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Increased inorganic nitrogen leaching from a mountain grassland ecosystem following grazing removal: A hangover of past intensive land-use?

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Abstract

Heathlands and grasslands occur in montane regions, naturally or due to anthropogenic land-use. These are typically nutrient-poor but exposure to elevated nitrogen deposition and intensive livestock grazing causes large-scale ecological change. We studied the long-term implications of grazing removal on soil and drainage water biogeochemistry and the implications for nitrogen cycling in fifty-year replicated grazing exclosures on a montane grassland exposed to high rates of ambient nitrogen deposition. Evidence of 'ecosystem recovery' represented by successional change from graminoid to shrub-dominance after cessation of grazing was not reflected in the soil biogeochemistry. Cessation of grazing had a negative impact, with increased soil extractable and soil solution nitrate concentrations; an apparent shift towards a more nitrogen-rich, bacterially dominated microbial community; and the acidification of soils and leachate. The increase in nitrate leaching appears to have been counterbalanced by a decrease in dissolved organic nitrogen leaching, approximately maintaining the overall nitrogen balance of the system, whilst apparently altering ecosystem functioning. High rates of organic matter cycling and inorganic nitrogen uptake in grazed grassland may have sustained ecosystem N limitation under elevated nitrogen deposition. Grazing removal caused long-term over-supply of nitrogen from mineralisation of enriched organic matter, exacerbated by continued high nitrogen deposition, exceeding the uptake demand of heath vegetation and resulting in nitrification and nitrate leaching. This disequilibrium between vegetation and soil following grazing removal has implications for restoration after periods of intensive grazing. Grazing may not simply leave a legacy of nutrient enrichment but its cessation may trigger nitrogen saturation and soil and freshwater eutrophication and acidification which counteract the immediate benefits of natural vegetation recovery. Long term, nitrogen saturation of abandoned grasslands is likely to reduce ecosystem resilience to invasion by nitrophilous species, pathogen attack and vulnerability to environmental pressures such as climate change. We conclude that partial and/or phased reduction in grazing levels may permit the more synchronised recovery of soils and vegetation, thereby avoiding imbalances between nitrogen supply and nitrogen demand and detrimental ecological effects.

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Introduction

Atmospheric concentrations of reactive nitrogen (N) have increased globally in the last few decades (Galloway *et al.,* 2008). Increases in the United Kingdom (UK) have largely occurred since the 1950s, due to a combination of nitrogen oxide emissions, primarily from road transport, and ammonia emissions from agriculture (RoTAP, 2012). Elevated N inputs have the potential to significantly alter nutrient poor systems throughout much of the world, and concern about the cascade of ecosystem impacts has been expressed by scientists, land managers and politicians (e.g. Wright *et al.,* 2001; Sutton *et al.,* 2011). Mountain ecosystems are generally considered to be extremely sensitive to environmental changes (e.g. Hagedorn *et al.,* 2010) and may be particularly sensitive to elevated N inputs due to the predominance of low nutrient soils, unproductive vegetation communities adapted to low nutrient levels, and low capacity to sequester atmospherically-deposited N into thin, organic-poor soils (Britton & Fisher, 2007; Helliwell *et al.,* 2010; Curtis *et al.,* 2011). The risk of N deposition impacts on montane areas is exacerbated by the higher orographic precipitation and deposition from clouds, mist and fog, which result in high rates of N input compared with adjacent lowland areas (Fowler *et al.,* 2005). The addition of reactive N has the potential to cause increased growth rates, sward composition change and loss of biodiversity in vegetation adapted to nutrient poor conditions; in the UK, this has been demonstrated by progressive loss of biodiversity observed in acidic grasslands along a gradient of increasing N deposition (Stevens *et al.,* 2004; Stevens *et al.,* 2010; Payne *et a*l., 2011; Stevens *et al*. 2011) and in calcareous grasslands (van den Berg, 2011); by temporal shifts towards more nutrient-demanding species observed at a national scale in semi-natural upland systems in the UK Countryside Survey (Smart *et al.,* 2003); and by observed vegetation changes in a range of long-term nitrogen addition experiments in semi-natural ecosystems (Phoenix *et al.,* 2012). Approximately 60% of grassland species' change points are at levels of nitrogen deposition at

or below what was formerly believed to be the critical load to bring about such change (Payne *et al*., 2013), and nitrogen deposition has been a significant driver in local plant extinctions in a range of acid grassland communities (McLean *et al*., 2011).

In addition to terrestrial eutrophication effects, excess N can lead to 'nitrogen saturation' where inputs exceed biological demands (Aber *et al.,* 1998) so that inorganic N (nitrate, NO_3^- and ammonium, NH_4^+) is only partly retained through uptake and immobilisation. This leads to the leaching of NO_3^- from the soil to surface waters, thus contributing to acidification and associated biodiversity loss (Stoddard et al., 1994) or to changes in aquatic stoichiometry and nutrient limitation (e.g. Elser et al., 2009). NO_3^- is a significant contributor to acidification in the UK (Allott *et al.,* 1995), and is expected to become increasingly important in the future, potentially limiting or even reversing surface water recovery from acidification that has occurred following reductions in sulphur deposition (Curtis *et al.,* 2005).

Increased N flux may also have effects on soil carbon (C), although these effects are complex; increased productivity under higher N loadings may increase C inputs to the soil, reduce the degradability of the more recalcitrant components of plant litter and dissolved organic matter, or reduce the need for plants to invest resources in root exudates to 'prime' fungal breakdown of organic matter to provide nutrients (e.g. Högberg et al., 2007; Kopaček et al., 2013), whereas the removal of nutrient restrictions on microbial growth and respiration may increase soil C oxidation rates (e.g. Neff *et al.*, 2002; Janssens and Luyssaert, 2009). Net increases in soil C under elevated N have been observed in forests (e.g. De Vries *et al.,* 2009), heathlands (Evans *et al.,* 2006a), and in peatlands under low N loadings (Turunen *et al.,* 2004). However, data for grasslands are sparse. Where increases in soil C due to enhanced plant growth do not keep pace with rates of N accumulation, N deposition may also result in a decrease in soil C/N ratio. Empirical relationships have been observed between

C/N ratio and rates of NO_3^- leaching in European and North American forests (Gundersen *et al.,* 1998; Hannah *et al.,* 2002), but observed relationships for UK ecosystems appear relatively weak, and vary according to soil and vegetation type (Rowe *et al.,* 2006). Evans *et al.* (2006b) concluded that variations in the organic matter content of soils, and therefore their capacity to accumulate N over long periods, obscured spatial relationships between N deposition, soil C/N ratio and NO_3^- leaching in UK moorland catchments.

Land management can also have significant effects on the composition and functioning of upland vegetation (Gordon *et al.,* 2004). One of the main management activities within temperate grasslands, either for economic or conservation objectives, is grazing. In the uplands of Britain, free ranging sheep grazing is an integral part of land-management, and is a major contributor to the economy of many rural areas. Grazing normally maintains shorter average sward heights and a higher proportion of living compared with dead standing biomass, and has been shown to be the principal factor controlling vegetation change in this region, increasing the proportion of grasses and decreasing the proportion of shrubs and trees (Milne & Hartley, 2001). Succession from grasslands to heathlands often occurs as a result of long-term removal of grazing, demonstrated by long-term sheep exclusion experiments in the uplands (Miles, 1988; Hill *et al.,* 1992). There is already concern over the future of grazing in some parts of the uplands, with widespread agricultural abandonment having potentially adverse environmental impacts (MacDonald *et al.,* 2000). For example, abandonment usually leads to litter accumulation (Medina-Roldán *et al.,* 2012) and associated negative effects on photosynthetic efficiency. The total area of managed rough grazing in Wales, primarlily grazed upland and montane ecosystems, reduced by around 25% between 1967 and 2009 (Hughes, 1973; StatsWales, n.d).

Grazing can affect nutrient cycling both directly (by removing, adding nutrients or transforming nutrients) and indirectly, by altering vegetation composition. Heathlands are

characterised by high nutrient turnover time in plants and soil and low levels of plant-available nutrients (Aerts & Chapin, 1999). In contrast, grasslands are associated with more rapid N cycles and greater plant available nutrient levels (Heil & Bobbink, 1993). Evidence also exists to suggest that long-term changes in grazing pressure can impact on soil pH (Marrs *et al.,* 1989) and rates of ammonium mineralisation, soil microbial activity and microbial biomass N (Medina-Roldán *et al.,* 2012). Given this strong link between vegetation composition and land-management, it seems likely that there will be strong interactive effects of land-management and N deposition on the structure and functioning of montane ecosystems (Van der Waal *et al.,* 2003; Britton *et al.,* 2005; Helliwell *et al.,* 2010). At present there is a lack of information on the effects of grazing on soil and water chemistry in upland ecosystems, although Helliwell *et al.* (2010) recently found little effect of grazing on soil solution chemistry in upland areas of Scotland. Here, we present the results of a study of the impacts of grazing removal on the biogeochemistry of a mountain grassland in a high N deposition area of Wales, based on a unique 50 year replicated grazing exclusion experiment. Due to the exceptionally long duration of the experiment, we were able to examine the biogeochemical behaviour of sites that had adjusted to different grazing levels, rather than the short-term, possibly transient impacts of recent experimental grazing changes. Our objectives were therefore: 1) to examine changes in soil and soil solution biogeochemistry as a consequence of long-term grazing removal; 2) to specifically assess whether the cessation of grazing and subsequent vegetation succession increases or decreases ecosystem susceptibility to nitrogen saturation and nitrate leaching; and 3) to evaluate whether ecosystem responses varied by soil type, by comparing adjacent mineral and organo-mineral soils.

Material and methods

The study was based on a grazing exclusion experiment set up in 1957 (Hughes & Dale, 1970) in the Snowdonia National Park, a grassland-dominated montane area of North Wales, UK. We used six grazing exclosure plots, located at three locations within an altitudinal range of 350-450m above sea level in the Snowdon massif (53° 05′ N, 41° 02′ W). Snowdonia has variable geology, soils and climate (Perkins, 1978), and as such the original sites were established on a variety of soils, ranging from a peaty podzol through to a brown earth (Hill *et al.,* 1992) which originally supported around 2.5 to 5.0 sheep ha^{-1} (Hughes & Dale, 1970). The Snowdon Environmental Change Network (ECN) monitoring site, which is located within the same study area at an altitude of 488 m, has a mean annual temperature of 7.6 °C, receives around 4200 mm annual rainfall and an estimated 23.1 kg N ha^{-1} yr^{-1} total N deposition (Turner *et al.,* 2010).

Original experimental design

The original grazing exclusion experiment was undertaken at each of the three study sites, and on two soil types at each site. The two soil types at each study site were located between 200 and 500 m apart. The soils considered were peaty podzols (histic podzols in the WRB classification; FAO, 2006) and typical brown earths (WRB cambisols). For each of the six site × soil combinations, the exclusion experiment comprised twelve 2.8 m × 3.7 m plots, arranged in a randomised block design, with three treatments and four blocks. The treatments were as follows:

- Treatment 1: Normal grazing - Sheep allowed free access, although most of the sheep are removed from the mountain in winter

- Treatment 2: Summer only grazing - Sheep excluded from October to April

- Treatment 3:   No grazing - Sheep excluded throughout the year.

The original grazing experiment ended in 1981, at which time all treatments were ring-fenced, thus converting the summer-only and year-round grazing plots into single ungrazed exclosures which have been maintained since this time.

Experimental design of current study

We sampled locations within and outside the long-term grazing exclosures. Although the original experimental design included nine sites (Hughes & Dale, 1970), only six were used in the current investigation, due to uncertainties regarding fencing integrity during the intervening period for the other three sites. The six sites used within this study were located in three areas, Llyn Llydaw, Pen-y-Pass and Cwm Idwal. Soil and vegetation measurements indicate that any residual differences between plots subject to different pre-1981 grazing treatments have largely disappeared (S. McGovern, unpublished data), however for this study we sampled areas that lay within one of the original zero-grazing plots (i.e. treatment 3) within each exclosure, to ensure the maximum time since grazing removal. A grazed plot, of the same size as the ungrazed plot, was established for this study next to each of the six existing ungrazed plots. These plots were unfenced, and thus freely accessible to sheep grazing the surrounding area, and subject to normal livestock management practices. Current sheep density at the nearby Snowdon ECN monitoring site is currently around 2-2.5 sheep ha^{-1}, having decreased from a peak of around 4-5 sheep ha^{-1} in the late 1990s (Lloyd et al., 2011; McGovern et al. 2013).

Vegetation cover

Vegetation cover was recorded between July and August 2009 using 100 randomly placed pins (35 cm height x 2 mm diameter) per experimental plot. The individual points were located by coordinates derived from random number tables, and pins inserted vertically. Presence or absence of species were recorded at each pin, with presence occurring if any live part of a plant was touching the pin. All plants were identified to species level and split by functional group (cryptogams, forbs, graminoids, shrubs and trees). Plant functional data were converted to weighted percentage cover to analyse any shift in species composition between treatments.

Soil solution sampling and chemical analysis

Four Rhizon® soil water samplers (Rhizosphere Research Products, Wageningen, Netherlands) were placed in a representative area of homogeneous vegetation within each plot to reduce the possibility of vegetation effects within treatments. The rhizons® were placed in a square, with two metres between each rhizon®. The same layout was used within both the grazed plots and exclosures, with sampling sites located to avoid any possible lateral water transfers between different treatments. The samplers were installed within the top 30 cm of the soil at the start of December 2008 by excavating a hole using a steel rod, then inserting the sampler. Care was taken to avoid soil disturbance and to ensure a good connection between the porous surface of the sampler and the surrounding soil. Samplers were left to settle in for one month before sampling commenced, to minimise any disturbance effects. Soil solution was extracted in situ and sampled every month from January to

December 2009. To ensure sufficient sample was collected and a standard protocol was followed throughout the year, soil solution was sampled over a 48 hour period once a month, using a BD Vacutainer®. Samples from the four rhizons® in each plot were bulked prior to analysis. The sampler membranes have a pore size of 0.15 μm, therefore further sample filtering was not required.

All soil water samples were analysed directly for pH (Hanna Instruments; Leighton Buzzard, UK pH 209 pH meter) and electrical conductivity (EC) (Jenway 4010 EC meter; Bibby Scientific, Stone, UK). Dissolved organic C (DOC) and total dissolved nitrogen (TDN) were measured with a Shimadzu TOC-TNV analyser (Shimadzu Corp., Kyoto, Japan). NH_4^+-N in soil solution was determined colorimetrically by microassay (Downes, 1978; Mulvaney, 1996). Cl, NO_3^--N, PO_4^{3-}-P and SO_4^{2-} were determined on a Dionex DX120 Ion Chromatograph (Dionex Corp., Sunnyvale, USA). Cations were measured using a Perkin Elmer AAnalyst 400 Atomic Absorption Spectrometer (PerkinElmer, Inc., Waltham, MA, USA) and these were summed to provide total cation concentrations. Dissolved organic N (DON) was calculated as the difference between TDN and dissolved inorganic N (DIN). The acid neutralising capacity (ANC) was calculated as the sum of base cations minus the sum of strong acid anions i.e. $(Na^+ + Ca^{2+} + Mg^{2+} + K^+ + NH_4^+) - (NO_3^- + Cl^- + SO_4^{2-})$, expressed in μeq l^{-1}.

Soil sampling and chemical analysis

A 5cm diameter soil core was taken to 50 cm from the location of each sampler at the end of the experimental period (December 2009), and split by horizon. After removal of vegetation and roots these were bulked per plot. Soil pH and EC were determined on a 1:2.5 soil:water

extract using the same instruments used for the soil solution samples. NO_3^--N and NH_4^+-N were extracted using a 1:4 ratio soil:1M potassium chloride (KCl) extractant and determined colorimetrically by microassay (Downes, 1978; Mulvaney, 1996). Microbial C and N were determined using the fumigation-extraction technique (Brookes *et al.,* 1985; Vance *et al.,* 1987) and calculated using a K_{EC} value of 0.45 and 0.54 respectively (K_{EC} represents the efficiency of extraction of microbial biomass (Wu *et al.* 1990; Joergensen 1996)). Total C and N of soils were determined using a LECO Truspec® CN Analyser (LECO, Stockport, UK). Exchangeable cations were extracted using a 1:20 soil to 0.5M ammonium chloride ratio and analysed using a Perkin Elmer AAnalyst 400 Atomic Absorption Spectrometer. Exchangeable acidity was determined on a 1:20 soil:1M KCl extract by fixed point titration to pH 8.3 with 0.1M NaOH (Page *et al.,* 1982) using a Metrohm 888 Titrando (Metrohm UK Ltd., Runcorn, UK). Effective cation exchange capacity (CEC) was calculated as the sum of the exchangeable bases (Na^+, Ca^{2+}, Mg^{2+} and K^+) and exchangeable acidity. Base saturation was calculated as the percentage of cation exchange capacity occupied by the base cations (Na^+, Ca^{2+}, Mg^{2+} and K^+). Bulk density was measured using a $100cm^3$ metal ring. Percentage moisture was calculated after drying at 80 ^{o}C until no more weight loss was recorded (a temperature of 80 ^{o}C was used for consistency with historic measurements made at the same site). To ensure quality control, standard soils with known chemical concentrations were included within the analysis.

Statistical Analysis

To investigate the effect of grazing and soil type on vegetation functional group, soil and soil solution chemical parameters, an analysis of variance taking account of the split plot design and including a soil × grazing interaction term were run in R (R Development Core Team, 2010). [R code used for analysis: model<-aov(x~soil type*grazing treatment +Error(site/soil type))]. Additional paired t-tests were run to investigate the effect of soil type on soil

solution chemistry [model<-t.test(x-treatment, paired=T)]. Soil chemical values are expressed on a dry weight basis. To avoid errors of pseudoreplication resulting from analysis of repeated measurements made on the same samplers, monthly data were averaged to provide one value per treatment per plot, for each chemical parameter measured.

Results

Vegetation cover

Soil type had little effect on percentage cover of difference vegetation types. However, significant differences in vegetation were clearly evident between the grazed and ungrazed treatment plots (Table 1). Grazed plots are dominated by graminoids, and contain little cover of shrubs and trees. Ungrazed plots contain significantly more shrubs and cryptogams, with a correspondingly lower cover of graminoids.

Effects of soil type on soil biogeochemistry

Significant effects of soil type were mainly seen within the chemical composition of the soil (Table 2) rather than the soil solution (Table 3). The peaty podzol showed higher exchangeable acidity and higher cation exchange capacity, with a correspondingly lower base saturation. Soil pH was lower in the peaty podzol, but not to a significant level. The plots on the peaty podzol also had higher C and N concentrations, as well as a higher C/N ratio (18 versus 14 g g^{-1}), although again these differences achieved only a probability level of <0.1. No significant differences according to soil type were observed for extractable NH_4^+, NO_3^-, or microbial C or N. Average moisture content appeared slightly higher in the podzol, and bulk density lower, but due to high variability within each soil type these differences were not significant.

Soil type had little effect on the chemical composition of the soil solution. Although both K^+ and Na^+ showed significant differences between the soil types (individual cation data not shown), total base cation concentration was not significantly different between soil types, and no differences were observed for DOC, nitrogen species, pH or major anions (Table 3).

Effects of grazing on soil biogeochemistry

Grazing exerted significant effects on a number of soil and soil solution variables (Tables 2-3). The only significant soil × grazing interaction observed for any measured variable was for K^+, and these results were therefore omitted from the tables. For the soils data, grazing effects were most pronounced on soil extractable NO_3^- concentrations, with higher concentrations observed in the ungrazed treatment for both soil types. Proportional increases in the ungrazed relative to the grazed plots were large, averaging approximately 250% for the brown earth, and 75% for the peaty podzol. On the other hand, NH_4^+ concentrations were unchanged. There was a significant negative effect of grazing cessation on pH (a mean decrease of 0.2 units for the brown earths, and 0.16 for the peaty podzols) which, since total cations were not significantly different, appears attributable to the higher NO_3^- concentrations. Both soil C and N showed large changes between grazed and ungrazed plots for each soil type, but the directions of change differed, with both C and N more than twice as high in the ungrazed relative to the grazed brown earth plots, but lower in the ungrazed versus grazed peaty podzol plots. Due to the different directions of change, there was no significant overall grazing effect on either measurement. In addition, because proportional differences in C and N between grazing treatments were similar in each soil type, there was no change in soil C/N ratio. In contrast, there was some indication of lower microbial C in the grazing removal plots (p = 0.09), but no accompanying change in microbial N. Consequently, there were large reductions in microbial C/N ratio, from around 14 in both sets of grazed plots to 9.2 in the ungrazed brown earth plots, and 10.6 in the ungrazed peaty podzol plots.

Soil solution NO_3^- changes mirrored those in extractable NO_3^-, with large, highly significant (p = 0.01) differences between ungrazed and grazed plots for both soil types. For

both soil types, mean NO_3^- concentrations were around five-fold higher in the ungrazed relative to the grazed plots (Figure 1a-b). These differences were significant on an individual soil type basis for the brown earth, but not for the peaty podzol, due to similar concentrations found at one pair of grazed and ungrazed treatment plots. However mean NO_3^- concentrations were elevated in grazed versus ungrazed plots in at least five of the monthly samples collected from each soil type, with the largest differences observed in the first part of the year (Figure 1a-b)

In contrast to the higher NO_3^- concentrations in ungrazed plots, DON concentrations were significantly lower in soil the ungrazed plots. Again, differences were mostly confined to the first part of the year, and were more evident for brown earths compared to peaty podzols (Figure 1c-d). Compared to NO_3^-, which showed grazing-related differences in around half of the monthly samples, differences in DON were only pronounced in a small number of individual sample sets. As a result of the opposing changes in NO_3^- and DON, TDN concentrations were not significantly affected by grazing (NH_4^+ concentrations were also unchanged with grazing). Mean pH was lower in the ungrazed plots, by 0.18 and 0.14 units under brown earths and peaty podzols respectively (Figure 1e-f). Differences in pH were sustained from January until June for both soil types, after which values in grazed and ungrazed plots were very similar. Similarly, ANC was significantly lower in ungrazed plots, by an average of 70 and 50 $\mu eq\ l^{-1}$ for the respective soil types. Base cations, SO_4^{2-}, Cl^- and DOC were not significantly affected by grazing.

Discussion

The long-term experimental exclusion of grazing livestock led to a succession from grassland to heathland communities within the exclosures, but there was limited evidence of further succession to woodland, probably due to the lack of suitable propagules in the locality. Fifty years after the cessation of livestock grazing, we observed significant differences between the soil and soil solution chemistry of grazed and ungrazed plots. Most notably, grazing removal led to clear and substantial increases in soil extractable NO_3^- concentrations, and in NO_3^- leaching, in both soil types studied. This increase in NO_3^- appears to have been largely offset in terms of the ecosystem N balance by a reduction in DON leaching, although without any clear accompanying change in DOC loss, and with little or no change in soil C/N ratio. There is evidence that the size of the microbial C pool has declined, whereas the microbial N pool has not, leading to large reductions in microbial C/N ratio. This change, from values characteristic of a fungal-dominated community to one dominated by bacteria, is consistent with measurements made over a decade earlier on the same experiments by Bardgett *et al.* (1997), who recorded both an overall reduction in microbial biomass, and a 25% reduction in fungal to bacterial phospholipid fatty acid (PLFA) concentrations, in the ungrazed plots. The authors attributed these changes to the reduced supply of readily utilisable substrate from sheep faeces, and to reduced root exudation. Our results support this, and further suggest that reductions in root exudation can be attributed to the over-supply of mineral N in the mature heathland ecosystems, reducing the need for plant 'priming' of organic matter decomposition to obtain N to support biomass growth (Högberg *et al*., 2007). This corroborates de Vries et al. (2012) who found that in extensively managed grassland systems, nitrogen retention was much higher in species poor intensively managed grasslands due to an increased abundance of fungi relative to bacteria.

The data therefore suggest that, although the overall N balance of the system may not have changed following grazing removal, there may have been major shifts in microbial community structure, as well as in the form of N loss. The increased NO_3^- leaching suggests that the supply of inorganic N from mineralisation of N-enriched organic matter, augmented by continuing high N deposition, exceeds plant and microbial N demand, allowing nitrification and subsequent NO_3^- leaching to occur. In the absence of other significant changes in soil solution base cations or acid anion concentrations, this increase in NO_3^- leaching has significantly acidified the soil, reducing both soil and leachate pH, and leachate ANC.

We suggest that the removal of grazing may have triggered increased NO_3^- leaching through a number of linked processes. Firstly, the mountains of Snowdonia have adapted to many centuries of grazing, leading to grassland dominance in most areas accessible to livestock. Graminoid vegetation has a characteristically low C/N ratio, due to the absence of woody biomass, which leads to the formation of a low C/N organic soil (e.g. Rowe *et al.,* 2006). Grazed grassland ecosystems are characterised by a relatively rapid N cycle, with high N inputs from livestock, and high mineralisation rates balanced by high vegetation N uptake, which is intensified by grazing pressure (Van Vuuren & Van der Eerden, 1992). Comparatively high DON leaching rates may be considered as a 'leak' in this N cycle, as has been suggested elsewhere (e.g. Hedin *et al.,* 1995), reducing the rate of ecosystem N enrichment due to elevated N deposition. Our data suggest that this leakage of DON from grazed plots occurs discontinuously, and mainly during the winter period, but further measurements would be required to confirm this. Additionally, since DON (unlike NO_3^-) is susceptible to adsorption and retention in mineral horizons (e.g. Kalbitz et al., 2000), we cannot be certain whether high DON concentrations in shallow organic soil solution necessarily translate into high rates of ecosystem DON loss.

In the exclosure plots, it is clear that the removal of grazing pressure has permitted succession to dwarf shrub dominance, but that this has not (despite the 50 year duration of the experiment) caused soil C/N ratios to revert to the higher values characteristic of dwarf shrub ecosystems (e.g. Rowe *et al.,* 2006). With the rate of N mineralisation from the low-C/N, former grassland soil now out of equilibrium with the lower uptake demand of the high-C/N, woody dwarf shrub vegetation (which has reached full maturity after 50 years of grazing removal), the system may have become inherently unstable with regard to nitrogen cycling. Alongside the cessation of N offtake in livestock biomass, an inferred shift from an N-poor fungal to an N-rich bacterial- dominated microbial community is likely to have favoured mineralisation and nitrification over immobilisation. With atmospheric N loadings remaining high, these changes have led to elevated NO_3^- leaching (Figure 2).

These results have significant implications for the restoration, or extensification, of upland ecosystems that have historically been subject to high grazing pressure. Measurement data clearly show that areas which have remained under heathland vegetation retain high C/N ratios and low levels of NO_3^- leaching (e.g. Pilkington *et al.,* 2005; Rowe *et al.,* 2006) unless exposed to exceptionally high N deposition loadings (e.g. Curtis *et al.,* 2004). However, it seems clear that removal of grazing will not necessarily lead to a return to these pre-disturbance conditions, despite the re-establishment of heathland vegetation, for many decades. To a degree, these findings mirror the conclusions from a number of N addition experiments (e.g. Vinton & Burke, 1995; Power *et al.,* 2006), which showed persistent effects of ecosystem nutrient enrichment for a period of years to decades following the cessation of N addition. A strong parallel can be drawn with studies in the USA indicating that land-use history may be a more important determinant of current N leaching from forests than present day N deposition or species composition (Goodale *et al.,* 2000; Goodale & Aber, 2001), a finding also suggested by a survey of oak woods across Wales in which lower C/N ratios and

higher NO_3^- leaching were associated with woodlands established on former grassland sites, rather than those receiving higher N deposition (Williams *et al.,* 2000). In montane areas of Snowdonia, Britton *et al.* (2005) concluded that N deposition and historic over-grazing would retard ecological recovery following grazing removal. Our results further suggest that the transition of heavily grazed grasslands to ungrazed heathland or woodland vegetation may not simply result in a persistence of N saturation, but might actually create the conditions in which N saturation, and therefore NO_3^- leaching, are triggered. These findings thus reinforce the importance of 'land-use legacies', as described by Foster *et al.* (2003), for ecosystem functioning, and also raise the possibility of a 'nitrogen hangover' effect, whereby the detrimental consequences of long-term N enrichment occur after, rather than during, the period of intensive land-use.

In some areas of countries such as the UK and Netherlands that have been subject to intensive land-use or high rates of N deposition, the legacy effects of ecosystem nutrient enrichment have been countered through major restoration interventions, such as turf-stripping (e.g. Dorland et al., 2004) or above-ground biomass removal (e.g. Mitchell et al., 2000; Härdtle et al., 2006). While potentially viable for small areas of high (or potentially high) conservation value, such as lowland heaths or wetlands, this approach clearly would not be appropriate at the scale of large upland grassland landscapes. Therefore, it appears that the complete removal of grazing from nutrient-enriched grassland sites will lead to an unavoidable trade-off between the restoration of heathland or woodland vegetation to meet habitat biodiversity goals on one hand, and the detrimental ecological impacts of NO_3^- leaching and acidification on the other. Likely impacts include damage to aquatic biota, offsetting biological recovery from acidification that has occurred in response to declining sulphur deposition (e.g. Ormerod and Durance, 2009; Monteith et al., 2012; Malcolm et al., 2012), and increased susceptibility of former grassland sites to invasion by nitrophilous

species able to exploit the enhanced mineral N availability (Miles, 1988; Hester *et al*., 1991). The latter has been observed at a broad scale in the UK Countryside Survey (Smart et al., 2003), although not in the present study (possibly due to the relative isolation of the exclosure plots). One possible mitigation option to avoid these detrimental consequences might be a more gradual (or partial) reduction of grazing intensity, rather than complete cessation. This could permit the more gradual adaptation of the ecosystem to changes in uptake demand, nitrogen turnover rates and vegetation stoichiometry, thereby avoiding the full-scale microbial community shifts and N saturation implied by our results.

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Table 1 Weighted percentage cover by plant functional type from grazed and ungrazed treatment plots in Snowdonia. Values represent mean ±

sem ($n = 3$). Significant p values are shown in bold.

| | Brown Earth | | | | Peaty Podzol | | | | p | |
	Grazed		Ungrazed		Grazed		Ungrazed		Soil Type	Grazing
Cryptogam	14.29	± 5.250	19.70	± 3.898	15.795	± 2.231	30.486	± 3.789	0.432	**0.033**
Forbs	29.41	± 3.783	17.73	± 8.941	20.70	± 2.834	17.79	± 3.870	0.608	0.241
Graminoids	54.36	± 3.081	29.25	± 14.367	56.36	± 5.807	15.63	± 8.602	0.351	**0.018**
Shrubs & Trees	1.95	± 1.597	33.31	± 5.095	7.15	± 5.095	36.09	± 15.451	0.752	**0.014**

Table 2 Chemical, physical and biological characteristics of soil from grazed and ungrazed treatment plots in Snowdonia. Values represent mean $\pm$ 1 sem ($n = 3$). Significant p values are shown in bold.

	Brown Earth		**Peaty Podzol**		*p*	
	Grazed	Ungrazed	Grazed	Ungrazed	Soil Type	Grazing
Soil C (g/100g^{-1})	4.23 $\pm$ 1.01	9.17 $\pm$ 4.94	25.32 $\pm$ 4.51	18.52 $\pm$ 4.01	0.060	0.728
Soil N (g/100g^{-1})	0.29 $\pm$ 0.06	0.61 $\pm$ 0.27	1.39 $\pm$ 0.28	1.01 $\pm$ 0.23	0.098	0.839
Soil C/N	14. $\pm$ 0.85	13.92 $\pm$ 1.49	18.48 $\pm$ 0.79	18.33 $\pm$ 0.25	0.067	0.664
NH$_4^+$ (mg/100g^{-1})	0.20 $\pm$ 0.04	0.39 $\pm$ 0.16	0.15 $\pm$ 0.03	0.19 $\pm$ 0.04	0.407	0.159
NO$_3^-$ (mg/100g^{-1})	1.51 $\pm$ 0.89	5.32 $\pm$ 0.59	4.11 $\pm$ 1.37	7.10 $\pm$ 0.64	0.186	**0.029**
Microbial C (μg/g)	2086 $\pm$ 384	1598 $\pm$ 278	3679 $\pm$ 1097	1622 $\pm$ 230	0.434	0.094
Microbial N (μg/g)	157.3 $\pm$ 32.21	189.1 $\pm$ 41.5	254.5 $\pm$ 79.0	188.7 $\pm$ 58.6	0.469	0.568
Microbial C/N	13.51 $\pm$ 0.91	9.20 $\pm$ 2.13	14.69 $\pm$ 1.09	10.58 $\pm$ 3.20	0.237	0.111
pH	4.86 $\pm$ 0.10	4.66 $\pm$ 0.20	4.22 $\pm$ 0.07	4.06 $\pm$ 0.08	0.058	**0.030**
Total cations (mEq/100g^{-1})	0.95 $\pm$ 0.13	1.32 $\pm$ 0.59	1.10 $\pm$ 0.49	0.61 $\pm$ 0.04	0.244	0.792

Exchangeable acidity (mEq/100g^{-1})	1.48 ± 0.51	2.84 ± 1.24	8.67 ± 0.22	9.78 ± 1.10	**0.012** 0.173
CEC (mEq/100g^{-1})	2.43 ± 0.57	4.15 ± 0.81	9.77 ± 0.70	10.39 ± 1.09	**0.011** 0.233
Base saturation (%)	43.44 ± 10.44	39.93 ± 20.13	10.68 ± 4.06	6.02 ± 0.83	**0.028** 0.213
Moisture content (%)	1.46 ± 0.23	2.22 ± 0.98	3.18 ± 0.33	3.13 ± 0.53	0.106 0.531
Bulk density (g cm^3)	0.56 ± 0.11	0.47 ± 0.09	0.13 ± 0.01	0.25 ± 0.11	0.161 0.827

Table 3 Chemical characteristics of soil solution leached from grazed and ungrazed treatment plots in Snowdonia. Values represent mean ± 1 sem ($n = 3$). Significant p values are displayed in bold. Values (except pH and ANC, in µeq per litre) are shown in µmol per litre.

| | Brown Earth | | | | Peaty Podzol | | | | p | |
	Grazed		Ungrazed		Grazed		Ungrazed		Soil Type	Grazing
DOC	1819.12	± 136.22	1527.37	± 168.74	1852.90	± 19.23	1896.50	± 214.62	0.249	0.530
TDN	145.58	± 25.97	90.54	±15.93	101.71	± 6.43	129.74	± 24.08	0.898	0.557
NH_4^+-N	21.41	± 2.74	19.72	± 2.72	17.19	± 1.57	27.47	± 9.34	0.842	0.418
NO_3^--N	6.65	± 3.84	38.69	± 9.78	7.74	± 3.56	49.88	± 16.43	0.764	**0.010**
DIN	28.06	± 6.58	58.41	± 12..00	24.93	± 2.29	77.35	± 17.62	0.470	**0.016**
DON	117.53	± 23.60	32.13	±4.88	76.78	± 8.32	52.40	± 8.61	0.488	**0.018**
pH	6.15	± 0.05	5.97	± 0.12	5.81	± 0.01	5.67	± 0.004	0.149	**0.024**
Total base cations	113.29	± 19.33	94.11	± 4.07	85.98	± 3.00	96.21	± 9.17	0.245	0.906
SO_4^{2-}	66.25	± 12.13	82.37	± 10.47	66.11	± 14.94	80.80	± 0.50	0.884	0.229
Cl^-	140.29	± 12.82	139.25	± 13.86	140.88	± 8.99	155.27	± 8.23	0.666	0.237
ANC	-80	± 014	-150	± 14	-110	± 26	-160	± 14	0.331	**0.030**

Fig. 1 Effects of grazing on mean nitrate, dissolved organic nitrogen (DON) and pH in monthly samples of soil solution from treatment plots in Snowdonia for brown earth and peaty podzol soils. Error bars represent ± 1 s.e.m for each sampling interval (*n*=3).
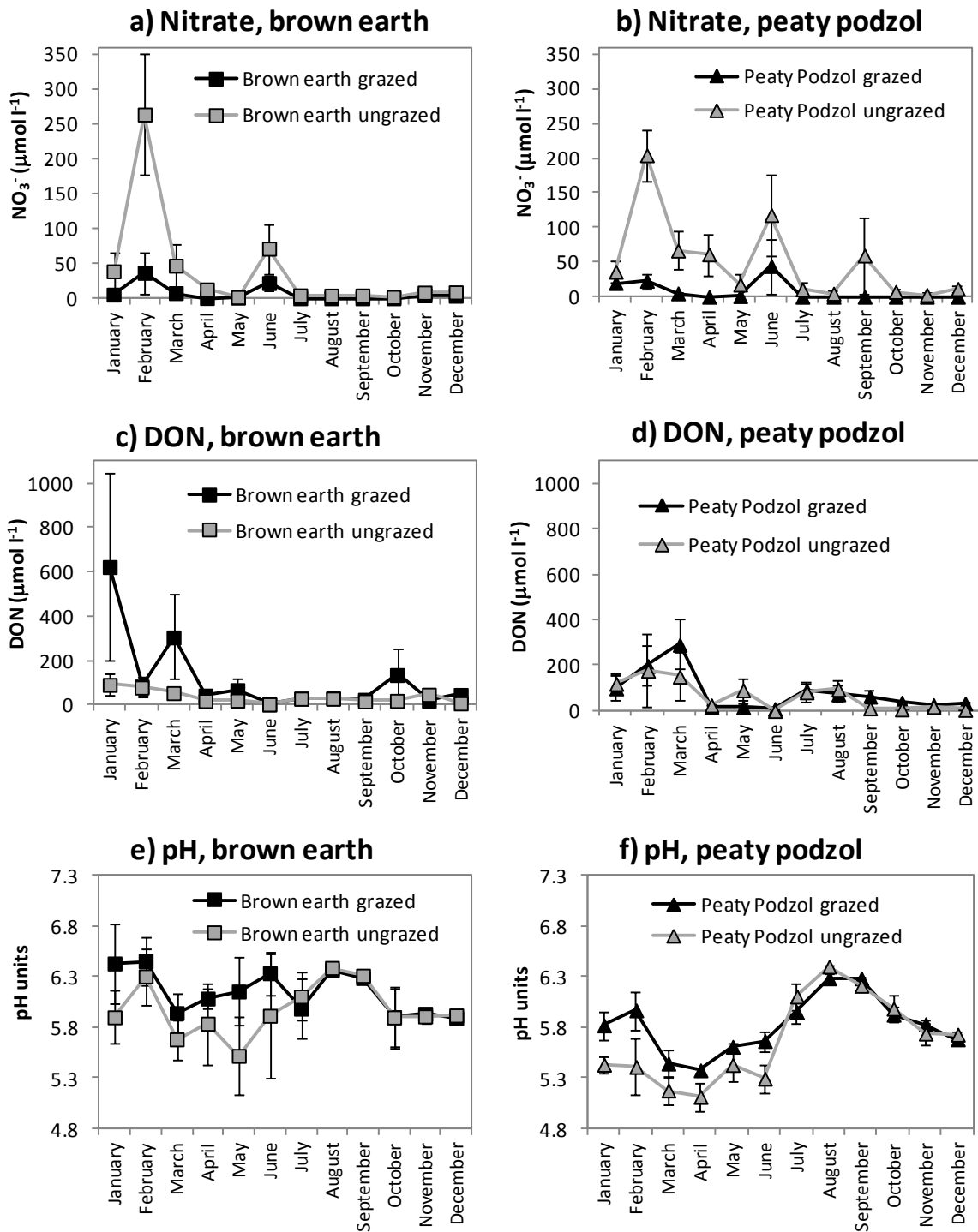
Fig. 2. Schematic representation of hypothesised changes in the nitrogen cycle during a transition from low-intensity grazed, low N-deposition grassland (early 20th century) to a more intensively grazed, high-N deposition grassland (mid-20th century within the exclosures, mid-20th century to present day outside exclosures), then to an ungrazed, high N-deposition, ecosystem following succession to shrub dominance (late 20th century to present day inside exclosures). Arrow thickness indicates the relative magnitude of each flux, as inferred from the measurements and site management information, and bulleted list below highlights key characteristics of the ecosystem during each period.