¹ (+120N) treatment is included to increase the N response gradient. A further 16
113 plots incorporate phosphorus additions however, these are not used in this study. After 10 years of
114 treatments, the cumulative additional N by treatment are 0, 100, 200, 400 and 1200 kg N ha⁻¹y⁻¹
115 above ambient N deposition i.e. the lowest N treatment adds 10 kg N ha⁻¹ yr⁻¹, so after 10 years

116 this is an additional 100 kg N input. Each treatment is replicated 4 times in a randomized block
117 design of overall size 20 x 20 m, with 20 plots in total used in this study. Earlier responses to N on
118 these plots of biological and chemical indicators have been reported by Edmondson et al. (2010).

119
120 The site has probably been a heathland since at least AD 1700, with active management as a grouse
121 moor by fire and grazing since the 1800s (Cawley, 2000). Over recent years the intensity of
122 management, including burning, has been less intense and the focus has been on attracting black
123 grouse (*Tetrao tetrix*) by cutting sections of heather to provide feeding close to nesting locations.
124 The last management to the actual plots was a burn in 1988, 10 years before the experiment started.
125 By the time soil cores were extracted in 2008, the plots were at the “mature to degenerate” stage,
126 dominated by *Calluna* but with gaps beginning to form due to senescence of the heather
127 (Gimingham, 1972).

128

129 **2.2. Plant biomass, canopy height and litter fall measurements**

130 Canopy height has been measured annually at the site since N additions began in 1998. It is
131 recorded at 16 locations in each plot; with 4 treatment replicates this provides 64 height
132 measurements for each N addition load (the mean height is presented in this study). Since a
133 destructive harvest is not possible, biomass was modelled by harvesting ten 1 x 1 m plant stands
134 of comparable aged and sized *Calluna* located off the plots and relating this measurement to the
135 canopy height of on- and off-plot plants using the equation:

136 Equation (1) **Biomass (g) = 1.94*(Canopy height)² - 128.85*Canopy height + 3017.8**

137 $R^2=0.98, P<0.001.$

138 A sample of ground plant tissue, incorporating recent shoot and leaf growth, per treatment level
139 was collected and analysed for %C and %N on a LECO Truspec Carbon and Nitrogen Analyser
140 (LECO Corporation, Michigan, USA). The plots represented a monoculture of *Calluna*, typical of
141 managed heathlands in the UK, and no changes in the species composition of vascular plants were
142 observed during the study period.

143

144 Annual litter productivity (litter fall) was measured at the site between May 1st 2007 and April 30th
145 2008. Five plastic plant pots (6.3 cm diameter) were set into the soil at random under the *Calluna*
146 canopy of each plot. Upon collection, the pots and accumulated litter were collected and the
147 contents dried and weighed. The results were used to calculate an annual rate of litter productivity
148 (litter fall).

149

150 **2.3. Soil carbon and nitrogen**

151 In July 2008, following 10 years of N addition, three 15 cm soil cores were collected from each
152 plot using a 3-cm diameter thin-walled steel corer. Since the experimental plots are all located
153 within an approximately 20 × 20 m square, there is general uniformity between the soil type and
154 horizon depths. Distinct soil horizons are apparent:

- 155 1. Litter (approx. 5cm). Loose surface litter, fresh and partially decomposed
- 156 2. OH1 (approx. 5cm depth) – the top organic layer of the soil consisting of fibrous roots and
157 partially decomposed organic matter
- 158 3. OH2 (approx. 5 cm depth) – the next layer of richly organic soil beneath OH1 consisting
159 mainly of humus/peat;

160 4. Gley (5-10 cm depth)– eluviated gley layer with little organic matter and a high mineral
161 content.

162 Difficulties in coring further into the mineral layer due to the presence of stones dictated a limit of
163 2 cm in the Gley horizon.

164

165 The individual soil horizons were dried at 80 °C for 24 hours. The depth of each soil horizon was
166 then measured and its mass recorded. A significant litter layer is found at Ruabon, however since
167 the coring technique tended to disturb the litter layer, litter depth was separately measured at 9
168 fixed locations in each plot. Following measurement and weighing, material from each horizon
169 was finely ground and a sub-sample analysed for C and N concentrations on the LECO Analyser.
170 This gave a total of 60 cores (3 per plot x 20 plots), with 12 at each N addition level, and 240
171 individual horizon profiles for C and N analyses. Chemical concentrations were then multiplied
172 by weight to give the pools of C and N of the litter layer and each soil horizon, the total C and N
173 pools, and the total organic (Litter + OH1 + OH2) C and N pools.

174

175 **2.4. Statistical analysis**

176 Data analysis was carried out in R version 3.01 (R Core Team, 2012). Due to heterogeneity of
177 variance in the data, the assumptions of regression such as normality and heterogeneity were not
178 always met. In these cases, notably the relationships between N deposition, litter and soil C, a
179 Generalised Additive Model (GAM) was fitted (Wood, 2011). When modelling biomass from
180 canopy height, the assumptions of regression were met and a relationship was fitted using quadratic
181 regression. The upscaling to landscape level considered the +0 to +40N treatments only; no
182 experimental additions between +40N and +120N were available and the highest +120N addition

183 is not representative of deposition loads found in the UK. In this case, the assumptions of
184 regression were also met and a linear regression was used.

185

186 Treatment differences of C%, N%, C/N and profile depth were investigated using either ANOVA,
187 for normally distributed data, or Kruskal-Wallis tests after first being tested for normality using
188 the Anderson-Darling test. Post-hoc comparisons were carried out using the Tukey test (for
189 ANOVA) and Wilcoxon rank sum test with Holm P-value adjustment (for Kruskal-Wallis). All
190 figures were produced using ggplot2 (Wickham, 2009).

191

192 **2.5. Nitrogen deposition mapping to heathlands and upscaling**

193 Concentration based estimated deposition (CBED) for N (and other pollutants) is mapped for the
194 UK on a 5x5 km grid (RoTAP, 2012). Values are derived from measurements of air concentrations
195 of gases and aerosols, and concentrations in precipitation from the UK Eutrophying and Acidifying
196 Pollutants (UKEAP) network. The measurements are interpolated to generate concentration maps
197 for the UK. The ion concentrations in precipitation are combined with the UK Met Office annual
198 precipitation map to generate maps of wet deposition. The wet deposition includes direct
199 deposition of cloud droplets to vegetation, and an orographic enhancement factor for the
200 concentration of precipitation in upland regions due to the seeder-feeder effect (Fowler et al. 1988).
201 Gas and particulate concentration maps are combined with spatially distributed estimates of
202 vegetation-specific deposition velocities (Smith et al. 2000) to generate dry deposition. Figure 1
203 shows mean total N (wet + dry, oxidised + reduced) deposition for 2011-2013 for UK areas of
204 dwarf shrub heath habitat. The habitat distribution map has been generated for UK research on
205 the impacts of air pollution using critical loads (Hall et al. 2015) and is defined from the CEH land

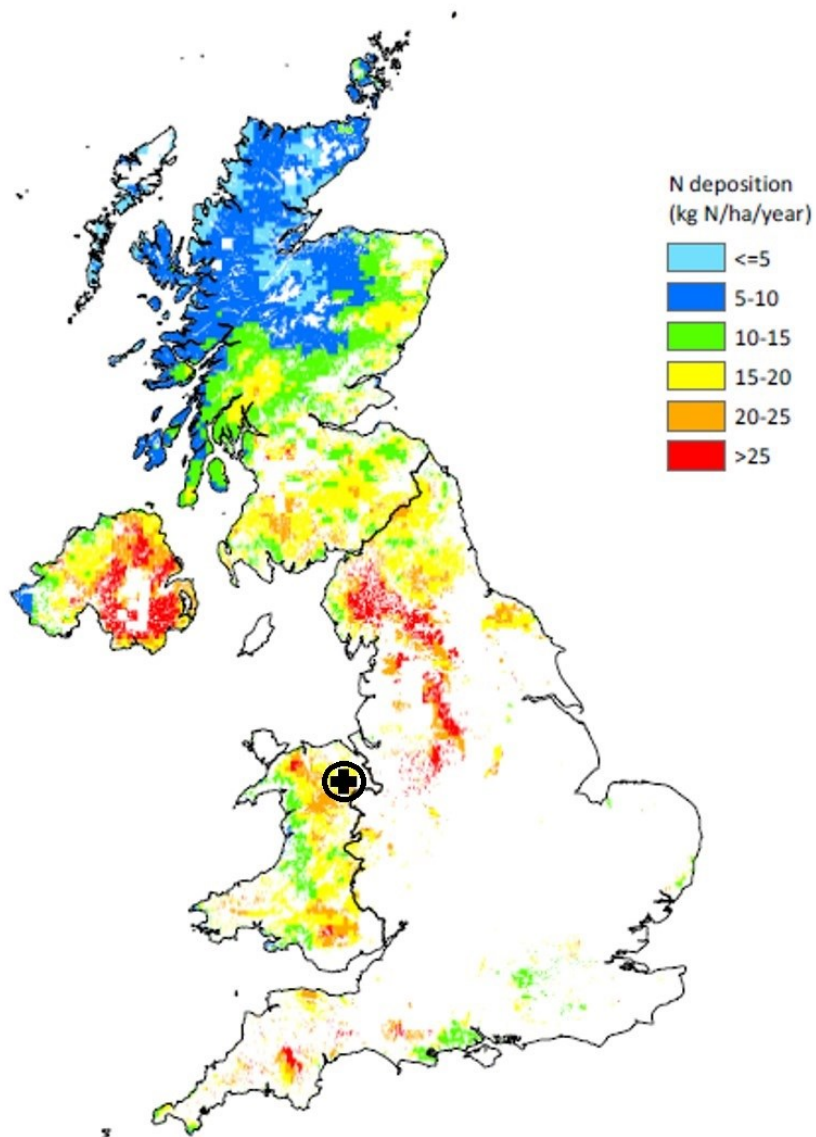
206 cover map 2000 (LCM2000: Fuller et al. 2002), further refined using ancillary data sets on species
207 distributions (Preston et al. 2002). The N deposition in Figure 1 is mapped for all 1x1 km squares
208 containing dwarf shrub heath. An estimate of C sequestration in litter and the organic component
209 of the soil (OH1 + OH2), by heathland area, was then modelled for each 1x1 km square, using the
210 linear relationship below, derived from the data gathered in this study:

211

212 Equation (2) **Organic Carbon sequestered (kg) per ha= 46613 + 575.6*N deposition**

213 $R^2=0.17, P=0.007.$

214 C sequestered at an assumed background, pre-industrial N load of 1 kg N ha⁻¹ yr⁻¹ for each 1 x 1
215 km square and the current N deposition load were calculated, with the increase in C sequestered
216 due to anthropogenic N being the difference between the amounts modelled at each deposition
217 load. In doing this, we assume that the relationship observed during experimentation is valid across
218 a broader geographic area and at a range of N deposition loads. Whilst this is an over simplification
219 and inherently flawed due to climatically-driven differences in plant growth and decomposition
220 rate, it enables an indicative magnitude of the likely response to N to be considered. In a study of
221 UK heathlands, canopy height was linearly associated with increasing N deposition across all sites
222 studied over a deposition range of 6 kg N to 33 kg N (Southon et al. 2013) suggesting that such a
223 response could exist at a landscape-scale.



224

225 **Figure 1. The study site on Ruabon Moor in North Wales (marked with a ⊕) over a UK dwarf shrub heath distribution**
 226 **map shaded by nitrogen (N) deposition. UK heathland N deposition range is 2.7 to 63.6 kg N ha⁻¹yr⁻¹.**

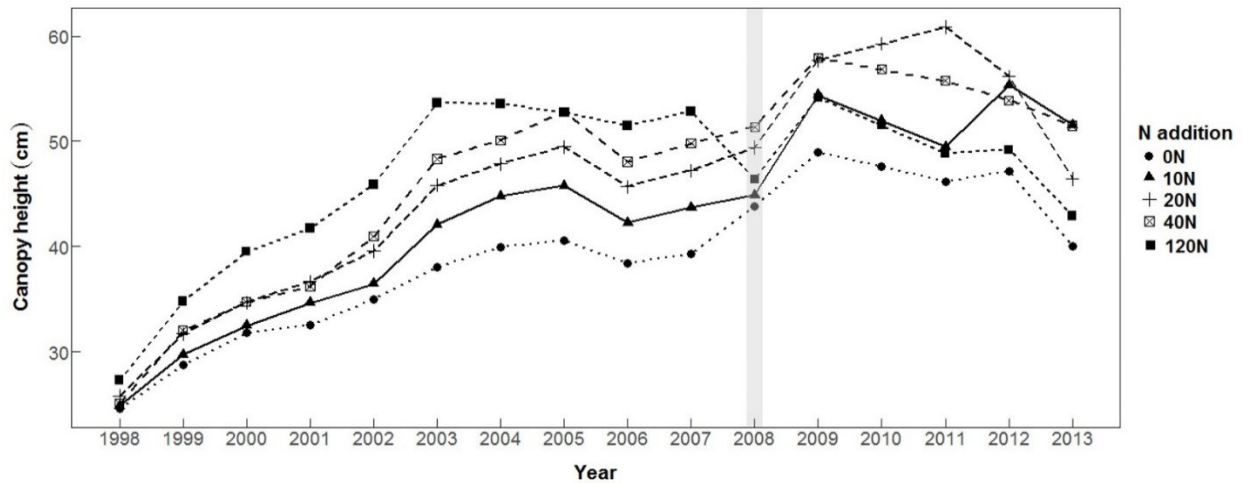
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228 **3. Results**

229 **3.1. Plant growth, biomass and litter fall**

230 *Calluna* showed a strong growth response following commencement of N additions in 1998, with
 231 increasing canopy height reflecting increasing N additions from the control (ambient N
 232 approximately 23 kg N ha⁻¹ yr⁻¹) up to +120N (Figure 2). With the +120N treatment canopy height
 233 stabilised by 2003 (5 years after treatment, and 15 years after the last management by fire) and

234 began to decline in 2009 after 11 years of treatment. This reflected a shift from plants in the mature
 235 to the degenerate stage, with active shoot growth declining and the canopy opening. In all other
 236 treatment plots stabilisation occurred later, around 2009, followed three years later by the decline
 237 phase.



238 Figure 2. Mean annual *Calluna* canopy height taken from 8 measurements per plot, 32 at each nitrogen (N) addition level.
 N treatment additions are: Control, +10 kg N, +20 kg N, +40 kg N, +120 kg N. Shaded bar illustrates when the sampling of
 the soil cores for CN analysis occurred.

239
 240 Total standing biomass C modelled from an off-plot harvest predicts increases in response to N
 241 addition, although incremental N above +20 kg N ha⁻¹ yr⁻¹ is not efficiently used, leading to falling
 242 incremental C per kg N added and increasing tissue N% (Table 1). However, at lower levels of N
 243 addition that are more relevant to those observed in the real-world, around 13 kg C are sequestered
 244 for every 1 kg increase in N deposition. On-site observations suggest much of this increased
 245 biomass is held in woody stems. Litter fall also increased in line with biomass from a mean of
 246 2534 kg ha⁻¹ yr⁻¹ in the control plots to 5272 kg ha⁻¹ yr⁻¹ in the +120N treatment, with an overall
 247 range of 1769 – 7487 kg ha⁻¹ yr⁻¹ (R²=0.49, P=0.003).

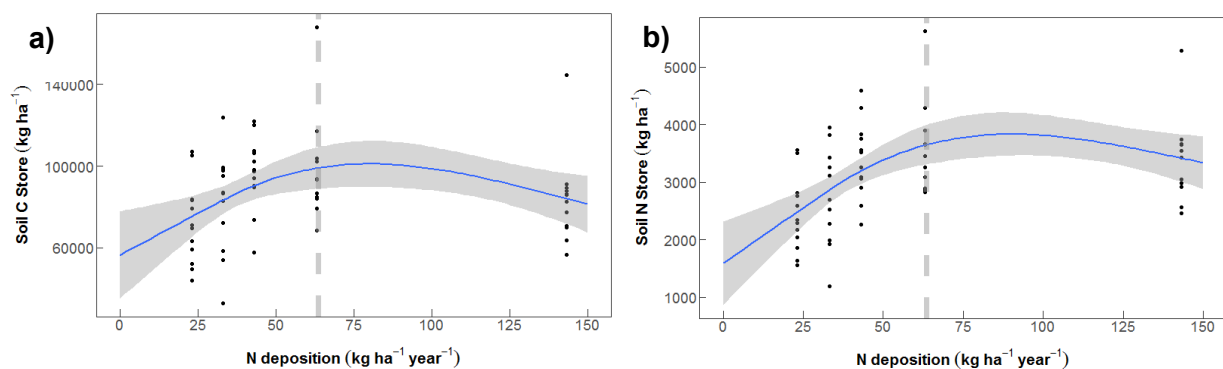
248 **Table 1. Summary of annual litter productivity (litter fall), biomass carbon (C) and nitrogen (N) stores and C**
 249 **sequestration/kg N modelled from off-plot harvest and calibrated by canopy height.**

N addition (kg ha ⁻¹ yr ⁻¹)	Total additional N (10 years treatment)	Annual litter fall (kg ha ⁻¹)	Biomass C (kg ha ⁻¹)	Biomass N (kg ha ⁻¹)	Biomass increase in C from ambient	Biomass ($\Delta C_{seq}/\Delta N$ kg C kg N ⁻¹)	Biomass C/N	Biomass N %
0	0	2534	5499	134.1	-	-	41.0	1.33
10	100	2766	6829	154.5	1330	13	44.2	1.24
20	200	4059	8015	230.3	2515	13	34.8	1.57
40	400	3750	8587	251.8	3088	8	34.1	1.60
120	1200	5272	9743	298.0	4244	4	32.7	1.67

250

251 3.2. Soil carbon and nitrogen CN pools

252 Surface C and N pools both show a trend of increasing C and N sequestration as N addition
 253 increases (Figure 3 a) and b)). This trend appeared to saturate with lower N additions above
 254 background N deposition producing greater increases in C and N sequestration, and the highest
 255 120 N addition failing to cause further C or N storage. This relationship between increasing N
 256 addition and C and N pools was significant when fitted by General Additive Model (Deviance
 257 explained=14%, F=4.43, P=0.013; Deviance explained=27%, F=10.58, P<0.001, respectively).



258

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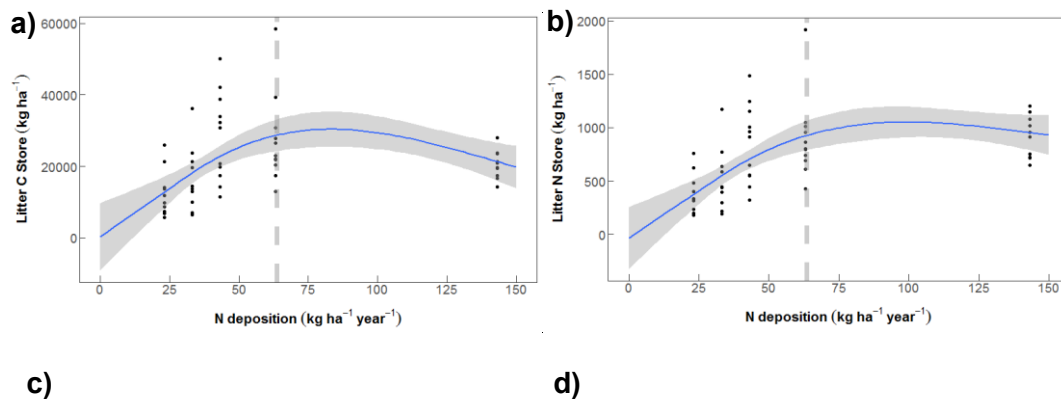
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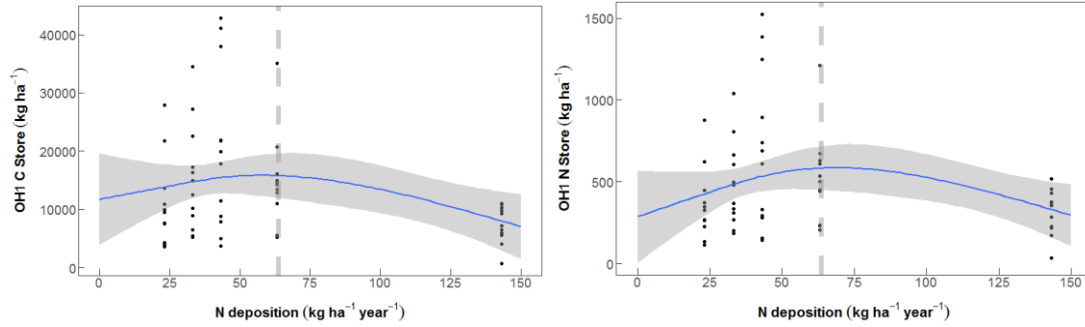
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Figure 3. Total soil pools of a) carbon (C) and b) nitrogen (N) at increasing N deposition (ambient + experimental N addition). Ambient nitrogen deposition is circa. 23 kg N ha⁻¹ yr⁻¹. Fitted using a Generalized Additive Model (GAM). Shaded

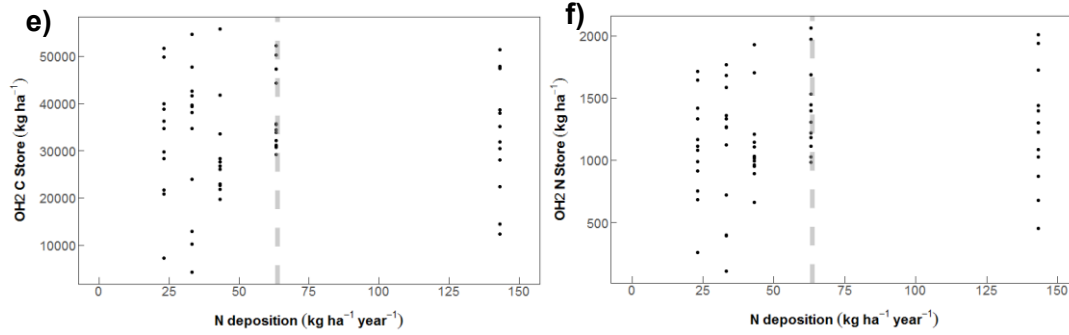
262 areas represent 95% confidence limits. N treatment additions are: Control, +10 kg N, +20 kg N, +40 kg N, +120 kg N.
263 Vertical dashed line shows the maximum modelled N deposition to UK heathlands.

264
265 Of all measured horizons, litter layer C and N pools increased the most strongly with increasing N
266 deposition, up to the +120N treatment (Figure 4 a) and b); Deviance explained =26.4%, F=10.02,
267 P<0.001 for C; Deviance explained=38.9%, F=18.59, P<0001 for N). At +120N, the litter C pool
268 declined to lower than that of both the +20 and +40N addition treatments (see Table 2 and Figure
269 4a). Litter N also appeared to saturate (Figure 4), although less sharply than litter C, reflecting the
270 large reduction in C/N at the highest +120N addition. The OH1 horizon also showed a positive
271 relationship between N deposition and C and N storage (Figure 4; Deviance explained =12%,
272 F=2.9, P<0.001 for C; Deviance explained=10.8%, F=2.65, P=0.05 for N), whilst the OH2 and
273 Gley horizons showed weak but non-significant, general trends of increasing soil C and N pools.
274 Across the sum totals of C and N in the litter, OH1 and OH2 soil horizons, considering the
275 difficulty in separating the profiles consistently, there was a significant relationship with N
276 deposition (Deviance explained=16.4%, F=5.13, P=0.009 for C, see Figure 7a; Deviance
277 explained=25.2%, F=9.52, P<0001 for N). It should be noted that, whilst the highest rate of
278 increase in soil C and N occurred in the litter layer, the largest total increase of C and N was in the
279 organic horizons (OH), reflecting the higher bulk densities of these horizons.

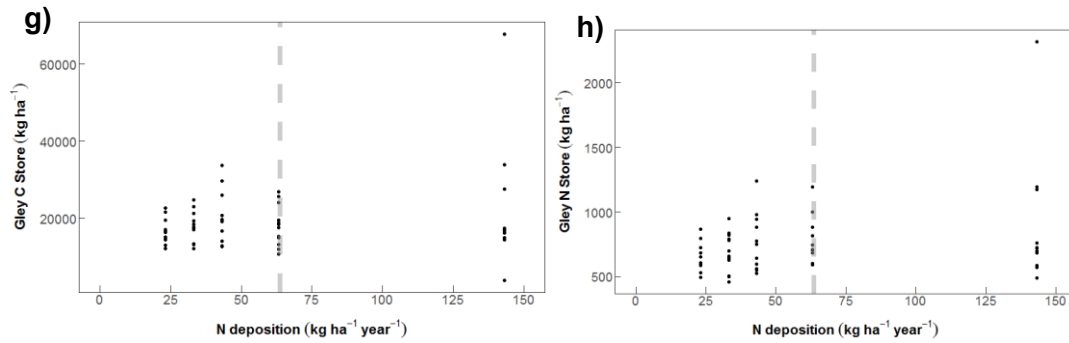




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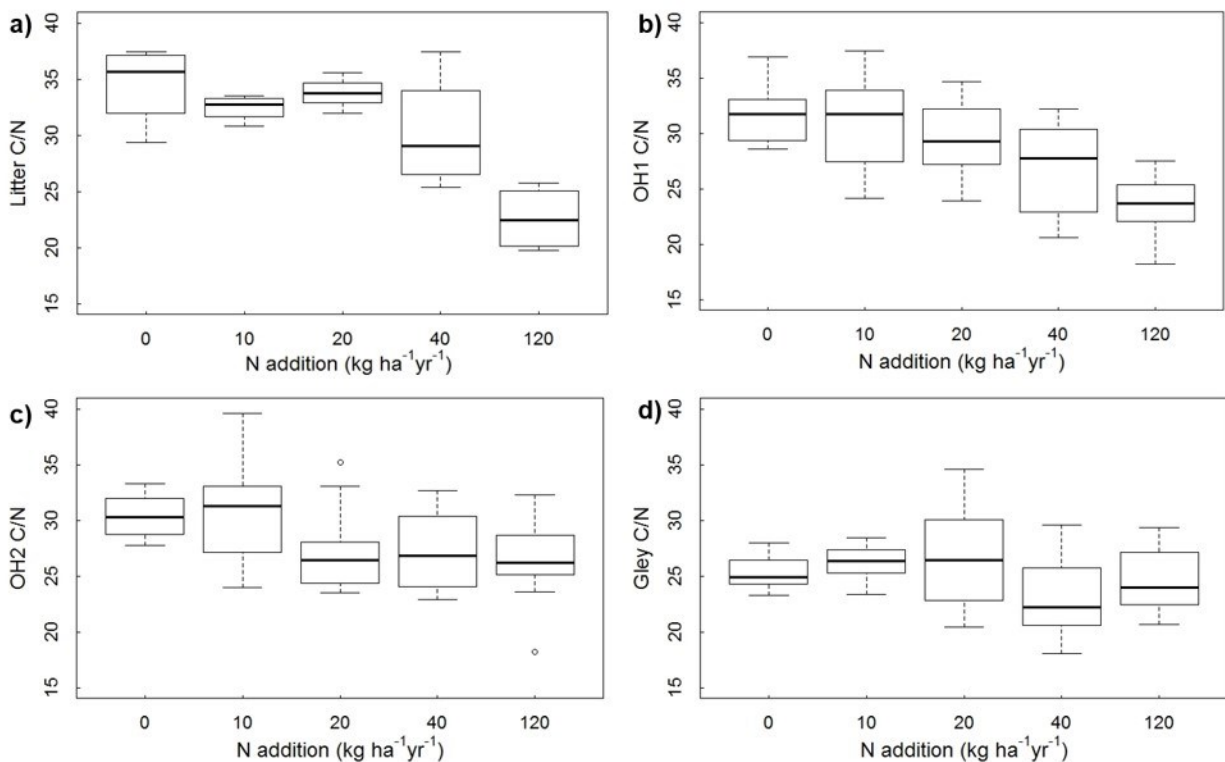
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285 **Figure 4. Soil carbon (C) and nitrogen (N) pools at increasing N deposition (ambient + experimental N addition), by horizon:**
 286 **Litter (a,b), OH1 (c,d), OH2 (e,f), and Gley (g,h). N treatment additions are: control (0) +10 kg N ha⁻¹ y⁻¹, +20 kg N ha⁻¹ yr⁻¹,**
 287 **+40 kg N ha⁻¹ yr⁻¹, +120 kg N ha⁻¹ yr⁻¹. Ambient N deposition is circa. 23 kg N ha⁻¹ yr⁻¹. Fitted using Generalized Additive**
 288 **Models (GAM). Shaded areas represent 95% confidence limits. Vertical dashed line shows the maximum modelled N**
 289 **deposition to UK heathlands.**

290

291 **3.3. Soil carbon and nitrogen concentrations**

292 The highest concentrations of both C and N were in the litter and organic horizons, with both
293 declining strongly in the gley horizon (Table 3). However, there is no significant effect of the N
294 treatment on litter or organic horizon C concentrations (Table 3), but there is a trend of higher C%
295 at the intermediate N treatment levels. The higher N treatments significantly increased N% in the
296 litter and OH1 horizons, and significantly reduced C/N in the litter layer (ANOVA, $F=9.7$,
297 $P<0.001$) and in both OH horizons (OH1:Kruskal-Wallis, $H=17.1$, $P=0.002$; OH2: Kruskal-Wallis,
298 $H=10.2$, $P=0.04$), particularly at the +120N addition. No significant differences in %C, %N or C/N
299 were found between treatments in the Gley horizon.



300

301 **Figure 5. Box plots showing carbon (C)/ nitrogen (N) ratio in the four soil horizons sampled: Litter (a), OH1 (b), OH2 (c)**
302 **and Gley (d). Median value shown by the horizontal line, the inter-quartile range by the outline of the box, smallest and**
303 **largest values that are not classed as outliers by whiskers with bar ends. Outliers more than 1.5 times from the inter-quartile**
304 **range are shown by circles.**

305

306 All N additions of +20 kg N ha⁻¹ yr⁻¹ or higher resulted in a deeper litter (Table 3), storing
 307 significantly more C and N than the control plots. The OH1 horizon increased significantly in
 308 depth, but only at the +20N addition, the other treatments were not significantly different. No
 309 significant differences existed in the OH2 horizon, and the Gley horizon was sampled to a fixed
 310 depth to ensure comparability across the cores.

311

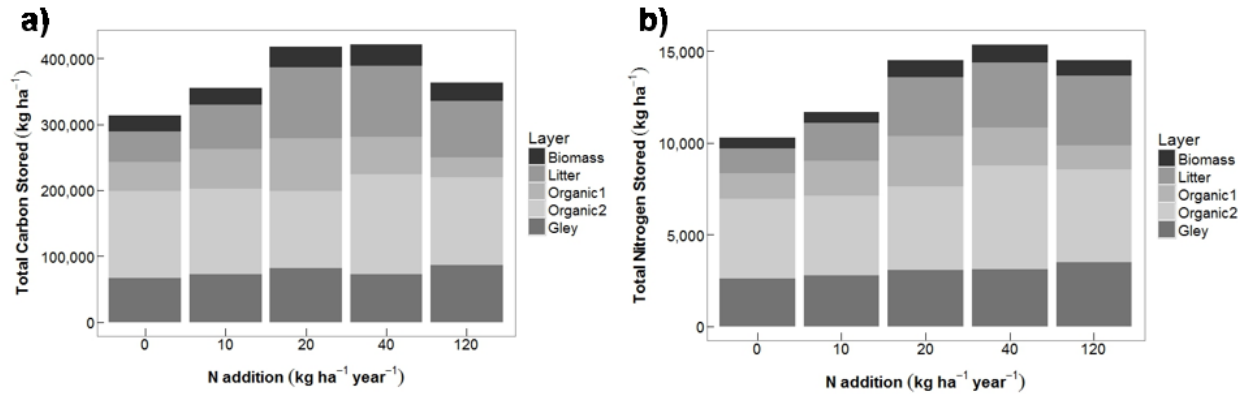
Table 3. Soil carbon (C) %, nitrogen (N) %, C/N ratio and profile depth for Litter, Organic 1, Organic 2 and Gley soil horizons. P values highlighted in bold represent significant differences. Individual deposition levels compared by post-hoc pairwise comparisons, values sharing a letter are not significantly different.

	C%			N%			C/N			Profile depth (m)		
	mean	Std. error	P	mean	Std. error	p	mean	Std. error	p	mean	Std. error	P
Litter												
Control	43.51 a	0.952		1.27 a	0.026		34.54 a	0.954		0.025 a	0.004	
+10	41.00 a	1.200		1.26 a	0.027		32.49 a	0.310		0.040 ab	0.006	
+20	46.17 a	0.166	0.300	1.37 a	0.011	<0.001	33.77 a	0.384	<0.001	0.055 b	0.008	0.001
+40	44.12 a	0.715		1.48 ab	0.044		30.23 a	1.370		0.058 b	0.007	
+120	40.13 a	1.799		1.78 b	0.056		22.58 b	0.760		0.051 b	0.003	
OH 1												
Control	46.56 a	0.612		1.48 a	0.004		31.72 a	0.732		0.019 ab	0.003	
+10	46.27 a	0.668		1.54 a	0.061		30.66 ab	1.222		0.019 ab	0.003	
+20	47.06 a	0.351	0.263	1.63 ab	0.059	0.002	29.38 ab	1.047	<0.001	0.027 a	0.004	0.025
+40	45.33 a	0.687		1.72 ab	0.075		26.83 bc	1.206		0.019 ab	0.002	
+120	44.49 a	1.326		1.92 b	0.088		23.52 c	0.721		0.012 b	0.002	
OH 2												
Control	39.33 a	1.669		1.30 a	0.043		30.38 ab	0.530		0.024	0.002	
+10	38.76 a	2.974		1.28 a	0.061		30.94 a	1.261		0.026	0.005	
+20	40.01 a	1.750	0.261	1.51 a	0.059	0.037	27.13 ab	1.065	0.005	0.020	0.002	0.117
+40	42.80 a	.977		1.58 a	0.075		27.42 ab	1.005		0.027	0.002	
+120	36.46 a	2.329		1.43 a	0.088		26.40 b	1.022		0.027	0.003	
Gley												
Control	9.66 a	0.694		0.38 a	0.024		25.32 a	0.416				
+10	16.13 a	0.601		0.55 a	0.022		28.08 a	0.428				
+20	13.60 a	1.358	0.167	0.51 a	0.045	0.277	26.71 a	1.414	0.074	n/a		
+40	11.49 a	1.384		0.49 a	0.049		23.09 a	0.975		fixed at 0.02 m for all		
+120	13.91 a	2.625		0.55 a	0.082		23.57 a	1.620		Cores		

312

313 The increase in soil C per unit of N added ($\Delta C_{seq}/\Delta N$, in kg C per kg N) is very high at the lowest
 314 N addition loads, peaking at 121 kg C kg N⁻¹ in the +20N treatment (see Table 2), with 62 and 10

315 kg C/ kg N at +40N and +120N, respectively. Across all the plots and both C and N pools, the
 316 increases were most obvious in the biomass, litter and organic soil horizons (Figure 6).

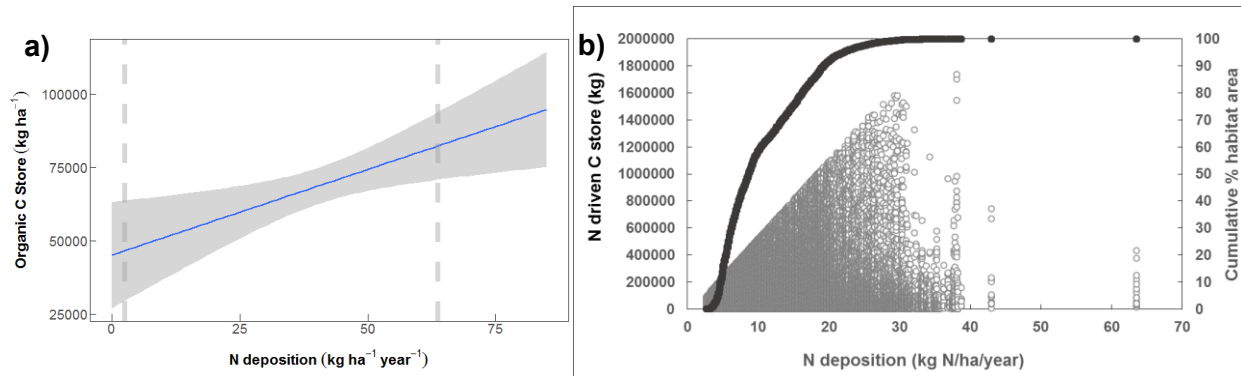


317
 318 **Figure 6.** Amounts of (a) carbon (C) and (b) nitrogen (N) in plant biomass and each soil horizon, plotted with biomass at
 319 the top descending through the soil profiles.

320

321 3.4. UK heathland soil carbon

322 Using the relationship between litter and organic soil (OH1 and OH2) C and N deposition (Figure
 323 7a), we can extrapolate the results of the experimental plots to a UK-wide scale to provide an initial
 324 estimate of the gains in C that are stimulated by N deposition in heathland over a typical
 325 management cycle of around 20 years (Figure 7b). Based on a UK heathland area of approximately
 326 2.5 million hectares, the total heathland C store for the top 15 cm of litter and soil (including
 327 mineral layer) is an estimated 172 Mt C, and for the litter and organic component only, it is 130
 328 Mt C. If we assume a pre-industrial N deposition of 1 kg N ha⁻¹ yr⁻¹ (based on contemporary
 329 measurements in northern Sweden – DeLuca et al. 2008), then the additional C sequestered due to
 330 contemporary N deposition above this level is 14 Mt, or around 0.7 Mt C per year over a 20 year
 331 management cycle.



332

333 **Figure 7. a) The fitted Generalized Additive Model (GAM) relationship between the organic C store (Litter + OH1 + OH2)**
 334 **and nitrogen (N) deposition (ambient + experimental N addition). Ambient N deposition is circa 23 kg ha⁻¹ yr⁻¹. Shaded area**
 335 **indicates 95% confidence limits, vertical dashed lines show the minimum and maximum modelled N deposition to UK**
 336 **heathlands. b) Area weighted N driven C sequestration totalled across plant litter, and OH1 and OH2 soil horizons is plotted**
 337 **on the primary y-axis – dark grey dots. Cumulative heathland habitat area at increasing N deposition on the secondary y-**
 338 **axis – solid black line.**

339

340 4. Discussion

341 Consistent with findings in forest ecosystems and previous heathland data, measured heathland
 342 litter and soil C storage increased with N addition at the Ruabon experimental site. However, we
 343 also found that this N-induced C accumulation appeared to saturate at high deposition loads,
 344 decreasing from 101 and 121 kg C kg N⁻¹ at +10N and +20N, to just 10 kg C kg N⁻¹ at +120N.
 345 This saturation was reflected in the falling soil C/N stoichiometry, particularly at the highest
 346 +120N addition. Of the total C accumulated by the ecosystem, an average of 8% was in plant
 347 biomass, 23% in litter, 54% in the organic soil, and 15% in the mineral soil.

348

349 The results suggest that at the lower levels of N deposition, C sequestration through photosynthesis
 350 exceeds C lost through autotrophic and heterotrophic respiration. When the soil cores were
 351 sampled in 2008, the site had last been managed 20 years earlier, and the plots were between their
 352 mature (~15 years) and degenerate (~25 years) stages of growth (Gimingham, 1972). *Calluna*
 353 biomass is at its most productive, with the greatest annual increments, during the mature stage

354 (Gimingham, 1972). Therefore, for N-induced C pools in shrublands to be sustainable in the
355 longer-term, ecosystems should be managed in a way that enables the vegetation to remain in this
356 active 'mature' growth phase. Without management the C sink could saturate during the
357 degenerate stage within a short timescale.

358

359 Growth measurements at the study site on older plots demonstrated the same pattern of rapid
360 growth response to N addition in the years following commencement of treatments (Carroll et al.
361 1999), followed by a slowing of response. The authors suggested that N deposition seemed to
362 advance the physiological age of *Calluna* (Carroll et al. 1999), with plants receiving higher levels
363 of N deposition moving through the growth phases sooner. This is reflected in the 6-year earlier
364 stabilisation in canopy height at the highest N addition (Figure 2) as the canopy opens and supports
365 the saturating response observed in the soil and litter C and N pools. Observations at the site reveal
366 that the +10N and +20N plants were in the mature and mature-degenerate growth-phases of the
367 *Calluna* life cycle, whereas the highest +120N plants were notably more degenerate. Koptittke et
368 al. (2012) measured C stock in the vegetation and soil at 11, 18 and 27 years post-management at
369 a Dutch lowland heathland and found that biomass peaked at around 18 years and fell back in the
370 older plots, although still remained greater than in the 11 year old plots. In their study, soil organic
371 C stores did not follow the same pattern, although C in the upper mineral component peaked in the
372 18 year old plots. Biomass C values in the control plots of around 10000 kg ha⁻¹ were at the lower
373 end of the range found in some studies (e.g. Santana et al. 2016 – range circa 8000-18000 kg ha⁻¹
374 ¹) but at the upper end of others (e.g. Milne et al. 2002 – range circa 3000-9000 kg ha⁻¹).

375

376 Modelling of C stocks on heather moorland growing on blanket peat suggests a strong relationship
377 between canopy height and gross photosynthesis (Dixon et al. 2015). However, as plants became
378 taller, the relationship between canopy height and ecosystem respiration became stronger,
379 suggesting that C stocks were not sustainable in the long-term without active ecosystem
380 management (Dixon et al. 2015). In the current study, growth in the ambient and low N plots
381 saturated 21 years after management and in the high N plots 15 years after management. A spatial
382 survey study across UK heathlands has demonstrated a link between N deposition and canopy
383 height (Southon et al. 2013), suggesting that there is potential for N-driven increases in C
384 sequestration in heather biomass at a regional scale. The increase in plant biomass in the current
385 study has contributed directly to larger litter stores in the N addition plots, and in turn to greater
386 sequestration of organic soil C. The mechanism for this appears to have been the markedly higher
387 litter fall observed in the elevated N plots. With a range of 1700-7500 kg ha⁻¹ yr⁻¹, this is similar
388 to work on older plots at the study site at the site (Carroll et al. 1999 – range circa 3800 – 6600 kg
389 ha⁻¹ yr⁻¹; Pilkington et al. 2005b – range circa 3200 – 7200 kg ha⁻¹ yr⁻¹), and the mean values from
390 the control plots similar to those in other studies (e.g. Chapman 1967 – 3160 kg ha⁻¹ yr⁻¹; Trinder
391 et al. 2008 – 2760 kg ha⁻¹ yr⁻¹). However, at a landscape-scale, grazing will influence biomass and
392 therefore also litter (Smith et al. 2015).

393

394 With a range of 13-138 kg C kg N⁻¹, soil C sequestration calculated from this study is more
395 variable, and on average higher, than that calculated by de Vries et al. (2009) (20-34 kg C kg N⁻¹)
396 from the smaller “old” plots at Ruabon. This appears primarily due to higher rates measured in the
397 litter layer in this study and the lower N additions in this study; C sequestration appears to saturate
398 at higher N. We also find higher N-driven soil C storage rates in this heathland than those

399 calculated from most forest data (e.g. Pregitzer et al. 2007; Hyvonen et al. (2008), but see Magnani
400 et al. 2007). There is however, considerable variability in the published data from forests with
401 Pregitzer et al. (2007) presenting an average C sequestration of 23 kg C kg N⁻¹ following 10 years
402 of N, although the study included a much larger maximum of 63 kg C kg N⁻¹. This figure was also
403 after removal of the litter layer. Hyvonen et al. (2008) presented a soil C sequestration range of 3
404 - 20 kg C kg N⁻¹, however, total N added was in the range 600 – 1800 kg N ha⁻¹ yr⁻¹ over 14-30
405 years. In this study, the total amount of N added to the system was 100 – 1200 kg ha⁻¹ yr⁻¹ over 10
406 years. Decomposition processes will therefore play a significant role in controlling the proportion
407 of plant or tree litter that remains in a system over the medium to longer term and this may mean
408 that figures quoted from relatively short-term studies exaggerate the long-term storage potential
409 that elevated N deposition provides. However, heathland soil is often waterlogged or partly
410 anaerobic, and vegetation is dominated by ericaceous shrubs that are high in lignin (*Calluna* at
411 Ruabon) (Berg and Laskowski, 2006) – both of these factors slow decomposition rates and increase
412 the potential for net C accumulation.

413

414 Extrapolation of the experimental data to a heathland area of just under 2.5 million hectares gives
415 an estimated pool of 172 Mt C in the top 15 cm of soil for all UK heathlands, and 120 Mt C in the
416 litter and organic component of the soil. Both these figures compare well to an estimated 120 Mt
417 C based on UK Countryside Survey data (Ostle et al. 2009), which may be slightly lower than our
418 value since it includes on balance more heathlands located further north in the UK, where growth
419 rates are likely slower. Based on our experiment we estimate that 14 Mt C, or 8% of the total, has
420 accumulated in UK heathland as a result of enhanced N deposition over a 20-year management
421 cycle. This equates to average figure of 0.7 Mt C, or 2.52 Mt CO₂e per annum and represents

422 0.44% of UK annual GHG emissions of 568 Mt CO₂e (UK National Statistics, 2013). As the
423 response appeared to saturate as the canopy moved into the degenerate stage (observed in the
424 highest +120N treatment), management interaction is required to sustain growth rate; in areas
425 without regular management, C sequestration rates are likely to be much lower. The absence of
426 data from other UK habitats such as bogs or grasslands means that it is difficult to put these N
427 driven increases in heathland C in context. In bogs, experimental N deposition initially increased
428 C sequestration but as shrub cover increased, C losses became greater (Bubier et al. 2007) and,
429 whilst owing to a larger surface area, grasslands may hold a greater overall C-store, faster
430 decomposing processes could mean less C entering the soil from plant litter. Further research
431 should aim to elucidate responses to N in these ecosystems.

432

433 Whilst our modelling clearly oversimplifies responses, as many other factors not least climate, are
434 likely to affect plant growth and C storage at a countrywide-scale, it highlights the potential
435 magnitude of N-driven C sequestration in heathlands. The total heathland organic C store of around
436 120 Mt C or 432 Mt CO₂e represents around 76 % of annual UK GHG emissions. This represents
437 just under 8% of UK soil C in the top 15 cm soil (Carey et al. 2008) and in this context the long-
438 term stabilisation of this pool is important. This stabilisation will depend upon climate and
439 management intensity e.g. shallow burning or cutting of biomass compared to intensive burns to
440 the litter layer.

441

442 There may also be alternative succession scenarios for heathlands that could stabilise and enhance
443 this pool. In the UK, heathlands are often a plagio-climax community that in drier areas would
444 usually shift to woodland if unmanaged. It is also worth noting that heathland ecosystems on

445 organo-mineral soils, such as Ruabon, lie on an ecological continuum that extends to *Calluna-*
446 *Sphagnum* blanket bog over deep peat on poorly drained areas nearby. Thus it is possible that
447 increased organic matter accumulation due to N addition could shift some heathland ecosystems
448 more towards C-accumulating peatland ecosystems (e.g. Turunen et al., 2004), provided that N
449 deposition levels and/or management practices are not so intensive as to restrict the growth of peat-
450 forming species (e.g. Evans et al., 2014). Both these successions would provide long-term
451 stabilisation of soil C with little management interference.

452

453 It must also be remembered that N deposition has been associated with large-scale reductions in
454 biodiversity. In heathlands specifically, N deposition is linked with falls in species richness of up
455 to 40%, and shifts in species composition (Southon et al. 2013; Field et al. 2014), with lower plants
456 such as bryophytes and forbs proving particularly sensitive in both experiments and gradient
457 surveys at the expense of faster growing grasses and shrubs (Edmondson et al. 2013; Southon et
458 al. 2013).

459

460 **5. Conclusion**

461 Plant litter, organic soil C accumulation and canopy height at Ruabon show clear positive
462 responses to moderate levels of N deposition, suggesting that C sequestration of ericaceous
463 ecosystems is increased by anthropogenically-enhanced N deposition. The amount and duration
464 of this extra C storage will depend on many factors, including climate, management, the level of
465 long-term N deposition, and the level of N saturation capacity of the ecosystem. The relationship
466 between N and plant growth are reflected at the landscape scale, suggesting the potential for N-
467 driven increases in C sequestration at levels of N deposition found across the UK and Western

468 Europe. However, in relation to the CO₂ equivalents released by human activity, the gains in C
469 storage are relatively modest. When considered in the context of falling biodiversity and altered
470 species composition in heathland, policy focus should remain on reducing N emissions.

471

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479

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648 **Table 2. Summary of soil carbon (C) stores with C sequestration per kg nitrogen (N) addition over the 10 year duration of the**
 649 **experiment.**

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N addition (kg ha ⁻¹ yr ⁻¹)	Total additional N (10 years treatments)	Total Soil C			Litter C			Organic Horizons Total C (OH1 + OH2)			Eluviated gley Horizon C (EAG)		
		(kg ha ⁻¹)	ΔC from ambient	kg C kg N-1	(kg ha ⁻¹)	ΔC from ambie nt	Δ% from ambie nt	(kg ha ⁻¹)	ΔC from ambient	Δ% from ambient	(kg ha ⁻¹)	ΔC from ambient	Δ% from ambient
0	0	72289	-	-	11619	-	-	44123	-	-	16547	-	-
10	100	82421	10131	101	16756	5137	44	47612	3489	8	19763	3216	9
20	200	96586	24297	121	26935	15316	132	49201	5078	12	20402	3855	24
40	400	97190	24900	62	26884	15264	131	52204	8081	18	18102	1555	9
120	1200	83847	11557	10	21341	9722	84	40784	-3340	-8	21722	5175	31

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