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

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Abstract

The large increases in reactive nitrogen (N) deposition in developed countries since the Industrial Revolution have had a marked impact on ecosystem functioning, including declining species richness, shifts in species composition, and increased N leaching. A potential mitigation of these harmful effects is the action of N as a fertiliser, which, through increasing primary productivity (and subsequently, organic matter production), has the potential to increase ecosystem carbon (C) storage. Here we report the response of an upland heath to 10 years of experimental N addition. We find large increases in plant and soil C and N pools, with N-driven C sequestration rates in the range of 13-138 kg C kg N^{-1}. These rates are higher than those previously found in forest and lowland heath, mainly due to higher C sequestration in the litter layer. C sequestration is highest at lower N treatments (10, 20, and 40 kg N ha^{-1} yr^{-1} above ambient), with evidence of

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

1. Introduction

Since the Industrial Revolution and throughout most of the 20th century the level of reactive nitrogen (N) in the atmosphere (primarily NH_3, NH_4^+, NO_x, NO_3^-, and organic N) has increased due to fossil fuel burning and agricultural intensification (Galloway et al., 2004). Between 1860 and 1990, there was a 10-fold increase in reactive N, with a further doubling predicted by 2050 (Galloway et al., 2004). This greatly enhanced atmospheric N deposition has had profound effects on ecosystem functioning, including reduced terrestrial plant species diversity, altered species composition, and leaching of N to freshwater habitats following N saturation (Stevens et al. 2004; Clark and Tilman, 2008; Dise et al. 2011; Phoenix et al. 2012; Field et al. 2014).

Potentially counteracting these negative effects of elevated N deposition is increased carbon (C) sequestration into ecosystems through enhanced plant growth (Yue et al. 2016) and, in some cases, a retardation of long-term decomposition rates (Berg and Laskowski 2006), thereby mitigating rising atmospheric CO_2. In forests, both regional-scale N-gradient studies and N-addition experiments have demonstrated N-driven increases in ecosystem C storage ranging from 12-36 kg C kg N^{-1} ha^{-1} (De Vries et al. 2006; Hyvönen et al. 2007; Pregitzer et al. 2007). However, most of the additional C stored in forests in response to N deposition is in new tree biomass rather than soil (Nadelhoffer et al. 1999), with high rates of soil C turnover further suggesting that Forests represent poor long-term soil C stores (Tipping et al. 2010; Mills et al. 2014). In contrast, ecosystems such as bogs and heathlands, that primarily sequester new C in soil can be significant C sinks for hundreds or thousands of years (Dise, 2009). This is due to high moisture levels and vegetation rich in recalcitrant compounds (e.g. *Sphagnum* mosses and ericaceous plants such as *Calluna*) limit decomposition rates, causing a build-up of soil organic matter (Anderson and Hetherington, 1999; Berg and Laskowski, 2006).

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

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

Here we report the first detailed analysis of organic and mineral soil C content in response to experimental N addition on an upland heath ecosystem. We also upscale the data to estimate the magnitude of N induced C sequestration at a landscape scale. We hypothesise that 1) N addition increases the rate of sequestration of C in the organic and mineral soil horizons, 2) that C/N stoichiometry is not fixed and therefore the rate of C sequestration will vary in response to N addition, and 3) N-induced sequestered C in heathland is a potentially significant sink in relation to the CO_2 equivalents ($CO_{2\ eq}$) emitted in the UK through human activities.

2. Methods

2.1. Study site

Ruabon Moor is an upland heath situated at an altitude of 480 m, approximately 6 km north of Llangollen in North Wales, UK (Figure 1, UK Grid Reference SJ224491). Annual precipitation is approximately 1000 mm, and total inorganic N deposition in 2008 was estimated as 23.1 kg N $ha^{-1}yr^{-1}$ from APIS (Air Pollution Information System) data, which uses the CBED model (APIS, 2008). The canopy is dominated by *Calluna*, although where burning has taken place or a gap in the canopy occurs naturally, *Vaccinium myrtillus* grows well before it is shaded out by *Calluna* regrowth. Understory vegetation consists mainly of the moss *Hypnum jutlandicum*; this combination of vegetation gives the site a British NVC classification of H12 *Calluna – Vaccinium myrtillus* heath (Rodwell, 1991) or a European EUNIS classification of F4.2. Soil is an iron pan stagnopodzol (F.A.O. Placic Podzol) (Evans et al. 2006). N additions (of 0, 40, 80 and 120 kg N $ha^{-1}yr^{-1}$) to 20 plots at the site began in 1989 (Caporn et al. 1995) and these 'old' plots (1 × 1 m) were used by de Vries et al. (2009) in their study of N-induced C sequestration. In 1998, 36 rectangular (2 × 2 m) 'new' plots were established. N as NH_4NO_3 solution is mixed with rainwater collected at the site and applied monthly to these new plots using a watering can at more realistic N additions of 0, 10, 20, 40 kg N $ha^{-1}yr^{-1}$ ('+0, +10N, +20N, and +40N', respectively). A higher 120 kg N $ha^{-1}yr^{-1}$ (+120N) treatment is included to increase the N response gradient. A further 16 plots incorporate phosphorus additions however, these are not used in this study. After 10 years of treatments, the cumulative additional N by treatment are 0, 100, 200, 400 and 1200 kg N $ha^{-1}y^{-1}$ above ambient N deposition i.e. the lowest N treatment adds 10 kg N ha^{-1} yr^{-1}, so after 10 years this is an additional 100 kg N input. Each treatment is replicated 4 times in a randomized block design of overall size 20 x 20 m, with 20 plots in total used in this study. Earlier responses to N on these plots of biological and chemical indicators have been reported by Edmondson et al. (2010).

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

2.2. Plant biomass, canopy height and litter fall measurements

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

Equation (1) $$\textbf{Biomass (g)} = \mathbf{1.94*(Canopy\ height)^2 - 128.85*Canopy\ height + 3017.8}$$

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

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

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

2.3. Soil carbon and nitrogen

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

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

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

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

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

2.4. Statistical analysis

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

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

2.5. Nitrogen deposition mapping to heathlands and upscaling

Concentration based estimated deposition (CBED) for N (and other pollutants) is mapped for the UK on a 5x5 km grid (RoTAP, 2012). Values are derived from measurements of air concentrations of gases and aerosols, and concentrations in precipitation from the UK Eutrophying and Acidifying Pollutants (UKEAP) network. The measurements are interpolated to generate concentration maps for the UK. The ion concentrations in precipitation are combined with the UK Met Office annual precipitation map to generate maps of wet deposition. The wet deposition includes direct deposition of cloud droplets to vegetation, and an orographic enhancement factor for the concentration of precipitation in upland regions due to the seeder-feeder effect (Fowler et al. 1988). Gas and particulate concentration maps are combined with spatially distributed estimates of vegetation-specific deposition velocities (Smith et al. 2000) to generate dry deposition. Figure 1 shows mean total N (wet + dry, oxidised + reduced) deposition for 2011-2013 for UK areas of dwarf shrub heath habitat. The habitat distribution map has been generated for UK research on the impacts of air pollution using critical loads (Hall et al. 2015) and is defined from the CEH land cover map 2000 (LCM2000: Fuller et al. 2002), further refined using ancillary data sets on species distributions (Preston et al. 2002). The N deposition in Figure 1 is mapped for all 1x1 km squares containing dwarf shrub heath. An estimate of C sequestration in litter and the organic component of the soil (OH1 + OH2), by heathland area, was then modelled for each 1x1 km square, using the linear relationship below, derived from the data gathered in this study:

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

R^2=0.17, P=0.007.

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

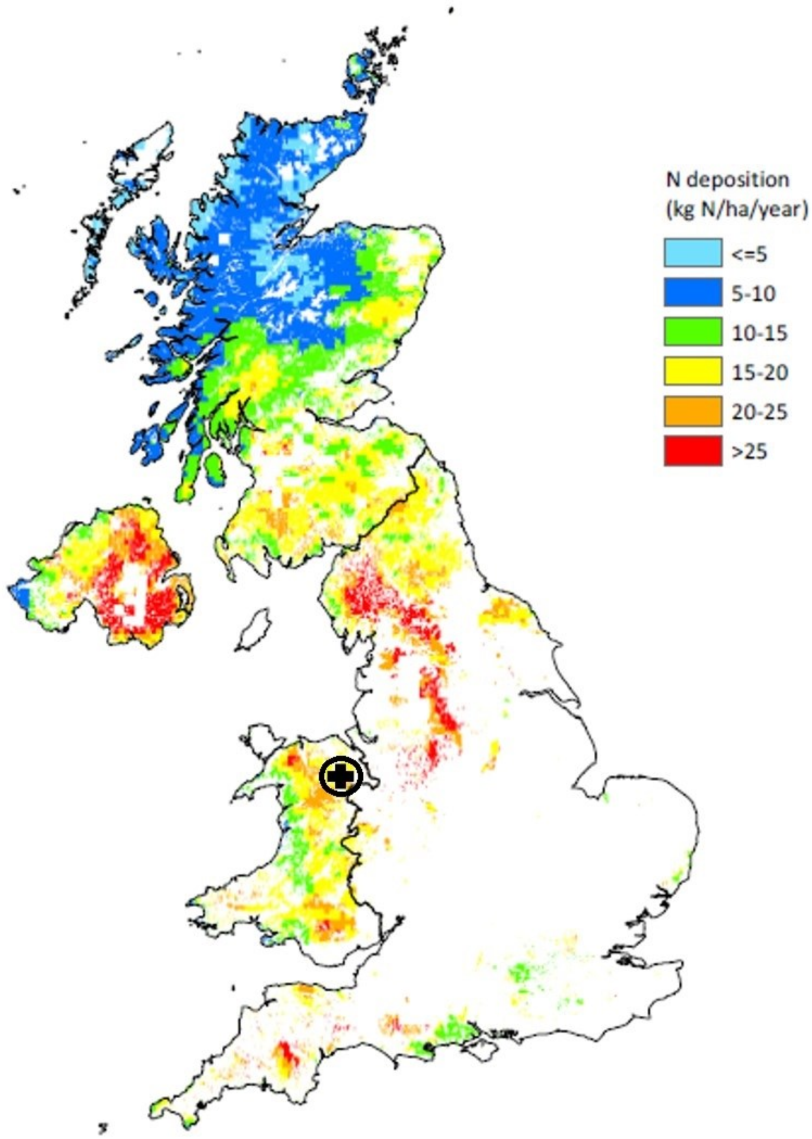


Figure 1. The study site on Ruabon Moor in North Wales (marked with a ⊕) over a UK dwarf shrub heath distribution map shaded by nitrogen (N) deposition. UK heathland N deposition range is 2.7 to 63.6 kg N ha^{-1}yr^{-1}.

3. Results

3.1. Plant growth, biomass and litter fall

Calluna showed a strong growth response following commencement of N additions in 1998, with increasing canopy height reflecting increasing N additions from the control (ambient N approximately 23 kg N ha^{-1} yr^{-1}) up to +120N (Figure 2). With the +120N treatment canopy height stabilised by 2003 (5 years after treatment, and 15 years after the last management by fire) and began to decline in 2009 after 11 years of treatment. This reflected a shift from plants in the mature to the degenerate stage, with active shoot growth declining and the canopy opening. In all other treatment plots stabilisation occurred later, around 2009, followed three years later by the decline phase.

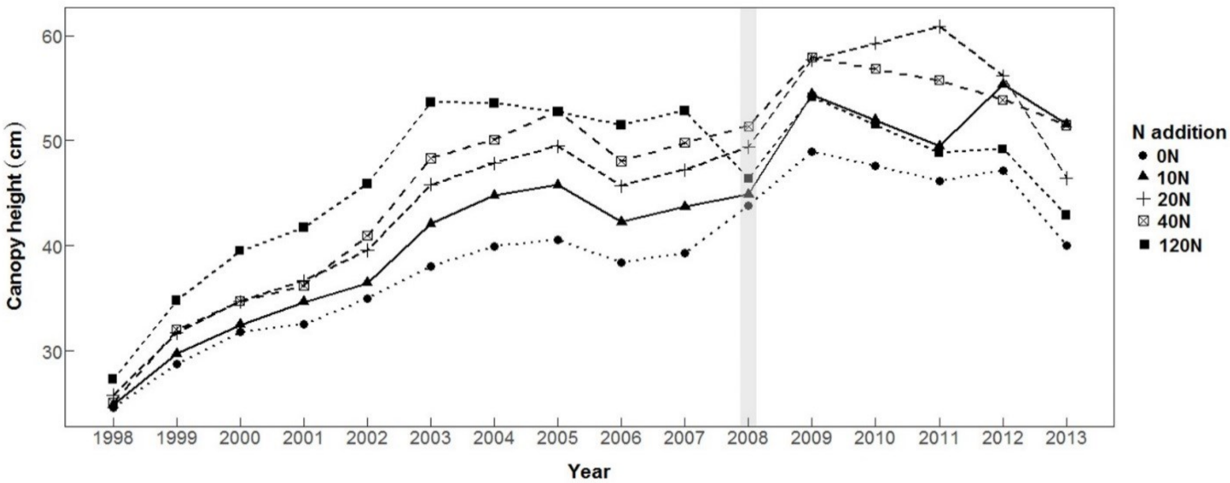


Figure 2. Mean annual *Calluna* canopy height taken from 8 measurements per plot, 32 at each nitrogen (N) addition level. N treatment additions are: Control, +10 kg N, +20 kg N, +40 kg N, +120 kg N. Shaded bar illustrates when the sampling of the soil cores for CN analysis occurred.

Total standing biomass C modelled from an off-plot harvest predicts increases in response to N addition, although incremental N above +20 kg N ha^{-1} yr^{-1} is not efficiently used, leading to falling incremental C per kg N added and increasing tissue N% (Table 1). However, at lower levels of N addition that are more relevant to those observed in the real-world, around 13 kg C are sequestered for every 1 kg increase in N deposition. On-site observations suggest much of this increased biomass is held in woody stems. Litter fall also increased in line with biomass from a mean of 2534 kg ha^{-1} yr^{-1} in the control plots to 5272 kg ha^{-1} yr^{-1} in the +120N treatment, with an overall range of 1769 – 7487 kg ha^{-1} yr^{-1} (R^2=0.49, P=0.003).

Table 1. Summary of annual litter productivity (litter fall), biomass carbon (C) and nitrogen (N) stores and C 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 (ΔCseq/ΔN (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

3.2. Soil carbon and nitrogen CN pools

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

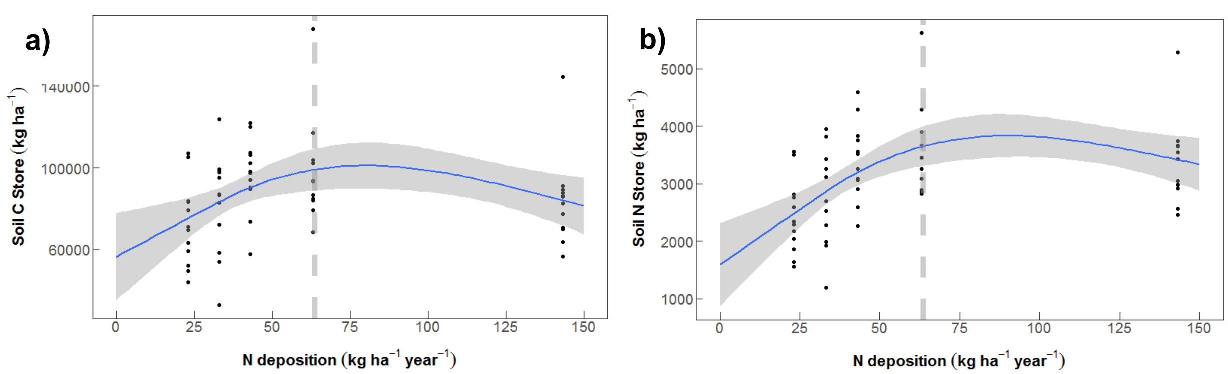
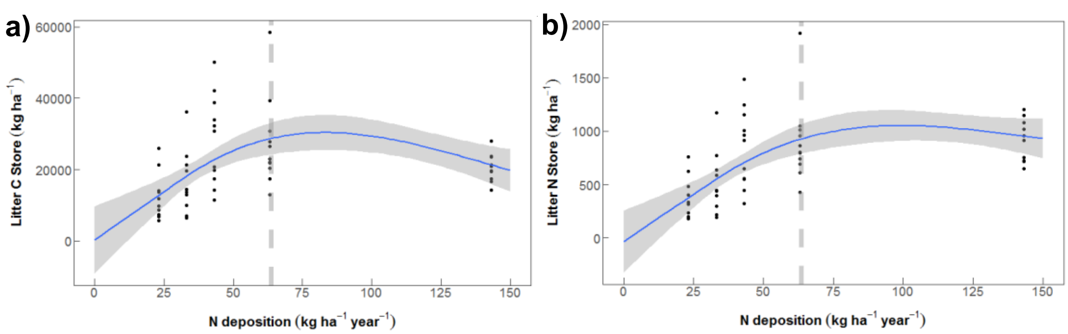


Figure 3. Total soil pools of a) carbon (C) and b) nitrogen (N) at increasing N deposition (ambient + experimental N addition). Ambient nitrogen deposition is circa. 23 kg N ha^{-1} yr^{-1}. Fitted using a Generalized Additive Model (GAM). Shaded

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

Of all measured horizons, litter layer C and N pools increased the most strongly with increasing N deposition, up to the +120N treatment (Figure 4 a) and b); Deviance explained =26.4%, F=10.02, P<0.001 for C; Deviance explained=38.9%, F=18.59, P<0001 for N). At +120N, the litter C pool declined to lower than that of both the +20 and +40N addition treatments (see Table 2 and Figure 4a). Litter N also appeared to saturate (Figure 4), although less sharply than litter C, reflecting the large reduction in C/N at the highest +120N addition. The OH1 horizon also showed a positive relationship between N deposition and C and N storage (Figure 4; Deviance explained =12%, F=2.9, P<0.001 for C; Deviance explained=10.8%, F=2.65, P=0.05 for N), whilst the OH2 and Gley horizons showed weak but non-significant, general trends of increasing soil C and N pools. Across the sum totals of C and N in the litter, OH1 and OH2 soil horizons, considering the difficulty in separating the profiles consistently, there was a significant relationship with N deposition (Deviance explained=16.4%, F=5,13, P=0.009 for C, see Figure 7a; Deviance explained=25.2%, F=9.52, P<0001 for N). It should be noted that, whilst the highest rate of increase in soil C and N occurred in the litter layer, the largest total increase of C and N was in the organic horizons (OH), reflecting the higher bulk densities of these horizons.



c) d)

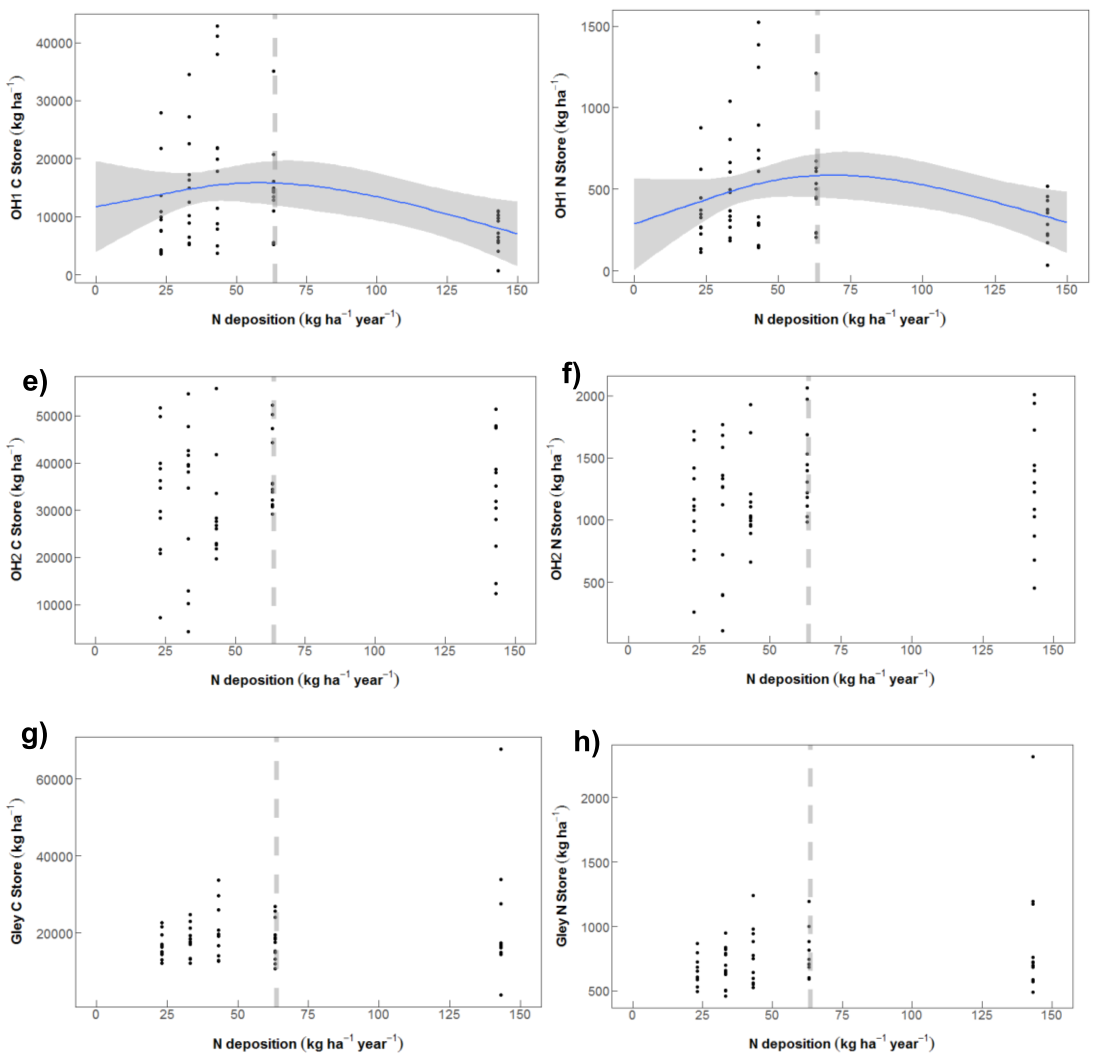


Figure 4. Soil carbon (C) and nitrogen (N) pools at increasing N deposition (ambient + experimental N addition), by horizon: Litter (a,b), OH1 (c,d), OH2 (e,f), and Gley (g,h). N treatment additions are: control (0) +10 kg N ha^{-1} y^{-1}, +20 kg N ha^{-1} yr^{-1}, +40 kg N ha^{-1} yr^{-1}, +120 kg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

3.3. Soil carbon and nitrogen concentrations

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

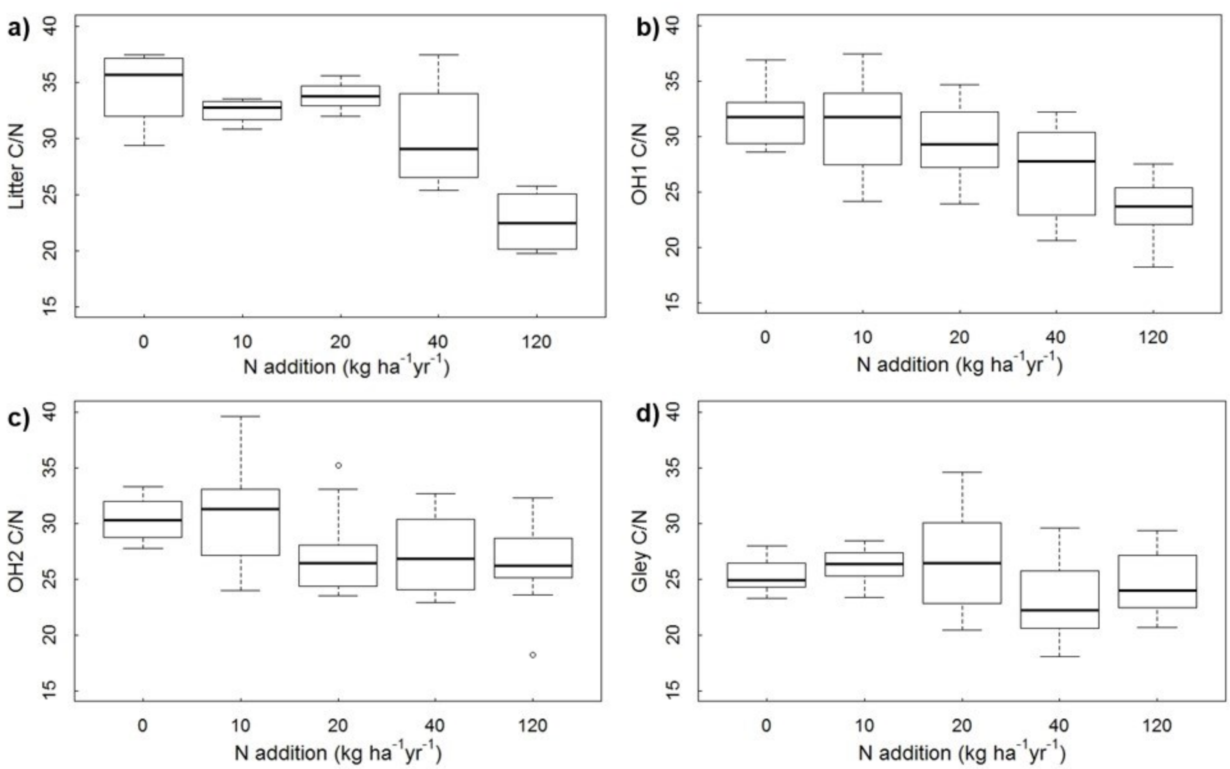


Figure 5. Box plots showing carbon (C)/ nitrogen (N) ratio in the four soil horizons sampled: Litter (a), OH1 (b), OH2 (c) and Gley (d). Median value shown by the horizontal line, the inter-quartile range by the outline of the box, smallest and largest values that are not classed as outliers by whiskers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

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

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 fixed at 0.02 m for all Cores		
+40	11.49 a	1.384		0.49 a	0.049		23.09 a	0.975				
+120	13.91 a	2.625		0.55 a	0.082		23.57 a	1.620				

The increase in soil C per unit of N added (ΔCseq/ΔN, in kg C per kg N) is very high at the lowest N addition loads, peaking at 121 kg C kg N^{-1} in the +20N treatment (see Table 2), with 62 and 10 kg C/ kg N at +40N and +120N, respectively. Across all the plots and both C and N pools, the increases were most obvious in the biomass, litter and organic soil horizons (Figure 6).

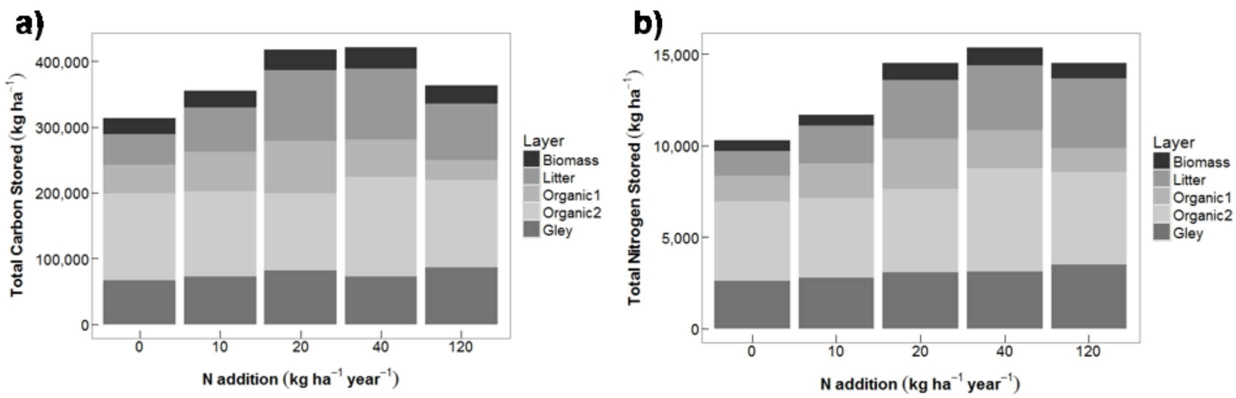


Figure 6. Amounts of (a) carbon (C) and (b) nitrogen (N) in plant biomass and each soil horizon, plotted with biomass at the top descending through the soil profiles.

3.4. UK heathland soil carbon

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

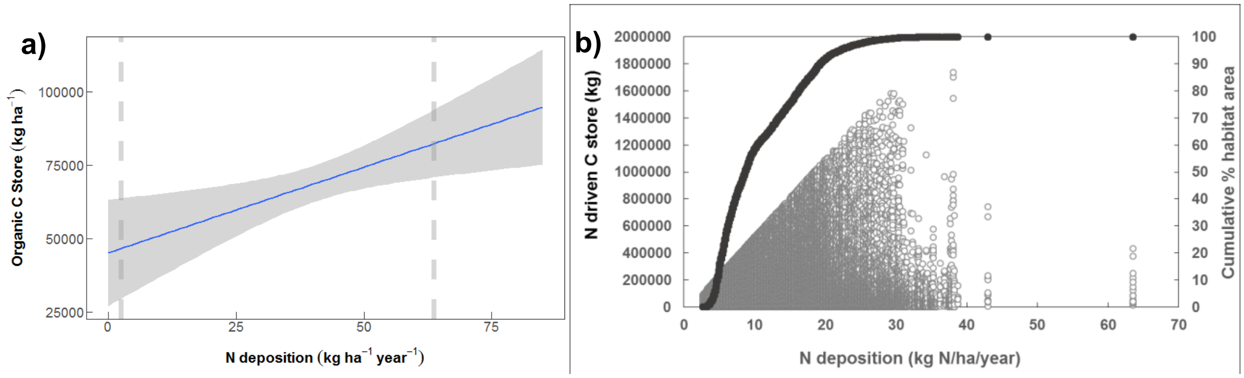


Figure 7. a) The fitted Generalized Additive Model (GAM) relationship between the organic C store (Litter + OH1 + OH2) and nitrogen (N) deposition (ambient + experimental N addition). Ambient N deposition is circa 23 kg ha^{-1} yr^{-1}. Shaded area indicates 95% confidence limits, vertical dashed lines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

4. Discussion

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

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

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

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

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

With a range of 13-138 kg C kg N^{-1}, soil C sequestration calculated from this study is more variable, and on average higher, than that calculated by de Vries et al. (2009) (20-34 kg C kg N^{-1}) from the smaller “old” plots at Ruabon. This appears primarily due to higher rates measured in the litter layer in this study and the lower N additions in this study; C sequestration appears to saturate at higher N. We also find higher N-driven soil C storage rates in this heathland than those calculated from most forest data (e.g. Pregitzer et al. 2007; Hyvonen et al. (2008), but see Magnani et al. 2007). There is however, considerable variability in the published data from forests with Pregitzer et al. (2007) presenting an average C sequestration of 23 kg C kg N^{-1} following 10 years of N, although the study included a much larger maximum of 63 kg C kg N^{-1}. This figure was also after removal of the litter layer. Hyvonen et al. (2008) presented a soil C sequestration range of 3 - 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 years. In this study, the total amount of N added to the system was 100 – 1200 kg ha^{-1} yr^{-1} over 10 years. Decomposition processes will therefore play a significant role in controlling the proportion of plant or tree litter that remains in a system over the medium to longer term and this may mean that figures quoted from relatively short-term studies exaggerate the long-term storage potential that elevated N deposition provides. However, heathland soil is often waterlogged or partly anaerobic, and vegetation is dominated by ericaceous shrubs that are high in lignin (*Calluna* at Ruabon) (Berg and Laskowski, 2006) – both of these factors slow decomposition rates and increase the potential for net C accumulation.

Extrapolation of the experimental data to a heathland area of just under 2.5 million hectares gives an estimated pool of 172 Mt C in the top 15 cm of soil for all UK heathlands, and 120 Mt C in the litter and organic component of the soil. Both these figures compare well to an estimated 120 Mt C based on UK Countryside Survey data (Ostle et al. 2009), which may be slightly lower than our value since it includes on balance more heathlands located further north in the UK, where growth rates are likely slower. Based on our experiment we estimate that 14 Mt C, or 8% of the total, has accumulated in UK heathland as a result of enhanced N deposition over a 20-year management cycle. This equates to average figure of 0.7 Mt C, or 2.52 Mt CO_2e per annum and represents

0.44% of UK annual GHG emissions of 568 Mt CO_2e (UK National Statistics, 2013). As the response appeared to saturate as the canopy moved into the degenerate stage (observed in the highest +120N treatment), management interaction is required to sustain growth rate; in areas without regular management, C sequestration rates are likely to be much lower. The absence of data from other UK habitats such as bogs or grasslands means that it is difficult to put these N driven increases in heathland C in context. In bogs, experimental N deposition initially increased C sequestration but as shrub cover increased, C losses became greater (Bubier et al. 2007) and, whilst owing to a larger surface area, grasslands may hold a greater overall C-store, faster decomposing processes could mean less C entering the soil from plant litter. Further research should aim to elucidate responses to N in these ecosystems.

Whilst our modelling clearly oversimplifies responses, as many other factors not least climate, are likely to affect plant growth and C storage at a countrywide-scale, it highlights the potential magnitude of N-driven C sequestration in heathlands. The total heathland organic C store of around 120 Mt C or 432 Mt CO_2e represents around 76 % of annual UK GHG emissions. This represents just under 8% of UK soil C in the top 15 cm soil (Carey et al. 2008) and in this context the long-term stabilisation of this pool is important. This stabilisation will depend upon climate and management intensity e.g. shallow burning or cutting of biomass compared to intensive burns to the litter layer.

There may also be alternative succession scenarios for heathlands that could stabilise and enhance this pool. In the UK, heathlands are often a plagio-climax community that in drier areas would usually shift to woodland if unmanaged. It is also worth noting that heathland ecosystems on organo-mineral soils, such as Ruabon, lie on an ecological continuum that extends to *Calluna-Sphagnum* blanket bog over deep peat on poorly drained areas nearby. Thus it is possible that increased organic matter accumulation due to N addition could shift some heathland ecosystems more towards C-accumulating peatland ecosystems (e.g. Turunen et al., 2004), provided that N deposition levels and/or management practices are not so intensive as to restrict the growth of peat-forming species (e.g. Evans et al., 2014). Both these successions would provide long-term stabilisation of soil C with little management interference.

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

5. Conclusion

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

Europe. However, in relation to the CO_2 equivalents released by human activity, the gains in C storage are relatively modest. When considered in the context of falling biodiversity and altered species composition in heathland, policy focus should remain on reducing N emissions.

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

		Total Soil C			Litter C			Organic Horizons Total C (OH1 + OH2)			Eluviated gley Horizon C (EAG)		
N addition ($kg\ ha^{-1}\ yr^{-1}$)	**Total additional N (10 years treatments)**	**($kg\ ha^{-1}$)**	**ΔC from ambient**	**kg C kg N-1**	**($kg\ ha^{-1}$)**	**ΔC from ambient**	**Δ% from ambient**	**($kg\ ha^{-1}$)**	**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