IDENTIFYING KEY ENVIRONMENTAL FACTORS AFFECTING PRIMARY PRODUCTIVITY AND ECOSYSTEM SERVICES IN THE UPLANDS

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SUMMARY

Land use intensity and reactive nitrogen (N) enrichment from atmospheric deposition are recognised as major threats to upland ecosystem function. Despite recent reductions in emissions of nitrogen oxides and ammonia from combustion and agricultural activities, N deposition rates on upland habitats from pollution remain above accepted critical loads. Increases in N availability can result in N saturation and lead to a reduced capacity for N retention which may be exacerbated by a shift in nutrient limitation status, from N limitation to phosphorus (P) limitation, which can constrain vegetation biomass production. Phosphorus limitation however is potentially an important mechanism constraining biodiversity loss associated with increasing nutrient availability and may moderate soil microbial and decomposer activity, which positively influences soil carbon (C) storage potential.

This thesis aimed to elucidate the effect of current drivers of environmental change on upland ecosystem processes utilising a landscape scale survey and experimental manipulation of nutrient availability. The results presented here include investigations into the interactive effects of livestock stocking rate and N deposition (Chapter 2), and the role of N and P availability in influencing upland ecosystem components and processes, including vegetation species composition, soil chemistry (Chapter 3), soil invertebrates, vegetation productivity and tissue chemistry (Chapter 4), and soil C flux (Chapter 5). It is hypothesised that increasing livestock grazing intensity and nutrient availability from N deposition, alter upland ecosystem processes resulting in C storage reductions in upland soil.

The results demonstrate a combined pressure from livestock grazing and N deposition which act in concert to modify upland vegetation and soil invertebrates and chemistry. Significant influence of N and P availability on ecosystem processes in upland habitats was also shown. Vegetation species composition was modified by the addition of P, with significant increases in cryptogam coverage. Microbial activity was enlarged, which was evident as higher soil C flux. These effects resulted in soil C content loss through changes in vegetation species composition, resulting in reductions in the direct input of organic matter from reduced root structures, and faster C cycling from increased decomposer activity.

It is concluded that the availability of P in upland habitats has strong controlling effects on ecosystem processes, which can reduce the ability of upland soil to retain C, and which continue to act in the long term due to the persistent nature of P in soil. Thus, the addition of P as a management tool to alleviate the effects of chronic N enrichment is not recommended. As a consequence, there is need for alternative approaches to upland land management which reduce the pressure from grazing livestock on sensitive upland habitats, particularly for habitats in exceedance of N deposition critical loads.

CONTENTS

LIST OF FIGURES

LIST OF TABLES

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CHAPTER 1

GENERAL INTRODUCTION

1.1 RATIONALE

Upland habitats are facing significant pressure from environmental change, driven by human activities, in the form of climate change (Orr *et al.,* 2008; Caporn & Emmett, 2009), land management intensity (Evans *et al.,* 2015) and the deposition of pollutants, particularly nitrogen (N) (Bobbink *et al.,* 2010; Field *et al.,* 2014). These drivers modify upland ecosystem processes and contribute to widespread and often severe declines in species richness and abundance, recorded across trophic levels (Dennis *et al.,* 2008; Maskell *et al.,* 2010).

The UK uplands support diverse habitats, some of which are of international conservation interest such as blanket bogs and heaths (Thompson *et al*., 1995; Parry *et al.,* 2014), and are highly important for biodiversity. In recent times the perceived importance of the upland environment has expanded to include vital ecosystem services such as carbon storage, water purification and flood mitigation (Haines-Young & Potschin, 2009; Van der Wal *et al.,* 2011). Ecosystem services represent the benefits human populations derive from ecosystem functions (Constanza *et al.,* 1997) and are important for both the amenity they offer and for driving public engagement in the protection of UK ecosystems (Bonn *et al.,* 2009). In the last fifty years, increasing demand for the production of food, timber, fibre and other resources such as recreation, has intensified the pressure on upland habitats and resulted in degraded landscapes (Holden *et al.,* 2007; Morecroft *et al.,* 2009), which may limit the future potential for delivery of essential ecosystem services (MEA, 2005; Bonn *et al.,* 2009; Van der Wal *et al.,* 2011).

Upland peatland soils are a major carbon (C) reservoir of significant national and international importance, which may be adversely affected as a result of these drivers. Soil C accumulation rates are currently at the lower end of amounts recorded for the last 150 years suggesting a downward trend (Billet *et al.*, 2010). An important factor for the ability of environments to capture C is nutrient status. Current rates of atmospheric N deposition and the presence of grazing livestock act together to modify the soil nutrient status of upland habitats (Hartley & Mitchell, 2005; Smith *et al*., 2015), but what impact this has on plant and invertebrate communities, and consequently on soil C turnover rate, remains uncertain.

There is substantial evidence that N enrichment can affect ecosystem processes and function through various pathways (Bobbink *et al*., 1998; Van der Wal *et al*., 2003; Stevens *et al*., 2004 & 2010; Medina-Roldan *et al*., 2012; Smith *et al*., 2015). Prolonged exposure to N enrichment in oligotrophic upland habitats can initiate a shift in nutrient limitation status from N limited to limitation by the next least available nutrient, which is typically phosphorus (P) (Phuyal *et al*., 2008; Crowley *et al*., 2012). Phosphorus availability may limit vegetation biomass production, restricting the ability of habitats to sequester additional reactive N inputs (Li *et al*., 2015), which could result in N saturation and lead to a reduced capacity for N retention (Emmett *et al*., 1995; Curtis *et al*., 2005). Alternatively P limitation may limit the impact of N enrichment on biodiversity by constraining ecosystem productivity and reduce the impact from increased competition (Olde Venterink, 2011; Ceulemans *et al*., 2014). Recognition of the link between aboveground and belowground processes has developed in recent years (Bardgett *et al*., 1993; Bardgett & Cook, 1998; Wardle *et al*., 1999a&b; Bardgett, 2002), and is important for determining the underlying mechanisms of change. A better understanding of the interaction of elements within ecosystems with regard to nutrient status is essential for determining the future response of upland habitats to current drivers of change.

1.2 THE UK UPLANDS

The UK uplands are dynamic, relatively high energy environments (Evans, 2009), internationally important for biodiversity, landscape and cultural significance, and locally important for socio-economic activities such as agriculture, tourism and recreation (Orr *et al.*, 2008; Reed *et al.*, 2009). Upland landscapes cover nearly one third of the UK land surface (Fielding & Haworth, 1999) and are built upon older, harder rocks, which are more resistant to the weathering effects of the harsh climate (Atherden, 1992). The uplands are highly valued in the UK and contain most of the large scale protected landscapes (Orr *et al.,* 2008), with nine out of the twelve English and Welsh national parks possessing upland habitat (Fielding & Haworth, 1999). Various definitions exist to describe the British uplands, ranging from altitudinal (i.e. above 300 m) (Millward & Robinson, 1980), climatological or environmental (prevailing weather conditions, soil or rock type) (Averis *et al*., 2004), to designations for low agricultural productivity land (i.e. Less Favoured Area - LFA) (Reed *et al.,* 2009). One theme is universal to these definitions: the uplands contain the highest landscapes in the UK.

The UK uplands are predominantly open, unenclosed and extensive tracts of land which are often erroneously considered to be ‘wild’, however the present shape and appearance of these landscapes can be attributed to the influence of hundreds to thousands of years of anthropogenic interference (Van der Wal *et al.,* 2011). Human societies began to alter the nature of the UK landscape markedly from around 5000 years before present (BP) (Atherden, 1992) with the removal of the mixed deciduous and coniferous forest which had dominated upland Britain, to make way for agriculture (Rackham, 1987). The deforestation of the UK was a slow but continuous process. By end of the Roman era in the UK (post 400 AD) the majority of upland tree cover had already disappeared (Simmons, 2003). Livestock have been grazed in the uplands since the first deforestation in Neolithic times (Bevan, 2009). This pressure from agriculture has been significant in driving and maintaining the open nature of this environment (Fuller, 1996). The current appearance of the UK uplands can be directly attributed to the agricultural management practices of burning and grazing by sheep, which are the principal influences maintaining the current open character of this landscape (Fuller, 1996; Fuller & Gough, 1999).

1.2.1 UPLAND ECOSYSTEMS AND HABITATS

The UK uplands can be split into two broad categories: montane and sub-montane (Fielding & Haworth, 1999). These categories encompass a broad range of habitat types but are particularly characterised by oligotrophic environments such as acid grasslands, heathlands and ombrotrophic wetlands such as blanket bogs. The nature of upland habitats is defined by varying climatological conditions (Averis *et al.*, 2004) and prevailing management approaches. The different habitats present across the upland landscape can be considered as a gradient of change subject to the prevailing site specific management and intensity, altered from bog, to heath, to grassland, by draining and grazing (Fuller & Gough, 1999). The climate of the uplands is dependent on the height of the landscape. Increasing altitude results in a reduced average temperature and amount of available sunshine which is significant for plant growth. The reduction in temperature relative to altitude is referred to as the lapse rate and is accepted to equate to 6^{o}C drop per 1000 metre rise (Fielding & Haworth, 1999). Additionally the upland climate has higher levels of precipitation and wind speed compared to lower regions (Millward & Robinson, 1980). Wind speed can also have a limiting effect on plant growth, and can serve to reduce effective temperature through wind chill (Fielding & Haworth, 1999). Wind speed has been calculated to increase with altitude between 6–9 ms^{-1} per 1000 metres (Grace & Unsworth, 1988). Thus such mechanisms govern the character of these landscapes.

Upland habitats are varied but can be generally categorised as heathland communities (Figure 1.1, A & B), mire or bog communities (Figure 1.1, C) or acid grassland communities (Figure 1.1, D) (Holden *et al.*, 2007). Heaths are characterised by dwarf shrub species including common or ling heather *Calluna vulgaris*, bell heather *Erica cinerea*, crowberry *Empetrum nigrum* and bilberry *Vaccinium myrtillus* (Rodwell, 1991). Mire or bog communities feature bog mosses *Sphagnum* spp, graminoids such as cotton grasses *Eriophorum* spp and purple moor grass *Molinia caerulea*, and dwarf shrubs including cross leaved heath *Erica tetralix* and bog cranberry *Vaccinium oxycoccos* (MacDonald *et al.*, 1998). Acid grasslands are dominated by graminoids including sheep's fescue *Festuca ovina*, mat grass *Nardus stricta*, bent grasses *Agrostis capillaris* and *A. canina*, purple moor grass *Molinia caerulea* and heath rush *Juncus squarrosus* (Fielding & Haworth, 1999) and forbs such as tormentil *Potentilla erecta* and heath bedstraw *Galium saxatile* (Stevens *et al.,* 2010).

The species composition and community structure of much of the vegetation found in UK uplands is unique in Europe (Averis *et al.*, 2004), with some habitats recognised internationally as of priority for conservation. Heathland habitats are increasingly rare globally, with seventy five per cent of the world's heather moorland found within the UK (Tallis *et al*., 1998; Holden *et al.*, 2007). Heathlands are regarded as important for biodiversity and support rare habitat specialist species.

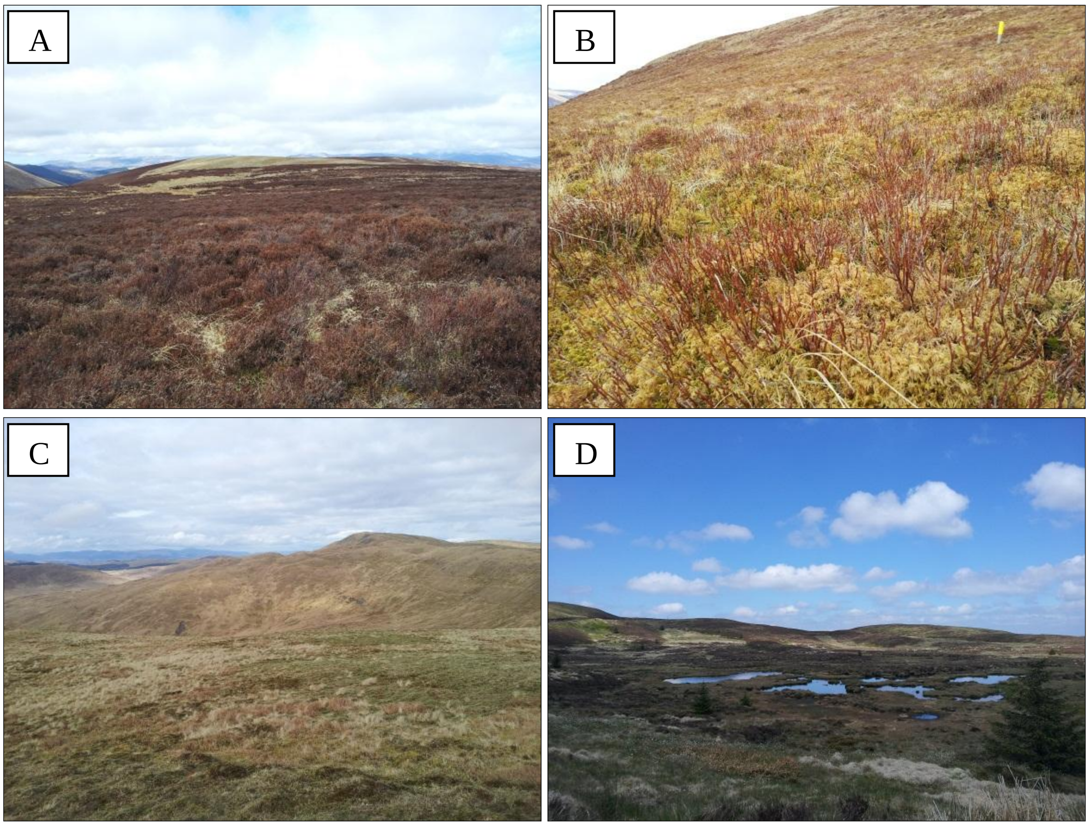

Figure 1.1 Examples of upland vegetation communities: A) dry heath, B) regeneration of dry heath after livestock grazing, C) grassland, D) blanket bog with pool complex (pictures taken by W. Stiles during fieldwork – summer 2013).

Of the 19 different heath plant communities, five are almost totally confined to the UK and Ireland, and a further six are better developed in the UK than anywhere else (Thompson *et al*., 1995). Blanket bogs and raised bogs are also habitats of conservation interest for the species assemblages they support and are designated under the EC Habitats Directive (Bain *et al*., 2011). Wetland habitats, such as upland bogs, are highly susceptible to perturbation from human activities, including burning, drainage and grazing (Parry *et al*., 2014), which can result in the degradation or loss of these habitats.

1.2.2 UPLAND SOIL CHARACTERISTICS

Acid or strongly base deficient soils such as peat are common in the upland environment due to the combined effects of a cool, wet climate, the relative scarcity of base rich parent material, and the presence of certain plant species such as *C. vulgaris*, which can have acidifying effects (Averis *et al.,* 2004; Holden *et al.,* 2007). Acidification happens naturally in the uplands as soils exposed to high levels of precipitation increase in inputs of exchangeable hydrogen (H^+) ions (or compounds which produce H^+) and lose soluble cations, such as calcium (Ca^{2+}) and magnesium (Mg^{2+}), through leaching (Rowell, 1994). In addition, in recent years the deposition of acidifying pollutants such as N & sulphur (S) have also altered soil biogeochemistry (Morecroft *et al.,* 2009). This impact has been shown to be diminishing nationally; amounts of S deposited in the UK reduced by 80% between 1986 and 2007 (RoTAP, 2012) (see figure 1.2, section 1.3.2), which has been confirmed by soil monitoring studies (Morecroft *et al.*, 2009; Emmett *et al.,* 2010; Kirk *et al.,* 2010) as an increase in UK soil pH, however, a recent re-visitation survey of the soils of Snowdon in Wales by McGovern *et al.,* (2013), showed a slight decrease in soil pH, suggesting that for upland soils acidification remains a problem. Soil pH level is directly correlated to fertility status as nutrients are more mobile in, and can therefore be readily leached from, low pH soil (Roem & Berendse, 2000). The fertility status of upland soil is traditionally low, mainly due to the effects of leaching and slow rates of nutrient cycling. Additionally, centuries of nutrient extraction from the production of wool, meat and timber has likely further reduced UK upland soil fertility status (Averis *et al.,* 2004).

Upland peatland soils typically have high organic matter contents and are a major C reservoir estimated to hold 2302 Mt C (Billett *et al.,* 2010). Values for peatland accumulation of C are varied and range from a rate of approximately –23 g C m^{-2} yr^{-1} (Gorham, 1991) to –56 to –72 g C m^{-2} yr^{-1}, with the latter figures considered more representative for current UK peatland (Billett *et al.,* 2010). Current accumulation rates however are reported to be lower than values seen in peat cores (–35 to –209 g C m^{-2} yr^{-1}) from the last 150 years (Billett *et al.*, 2010) suggesting a downward trend. Soil fertility and C content are intrinsically linked but the relationships are complex. Nutrient limitation can reduce C sequestration through reductions in plant growth and litter production (Goll *et al.*, 2012), whereas increases in fertility may stimulate decomposer activity resulting in C losses (Bragazza *et al*., 2006). The acidic nature of upland soil also limits soil organic matter (SOM) mineralisation rate, thus promoting C accumulation in low pH soil where decomposition is reduced (Worral & Evans, 2009).

The ability of upland soil to sequester and retain C is of significant importance (Emmett *et al.,* 2010). Recent reports of change in C content of UK soils is a contentious point, with UK wide studies reporting both losses (Bellamy *et al.,* 2005) and negligible change in levels (Emmett *et al.,* 2010) since 1978. However a recent review by Barraclough *et al*., (2015) of the national soil inventory (NSI) data used by Bellamy *et al*., (2005), showed that for organic soils in semi-natural habitats a decline of between 9-22% soil C is caused by the effects of changing temperature and rainfall rates between the two NSI surveys. This was driven by temperature induced changes in vegetation species composition and thus the input of organic matter to soil, which echoes the results of other replicated experiments (i.e. Ward *et al*., 2013 and 2014), highlighting the potential positive feedback between upland soil C stores and climate change.

1.3 ENVIRONMENTAL CHANGE IN THE UPLANDS

Upland habitats are highly susceptible to perturbation. Small changes to the hydrology, soils and ecology can have a significant influence on landscape appearance and function (Holden *et al.*, 2007). Environmental change in the uplands is driven by both natural and anthropogenic forces. Natural drivers of change act in both the short term, such as extreme weather events resulting in flooding, mass movement or erosion, and long term, such as gradual changes in climate influencing the process of blanket bog formation (Evans, 2009). Currently high rates of environmental change are being driven by the impact of anthropogenic activities (Condliffe, 2009; Reed *et al*., 2009) which modify upland ecosystems via three principal mechanisms: climate change, the atmospheric deposition of pollutants, particularly N, and land use or management practice change (Orr *et al*., 2008; Caporn & Emmett, 2009; Morecroft *et al.*, 2009; Burt & Holden, 2010; Stevens *et al.*, 2010). These drivers, acting in concert or independently, pose a significant threat to the health and function of upland habitats, and are responsible for widespread declines in biodiversity across trophic levels. A recent report on the biodiversity of the upland ecosystem showed that of 877 upland species assessed, 65% had decreased and of those 35% have decreased strongly (Burns *et al*., 2013). Such significant perturbation of any system is ultimately unsustainable, therefore the need to address these drivers is considerable and pressing. The design and implementation of suitable management approaches or amelioration techniques based on credible scientific evidence, but which are translatable and actionable (Anderson, 2014), are essential to reduce the impact of this change.

1.3.1 CLIMATE CHANGE

Warming of the climate system is now unequivocal, with almost the entire globe experiencing some surface warming, observed as increases in global average temperatures for the atmosphere and oceans, loss of snow and ice coverage and a rise in average global sea level (IPCC, 2013). The impact of anthropogenic warming on ecosystems is attributed to the emission of greenhouse gases (GHGs), such as carbon dioxide (CO_2) and methane (CH_4) (Rosenzweig *et al.*, 2007). In the last century (1880 to 2012) the Earth's average temperature has warmed by 0.85°C, a figure which has increased significantly since 1951 to a rate of 0.12°C rise per decade. Warming in the last 30 years has been substantial with the 30 year period between 1983 and 2012 recognised by the IPCC (2014b) as 'very likely' to have been the warmest in the Northern Hemisphere in the last 800 years and 'likely' to have been the warmest in the last 1400 years.

Climate change is predicted to have different effects at higher elevations than in lowland areas (Burt & Holden, 2010), with the uplands identified as a particularly vulnerable environment (IPCC, 2014a). Few long term climate records exist for the uplands which is potentially problematic as upland areas may not change in parallel to the lowlands (Pepin, 1995). Long term records from the Moor House station in the Pennines do show a similar trend of warming in the last seventy years to nearby lowland records, with the greatest warming observed in winter months suggesting reduced thermal seasonality (Holden & Adamson, 2002), however whilst the mean annual averages may appear in tandem, there is significant variation on a monthly and seasonal basis, making predictions for the uplands based on lowland data impractical (Burt & Holden, 2010; Holden & Rose, 2010).

The cooler climate is a defining feature of the uplands. Organisms adapted to this environment are likely to be more sensitive to the effects of a warming climate (Caporn & Emmett, 2009). Consequently, species are expected to migrate to higher latitudes and/or altitudes (Hickling *et al.,* 2006) or to select habitat types which support suitable microclimates (Gillingham *et al.,* 2012). This has several implications in a modified landscape such as the UK uplands, where habitat patches are often fragmented and isolated creating a barrier to movement (Holden *et al.,* 2007). Predictions of species movement from computer models suggest that movement will be affected most when the remaining habitats are in discrete, larger but more isolated patches, rather than scattered through a fine-grained landscape (Huntley and Baxter, 2002), which has clear implications for landscape and conservation strategies. It is expected this issue will result in the local or regional extinction of some species, with the greatest losses amongst those adapted to upland or high latitude habitats (Huntley and Baxter, 2002; Thomas *et al*., 2004). Plant community response to increasing CO_2 levels is a debated topic. A rise in atmospheric CO_2 has been reported to increase primary productivity (DeLucia *et al.,* 1999), resulting in greater biomass accumulation and C capture, or to have no significant effect (Berendse *et al.*, 2001). Studies have also shown that CO_2 uptake is directly affected by nutrient availability (Reich *et al.*, 2006; Reich & Hobbie, 2012), adding another level of complexity for oligotrophic habitats such as the uplands, exposed to N enrichment from deposition (see section 1.3.2). Changes in climate also affect species phenological traits, potentially resulting in asynchrony of ecological interactions. Disruptions to phenological timings can have grave consequences for ecosystem resilience, ecosystem service provision, and could further the potential for species extinctions (Thackeray *et al.*, 2010).

1.3.1.1 SOIL CARBON FLUXES

Peatland soil with high organic C content makes climate warming in the UK uplands of particular concern. A warming climate could convert these current sinks into sources of atmospheric C (Gorham, 1991; Worral *et al.*, 2004 & 2009), as the production of CO_2 increases significantly with even moderate rises in temperature (Dorrepaal *et al*., 2009). Carbon stored in soil is lost to the atmosphere in the form of CO_2 from SOM mineralisation (Dawson & Smith, 2007) and CH_4 from the anaerobic decomposition of organic matter by methanogenic microbes (Bubier & Moore, 1994; Cooper *et al*., 2014). Emissions of CO_2 are considerably larger than CH_4 (current (2011) atmospheric content mass for CO_2 = 828 Pg C and CH_4 = 3.7 Pg C), but the global warming potential of CH_4 is 28 times greater than CO_2, thus it is an important GHG (IPCC, 2013).

Stress from the effects of climate change is predicted to affect up to half the British peatlands by the end of the 21st century (House *et al*., 2010), with direct implications for C storage. The carbon stock of peatlands in the northern hemisphere is estimated to be 547 (473–621) Pg C, (Yu *et al*., 2010) which is 40% of global soil C stock in ~3% of the Earth’s terrestrial surface (Cooper *et al*., 2014). In the UK, 50% of terrestrial C is stored within peat soil (House *et al.*, 2010) in ~15% of the land area (Billet *et al*., 2010). Estimations of future C loss from UK soil, suggest a 12% fall in C stock would be equivalent in climatic effect to the total annual UK emissions of GHG from the burning of fossil fuels (House *et al*., 2010). Attempts to reduce additional factors contributing to C loss from soil reservoirs are therefore of immense importance.

1.3.2 NITROGEN DEPOSITION

Atmospheric deposition of reactive N is widely accepted to be a significant threat to biodiversity and ecosystem function (Bobbink *et al.,* 1998; Stevens *et al.,* 2004; Bobbink *et al.,* 2010; Stevens *et al.,* 2010; Field *et al.*, 2014). N deposition can increase soil acidification (see 1.2.2) (Morecroft *et al.,* 2009; Phoenix *et al*., 2012) and cause eutrophication (Smart *et al.,* 2003). Reactive N refers to all inorganic reduced forms of N (e.g., NH_3, NH_4^+), inorganic oxidized forms (e.g. NO_x, HNO_3, N_2O, NO_3^-) and organic compounds (e.g., urea, amines, proteins) (Bobbink *et al.*, 2010). Amounts of reduced and oxidised N deposition may vary considerably across the UK landscape, with reduced N deposition being more common in rural areas, especially in areas of intensive livestock production, and oxidised N deposition more common in urban areas (Stevens *et al.*, 2011). Nationally, the amounts are similar: in 2008, the quantities recorded for total N deposition were 175 Gg N yr^{-1} for reduced and 178 Gg N yr^{-1} for oxidised forms (RoTAP, 2012). Recently, emissions of N have reportedly fallen in the UK: nitrogen oxides have decreased by 62% since 1970, to 1.02 million tonnes and emissions of ammonia are reported to have decreased by 23% since 1980, to 270 thousand tonnes in 2013 (DEFRA, 2014 – see Figure 1.2). However a report by Emmett *et al.*, (2011) showed that large areas of the country still exceed the critical load and level for N deposition, and are predicted to continue to do so despite the reductions in emissions.

The availability of N is often a limiting factor for terrestrial ecosystems (Tamm, 1991) and for many this low availability results in plant communities with high species diversity (Bobbink *et al.,* 1998). In upland ecosystems the increase of reactive N has the potential to enrich soils, resulting in increased plant growth and therefore competition for resources such as light (Stevens *et al.*, 2010), which can

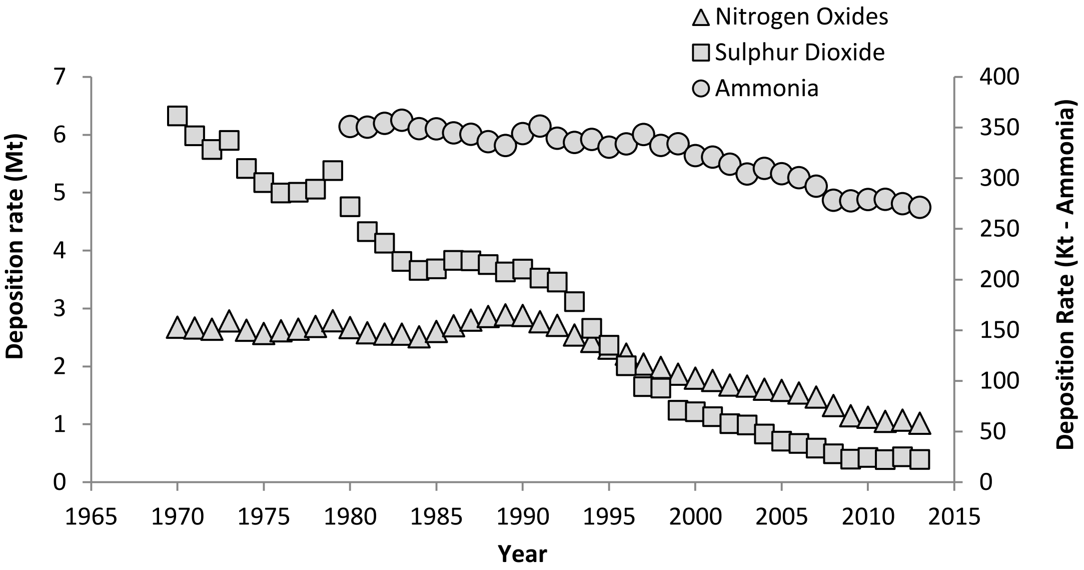

Figure 1.2 Trends in the emission of air pollutants, 1970 – 2013. Nitrogen oxides and sulphur dioxide measured in megatonnes (Mt), ammonia in kilotonnes (Kt) **(**DEFRA, 2014).

reduce plant species richness (Bobbink *et al.,* 1998; Stevens *et al.,* 2004); an effect confirmed by various studies of British and European vegetation (Smart *et al.,* 2003; Van der Wal *et al.,* 2003; Stevens *et al.,* 2004, & 2010; Maskell *et al.,* 2010; Field *et al.,* 2014). Additionally the invasion of nitrophilous species such as certain graminoids from the lowlands (Van der Wal *et al.,* 2003; Bobbink *et al.,* 2010) and the toxic effects of high levels of N on bryophytes such as *Racomitrium lanuginosum* (Pearce *et al.,* 2003; Armitage *et al.,* 2005) has the potential to alter community structure of upland vegetation. Existing critical load values for N deposition are potentially too high as changes in both species richness and ecosystem function indices have been shown to occur at low thresholds of N deposition (5-10 kg N ha^{-1} yr^{-1}), which in some cases is below current limits (Emmett *et al.,* 2011). Current critical loads also fail to allow for amplification caused by livestock grazing (Van der Wal *et al.,* 2003) (see section 1.3.4), which may have significant implications for conservation strategies and biodiversity targets. Whilst the impact on species richness is well documented, less well understood is the influence of N deposition on nutrient cycling. Increasing levels of N deposition have been shown to increase plant uptake of phosphorus (P) and potassium (K) (Rowe *et al.*, 2008; Jones & Power, 2011), suggesting that primary productivity could increase with higher levels of N and carbon dioxide (CO_2) regardless of nutrient limitation, which has potentially beneficial implications for C uptake (Law, 2013).

1.3.2.1 NUTRIENT AVAILABILITY EFFECTS IN UPLAND HABITATS

The increasing availability of N in upland habitats affects ecosystem function and alters ecosystem processes (Jones & Power, 2012; Southron *et al*., 2013). Nitrogen enrichment can increase soil microbial activity rates, via release from nutrient limitation and also through the inhibition of phenolic and polyphenolic compound production of some plants, which otherwise exert a suppressive effect on microbial activity (Witzell & Shevtsova, 2004; Bragazza & Freeman, 2007), this results in higher rates of decomposition; which is problematic particularly for soils that are important C stores with traditionally low rates of decomposition due to the low tissue nutrient content of sequestered plant material, such as peat (Bragazza *et al.*, 2008). Furthermore, the rate of decomposition also controls the availability of other nutrients such as P and K, as these are stored in SOM and released with mineralisation (Bragazza *et al*., 2013), thus increases in decomposition facilitated by increased N enrichment can alter numerous nutrient cycling pathways concurrently.

Increases in reactive N availability in conventionally N limited environments can initiate a shift in nutrient limitation from N to P limitation (Crowley *et al.*, 2012; Peñuelas *et al*., 2013; Rowe *et al.*, 2014). Where P limitation restricts vegetation biomass production (Li *et al*., 2015), ecosystems may be unable to sequester reactive N inputs, which could result in N saturation and therefore N losses through leaching (Emmett *et al.*, 1995; Britton & Fisher, 2007). The availability of P is negatively related to plant species richness independent of N deposition rate (Ceulemans *et al*., 2014). Phosphorus limitation therefore serves to constrain productivity and is likely to be a major regulator of ecosystem biodiversity in habitats where N is readily available such as those exposed to high rates of N deposition (Ceulemans *et al*., 2014) and may also serve to limit rates of decomposition within upland peaty soil, as microbial decomposer activity is also currently limited by P availability (Bragazza *et al*., 2012). Phosphorus deposition is also a potential pollutant, both from natural sources (Tipping *et al*., 2014) and from anthropogenic activities, primarily agriculture. The extent to which this impacts upland habitats however requires more research to determine.

Recently, P addition has been discussed as a potential management tool to alleviate the impact of chronic N enrichment from deposition (Armitage *et al.*, 2012; Blanes *et al*., 2012). The addition of P can increase N uptake and usage by improving the competitive ability of roots for N against free-living microbes (Blanes *et al*., 2012), or increasing enzyme action such as nitrate reductase activity (Arróniz-Crespo *et al*., 2008), resulting in greater vegetation growth and C sequestration. Whilst localised success with regard to heath restoration has been demonstrated with the addition of P (Armitage *et al.*, 2012), this approach requires much consideration, as P is highly persistent in soil and therefore has the potential to offer long term influence on ecosystem processes. Enhanced nutrient availability can also affect numerous ecosystem processes simultaneously, which can make predictions of change complex. As an example, Mack *et al*., (2004) found N and P addition in arctic tundra systems resulted in concomitant stimulation of both plant production and decomposition, but with larger effects observed for decomposition, therefore resulting in an overall net loss of C.

1.3.3 LIVESTOCK GRAZING

The latter half of the 20th century saw an unprecedented intensification of agriculture, responsible for the widespread loss of habitats and biodiversity (Morecroft *et al.,* 2009). In the UK uplands this intensification resulted in greater densities of sheep *Ovis aries* (Fuller, 1996). By 1990 sheep numbers in Scotland, England & Wales had more than doubled from 1950 levels to a high of 41.2 million, which can be attributed to the advent of farm subsidies in the form of headage payments (Fuller & Gough, 1999). More recently changes to agricultural policy, such as the introduction of Environmentally Sensitive Area schemes in England and Wales, have reduced sheep densities (Holden *et al.,* 2007). The total number of sheep and lambs in the UK as of December 2013 was 22.6 million animals (DEFRA, 2014b). Numbers, which for sensitive environments such as the uplands remain potentially unsustainable.

1.3.3.1 UPLAND ECOSYSTEM RESPONSES TO LIVESTOCK GRAZING

Livestock grazing has been shown to affect the structure and composition of the plant (Bullock *et al.*, 2001), avian (Fuller, 1996; Evans *et al.*, 2005), mammal (Evans *et al.,* 2006; Wheeler, 2008) and invertebrate (McFerran *et al.*, 1994; Dennis *et al.,* 2008) communities in upland habitats, all of which can be inter-linked via cascading effects (Evans *et al*., 2015). Grazing by large herbivores has been shown to directly modify plant community structure through preferential selection of species

(Anderson & Briske, 1995), the alteration of disturbance regimes (Bullock *et al.,* 2001), or by increasing nutrient availability through enrichment from dung and urine (Medina-Roldan *et al.,* 2012). Selective grazing can affect plant success through defoliation and through changes to species dispersal rate. This can be either beneficial, by aiding the transport of seeds to new areas (Pakeman & Small, 2009), or harmful, through the consumption of juvenile specimens resulting in a reduction in seed production (Holden *et al.,* 2007). These effects will ultimately result in a modified plant community. Pakeman, (2004) showed that augmented grazing intensity would lead to shifts in vegetation species composition towards increases in species exhibiting traits such as ruderal strategies, annual life histories, seasonal regeneration by seed, flowering and seed dispersal in early season, low growing with rosette leaf habit and high requirement for light. The greatest species diversity is expected to be supported by habitats possessing high heterogeneity of vegetation types and structure (Buchanan *et al.,* 2006; Pearce-Higgins & Grant, 2006), which is unlikely in the extreme to be facilitated by heavy grazing from sheep, highlighting some important considerations for future management plans (Evans *et al.,* 2006).

Change to the spatial heterogeneity of vegetation caused by grazing modifies habitat diversity and influences consumers in higher trophic levels (Adler *et al.,* 2001; Evans *et al*., 2015). The total biomass of foliar arthropods recognised as dietary components of moorland birds increases with grazing intensity reductions (Dennis *et al.*, 2008). Spider abundance in grassland is also significantly greater in the absence of grazing (McFerran *et al.,* 1994; Dennis *et al.,* 2015), thus greater prey abundance is positively correlated with less or no grazing presence, which is directly related to predator populations (Evans *et al*., 2015). However this is potentially variable depending on invertebrate order as Mysterud *et al.,* (2005) found no significant difference between abundances of Diptera and Hemiptera subject to grazing intensity, concluding that other factors, such as plant community composition and diversity, may have greater influence for these groups.

Upland landscapes have seen large declines in bird species, which is largely attributed to the impact of grazing by sheep (Fuller, 1996; Fuller & Gough, 1999). Studies have suggested that grazing effects on bird populations include simplification of vegetation structure reducing available nesting and forage opportunity (Evans *et al.,* 2006) and reductions in egg sizes and breeding success (Evans *et al.,* 2005). The indirect effects through impacts to food sources such as invertebrate prey species may also be significant (Buchanan *et al.,* 2006). Investigations into the effect of grazing intensity on small mammal populations (field voles, *Microtus agrestis*) have shown negative relationships (Steen *et al.,* 2005*;* Evans *et al.,* 2006; Wheeler, 2008). This has been attributed to both competition for food between large and small herbivores and reduced sward height offering less refuge from predation (Evans *et al.,* 2006). Population estimates of voles and their predators such as short-eared owl *Asio flammeus* and long-eared owl *Asio otus*, have reportedly doubled under reduced grazing (Wheeler, 2008). Therefore high sheep density in upland habitats influences populations of species across trophic levels.

Grazing also influences the composition and diversity of soil organisms (Bardgett *et al.,* 1993; Fu *et al.,* 2012) through the alteration of resource quality and quantity produced by plants, which affects soil biota communities (Wardle *et al.,* 2004; Klumpp *et al.,* 2009). Bardgett *et al.,* (1998) attribute this to two factors: (1) the alteration of quality and quantity of detritus and non-detritus inputs and; 2) the influence of soil physical and chemical characteristics on soil microhabitat quality. Studies have reported much variation in the soil biota populations subject to grazing intensity. Collembola populations have been shown to be both highly positively associated (Bardgett *et al.*, 2003), and highly negatively associated (King *et al.*, 1976) with higher grazing intensity. The microbial community (composed of bacterial and fungal microbes) can vary in bacterial-fungal ratio with different management regimes (Bardgett *et al.*, 1996; Yeates *et al.*, 1997), which affects populations of specialist fungal or bacterial feeding nematodes. Villenave *et al.*, (2011) showed that application of an intensive grazing regime induced a significant increase in numbers of bacterial feeders and a decrease of the relative abundance of fungal-feeding nematodes. A greater understanding of this relationship is necessary as nematodes may fulfil a regulatory role for microbial communities and nutrient dynamics in upland soils (Bardgett *et al.*, 1997).

1.3.3.2 LIVESTOCK GRAZING INFLUENCE ON UPLAND SOIL CHARACTERISTICS

The importance of soil C and N, and factors currently affecting this status in the upland ecosystem, have already been reviewed (see sections 1.2.2 and 1.3.1.1). Large herbivores have the potential to modify soil physical and chemical properties (Medina-Roldán *et al*., 2012 & 2012b) and can influence ecosystem function and processes (Milchunas and Lauenroth, 1993; Wardle *et al*., 2004; Bai *et al*., 2012). In areas of high livestock density soil can become compacted, increasing bulk density (Steffens *et al*., 2008) and reducing the potential for water infiltration (Holden *et al.*, 2007), the severity of which is dependent on soil type (Van Haveren, 1983). Livestock grazing can alter the cycles of soil C, N and other nutrients in grassland ecosystems (Li *et al.*, 2011; Bai *et al*., 2012), affecting C storage potential (Pineiro *et al.*, 2009). Grazing may affect the content of C in soil by varying the magnitude or the proportion of NPP (net primary productivity) reaching the soil, by changing soil N stocks and/or by altering SOM decomposition. SOM is the main reservoir of C and determines characteristics such as soil fertility, water retention, and soil structure (Pineiro *et al.,* 2010). Grazing impact on soil C and N remains controversial and has been reported to increase (Manley *et al*., 1995; Gao *et al.,* 2009; Li *et al.,* 2011), decrease (Derner *et al*., 2006; Steffens *et al*., 2008; Pineiro *et al.*, 2009) or have little or no effect on the size of both pools (Milchunas and Lauenroth, 1993; Martinsen *et al.*, 2011).

There are numerous pathways through which livestock can affect soil C status. Grazing can reduce SOM by limiting the amount of plant material returned to the soil through consumption (Manley *et al*., 1995), and can affect decomposition rates by altering plant species and therefore root traits (Smith *et al*., 2014) and hence their control on the soil microbial communities (Klumpp *et al.*, 2009). These processes may facilitate decomposition and accelerate the loss of soil C, however grazing-induced changes in species composition can also potentially enhance SOM by increasing below-ground biomass or root to shoot ratio (R:S), regardless of aboveground biomass level, thus increasing the proportion of belowground C and N even under heavy grazing (Schuman *et al.*,1999; Li *et al.*, 2011). Herbivore excretions can accelerate N cycling, but may decrease N inputs to the soil because of higher N volatilization and leaching from urine and dung patches (Pineiro *et al.*, 2006). Grazing may also change legume abundance and consequently N fixation rates, which alters N inputs to the soil (Allard *et al.*, 2003) affecting C accumulation (Knops & Tilman, 2000). Plant community composition may also be a key factor for determining the net effect of grazing on SOM levels, as both the net primary productivity (NPP) pathway and the N pathway are affected by changes in species composition. Grazing has been shown to increase forbs with low NPP and thus decrease C inputs to the soil (Pineiro *et al.*, 2010).

1.3.4 SYNERGISTIC EFFECT OF LIVESTOCK GRAZING AND N DEPOSITION

The relationship between N deposition and livestock grazing pressure is highly complex and ecosystem responses extremely variable (Hartley & Mitchell, 2005; Smith *et al.*, 2015). Nitrogen inputs take two main forms: oxidised (NO_y) and reduced (NH_x) (see section 1.3.1). NH_x inputs are the result of agricultural activities such as animal husbandry (from livestock wastes) and fertiliser application (Stevens *et al.*, 2011). Therefore livestock grazing impact is twofold: both as a contributor to reactive N enrichment and also from the physical impact of herbivore activity (i.e. Evju *et al.*, 2009). Whilst the presence of livestock may increase the rate of NH_x deposition, it could also limit the impact of this enrichment on vegetation growth through the removal of biomass (Stevens *et al.*, 2011). Increased competition for light, mediated by increased growth capability, is likely to be the most important driver of species compositional change with N deposition (Stevens *et al.*, 2010); thus grazing itself may limit the dominance of certain species, potentially reducing negative effects (Borer *et al.*, 2014).

Nonetheless, vegetation species composition could still suffer modification from the preferential selection of species by livestock (Anderson & Briske, 1995). Both N deposition and livestock grazing serve to increase the frequency of graminoids in a wide range of habitats (Mountford *et al.*, 1993; Van der Wal *et al.*, 2003; Hartley &

Mitchell, 2005). Modification of vegetation species composition towards species which are considered more palatable and thus more likely to be preferentially selected, such as grasses, coupled with augmented growth from N enrichment, results in increased attraction to herbivores, with consequent rises in trampling and biomass removal. This forms the basis of the multi-step feedback loop proposed by Van der Wal *et al.*, (2003) (Figure 1.3), which demonstrates the negative synergism of livestock grazing and N deposition on upland vegetation; an effect which is potentially greater than the complementary influence of herbivores, with regard to biomass removal limiting the stress from restricted light availability, suggested by Borer *et al.*, (2014). The shift in vegetation species is also particularly significant for peatlands, as a move from vegetation which produces recalcitrant litter to species which are less decomposition resistant such as grasses, can reduce or stop peat formation, thus inhibiting C sequestration (Berendse *et al.*, 2001).

In general, the effects of N enrichment from atmospheric deposition and livestock grazing on C sequestration are highly uncertain, but for peatland, this combination is expected to negatively influence C status. High levels of N enrichment have been shown to decrease litter C:N ratios, resulting in elevated decomposition rates through enhanced microbial activity (Mack *et al.*, 2004; Bragazza *et al.*, 2006). This coupled with decomposition rate modification from grazing, through shifts in vegetation species composition and therefore associated root traits (Smith *et al.*, 2014), towards species which are less decomposition resistant, suggests that the effects of N deposition and livestock grazing on peatland could potentially result in major C losses.

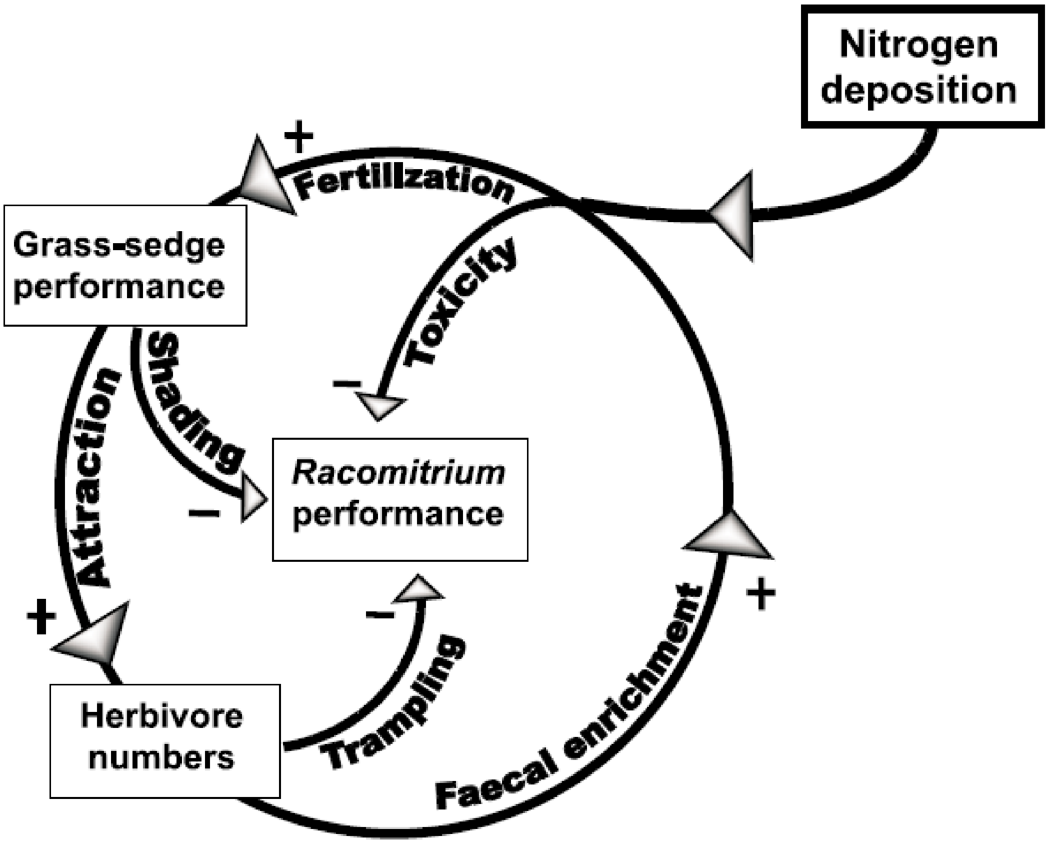

Figure 1.3 Conceptual model demonstrating a multi-step feedback loop for the combined effects of N deposition and livestock grazing, resulting in degradation of upland vegetation and habitats, as proposed by Van der Wal *et al.,* (2003).

1.4 THESIS OVERVIEW

Environmental change in upland habitats is increasingly driven by anthropogenic activity (MEA, 2005). The combined effects of climate change, atmospheric deposition of N, and land management intensity are causing degradation in upland habitats, reducing biodiversity and increasing susceptibility to future environmental perturbation (Morecroft *et al.,* 2009; Burns *et al*., 2013; Hautier *et al*., 2015). These current drivers are likely further intensified by mutual interactions (Hartley &

Mitchell, 2005; Helliwell *et al*., 2010; Smith *et al*., 2015), yet the impact of this on upland ecosystem processes remains unclear. A more detailed understanding of the ecosystem response to the synergism between livestock grazing and N deposition, and the effect on ecosystem processes, is therefore highly important for the design and implementation of effective management strategies which account for this interaction, to reduce future habitat degradation

The effect of nutrient enrichment in upland habitats on C storage potential remains controversial (Mack *et al*., 2004; Limpens *et al*., 2004; Bragazza *et al*., 2006; Fornara *et al*., 2013; Zhang *et al*., 2013). Partitioning causal effects in dynamic systems is highly complex, however understanding the role of nutrient availability with regard to ecosystem processes is crucial for the effective management of upland C storage. In addition, understanding how P availability affects upland biodiversity, ecosystem processes, and C storage potential, could greatly advance our understanding of long term ecosystem response to N enrichment from deposition as systems shift towards P limitation, and could offer valuable insights on ecosystem response to P availability, particularly where P addition is being considered as an amelioration measure to offset the impact of increasing N enrichment.

This thesis is comprised of an introduction and review of existing literature, four experimental chapters designed to test the aims and objectives detailed below (see 1.4.1) and a general discussion which provides synthesis. The experimental chapters are presented in format for publication in scientific journals; as a consequence some unavoidable repetition of introductory material exists between chapters.

1.4.1 AIMS AND OBJECTIVES OF RESEARCH

The aim of this research is to further understanding of the effect of land management intensity and N enrichment from atmospheric pollutant deposition, on upland ecosystem processes and the potential for ecosystem service delivery in the form of soil C storage. The primary aims of this thesis were:

1) Evaluate the impact of reactive N deposition and livestock grazing intensity, and their interaction, on upland ecosystem processes and biodiversity.
2) Investigate the long term impact of N and phosphorus (P) availability on upland vegetation structure and species composition.
3) Assess the influence of N and P availability on upland soil chemistry and biology.
4) Quantify the impact of N and P availability on soil GHG emissions and C storage potential.

1.4.2 EXPERIMENTAL CHAPTERS AND HYPOTHESES

The first experimental chapter (Chapter 2) details the findings of an inferential study into the combined impacts of livestock grazing intensity and N deposition rate at locations across the Cambrian Mountains, Mid Wales. The second, third and fourth experimental chapters will investigate the role of nutrient availability in the upland ecosystem with two randomised block nutrient addition (N and P) experiments, which have been run in both the short (one year) and longer term (1996 - 2012, with sampling undertaken three years after ceasing N inputs and 15 years after a single P application). These chapters investigated ecosystem responses to nutrient addition and specifically tested: soil C and N and vegetation community responses to N + P enrichment in the long term (Chapter 3); the response of vegetation biomass, soil nutrient status, and soil invertebrate communities to N + P enrichment in the short term (Chapter 4); Soil C flux modification, in the form of two GHGs (CO_2 and CH_4), with N + P enrichment in both the sort and the long term, to determine the impact of increasing nutrient availability on microbial activity and C storage potential in upland soil (Chapter 5).

It is hypothesised that increasing livestock grazing intensity and nutrient availability from N deposition, alter upland ecosystem processes resulting in C storage reductions in upland soil. This thesis will establish this by testing the following specific hypotheses:

- Chapter 2 - N deposition and grazing intensity have additive effects and result in: 1) lower vegetation height and biomass through offtake and species composition change (observed as higher graminoid cover); 2) lower belowground C and N pool size (driven by the top-down effects of vegetation species change and modification of microbial activity); 3) reduced abundance of a key upland invertebrate group (craneflies) as a consequence of reduced soil C and N pool size, as this relates to the availability of food resources.
- Chapter 3 - increased availability of N and P would: 1) alter vegetation species composition, with greater graminoid species cover found in N addition treatments and greater cryptogam species cover with P addition; 2) reduced soil C content resulting from differences in vegetation species composition and nutrient availability; 3) that these effects will be largest where P limitation has been reduced.
- Chapter 4 - release from P limitation increases N utilisation and therefore ecosystem productivity (observed as greater plant shoot extension) and soil faunal abundance, as nutrient availability increases food resources and thus

energy provision from the bottom-up. It is expected that through bottom-up regulation, increases in the abundance of soil fauna will be observed as increases in taxonomic richness, with shifts in the ratio between predators and prey (zootroph vs phytotroph/saprophytotroph), as predator numbers increase in proportion with increases in prey species availability. We also hypothesized a positive relationship between soil invertebrate abundance and vegetation leaf tissue nutrient concentrations, because soil fauna can increase nutrient mineralisation and uptake in plants.

- Chapter 5 - the addition nutrients (N and P) will increase CO_2 and CH_4 flux at upland sites, with the largest response in treatments where P is added, as microbial activity is expected to increase with alleviation from nutrient limitation.

CHAPTER 2

LIVESTOCK GRAZING AND NITROGEN DEPOSITION AS MAJOR FACTORS AFFECTING UPLAND ECOSYSTEM PROCESSES

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2.1 ABSTRACT

Upland habitats are under pressure from multiple drivers of environmental change acting concurrently which threaten key ecosystem services and processes. Although numerous studies have considered the impact of these drivers independently, relatively few studies have investigated the combined effect of multiple drivers acting simultaneously. This study investigated the mutual impact of livestock grazing and nitrogen (N) deposition on upland vegetation communities and soil chemical and biological components, across three key habitats (heath, calcifuge grassland and blanket bog) at nine locations in the Cambrian Mountain range, Mid Wales. Sites were subject to a range of grazing intensities (0 - 0.23 LGU ha^{-1} yr^{-1}) and N deposition rates (12.6 - 18.3 kg N ha^{-1} yr^{-1}). Livestock grazing and N deposition had significant effects on above-ground vegetation biomass, vegetation height and plant functional type cover, with increases in graminoid species cover and decreases in dwarf shrub species cover observed with higher grazing intensity. Altered vegetation species composition and physical structure was considered to relate to effects observed for both soil chemistry and biota. Total soil carbon (C) was lower at sites with higher rates of N deposition and grazing intensity suggesting increased rates of decomposition facilitated by augmented microbial activity, both from N stimulation and relief from polyphenolic compound inhibition via vegetation species change. Cranefly (Diptera: Tipuloidea) abundance was negatively related to N deposition rate through the associated reduction in resource availability from decreases in soil N. Field scale evidence of differences in vegetation characteristics, soil C and N cycling and the abundance of a key upland invertebrate group indicate that mutual effects of livestock grazing and N deposition are modifying upland ecosystem processes and the structure of upland habitats. Our findings suggest lower

livestock densities on sites exposed to N deposition amounts greater than current critical load limits may reduce impact to upland ecosystem processes.

2.2 INTRODUCTION

Habitats of the upland ecosystem are facing significant environmental pressure from the impacts of land use intensity (Evans *et al.,* 2015) and the deposition of pollutants, particularly nitrogen (N) (Bobbink *et al.,* 2010; Field *et al.,* 2014). These drivers have contributed to widespread and often severe declines in species richness and abundance recorded across trophic levels (Dennis *et al.,* 2008; Maskell *et al.,* 2010). Increasingly, the combined effects of these two drivers are recognised jointly (Van der Wal *et al.,* 2003; Hartley & Mitchell, 2005), yet critical loads, which indicate levels above which harm to ecosystems would be expected, still fail to consider possible synergism. A better understanding of the interaction of livestock stocking and N deposition (N_{dep}) rates on ecosystem processes is essential for the development of evidence-based management strategies.

Long term effects of an interaction between livestock grazing and N_{dep} on the upland ecosystem remain unclear. Grazing alters vegetation communities through increasing levels of disturbance (i.e. trampling), by the preferential selection of vegetation species (Anderson & Briske, 1995) and through changes to species competitive interactions via plant-soil feedbacks (Medina-Roldán *et al.,* 2012). Nitrogen enrichment can affect vegetation communities through direct toxicity, through changes to plant competitive interactions and soil pH levels, and through the increased susceptibility of plants to other environmental stresses (Bobbink *et al.,* 2010). The effects of N_{dep} are reported to be offset by the effects of livestock grazing (Borer *et al.,* 2014), however Van der Wal *et al.,* (2003) demonstrated a multiplestep feedback loop where grazing-induced vegetation species change, coupled with augmented growth from N enrichment, resulted in increased attraction to herbivores with consequent rises in trampling and biomass removal.

Both grazing and N_{dep}, independently and in combination, promote increased graminoid abundance in upland habitats (Welch, 1984; Hartley & Mitchell, 2005), with a potential shift in upland grasslands to less palatable grass species such as *Nardus stricta* (L.) and *Molinia caerulea* (L.) (McGovern *et al.*, 2011). The change in plant species composition, through the alteration of plant-soil interactions, modifies the soil environment altering the size of carbon (C) and N pools (Quin *et al.*, 2014; Ward *et al.*, 2014) and influencing soil biota (Bardgett *et al.*, 1999). Soil invertebrate species are susceptible to changes in the physical and chemical status of soil (Carroll *et al.*, 2011). Increasing nutrient availability can positively influence population size via the bottom-up enhancement of resources, linked to increased plant productivity (Van der Wal *et al.*, 2009). Craneflies (Diptera: Tipuloidea) are an integral component of the upland ecosystem, with a life cycle which crosses from below- to above-ground, linking the two (Coulson, 1962; Hadley, 1969). Larval mortality rates of craneflies respond to changes in soil conditions, particularly to varying levels of soil moisture (Carroll *et al.*, 2011), which can heavily influence the size of cranefly populations, and directly affect the potential food supply of species in higher trophic levels that are reliant on this group (Buchanan *et al.*, 2006).

Most studies have considered the impacts of livestock stocking rate and N_{dep} in isolation. Studies which have considered both factors in chorus showed either an interaction resulting in habitat degradation (Van der Wal *et al.*, 2003), or demonstrated a positive association in which the action of herbivory limits the impact of N enrichment (Borer *et al.*, 2014). Evidence of environmental effects when acting in combination is limited for the upland ecosystem, thus further study is required to better understand the ecosystem wide effects of any interaction between drivers. The relative effects of livestock grazing and N_{dep} on vegetation, soil chemistry and biology were studied across three upland habitats with particular emphasis on ecosystem function defined by the interactions between the soil chemical and biological components. We hypothesised that higher rates of N_{dep} and grazing intensity have additive effects and result in: 1) lower vegetation height and biomass through offtake and species composition change (observed as higher graminoid cover); 2) lower belowground C and N pool size (driven by the top-down effects of vegetation species change and modification of microbial activity); and 3) reduced abundance of a key upland invertebrate group (craneflies) as a consequence of reduced soil C and N pool size, as this relates to the availability of food resources.

2.3 MATERIALS AND METHODS

2.3.1 STUDY SITES AND EXPERIMENTAL DESIGN

The study was located across the Cambrian Mountains (latitude: 52.55º– 52.37ºN, longitude: 3.72º –3.81ºE and altitude: 480 – 675 m) during 2013-14 (Figure 2.1). Study sites covered a modest N deposition gradient of between 12.6 - 18.3 kg N ha^{-1} yr^{-1}, and were subject to livestock stocking rates from 0 – 0.23 livestock grazing units (LGU) per hectare per year (Table 2.1). Soil types are varied at each site and range from podzolic to deep peat and overlie a geology principally characterised by Ordovician mudstones, shales and coarse sandstones (BGS, 2015). For each site, study plots of 20 m x 20 m were delineated from three different habitats: heath, calcifuge grassland and blanket bog. Heath was categorised as having >25% dwarf shrub cover, calcifuge grassland as having <25% dwarf shrub cover, and blanket bog by characteristic vegetation (i.e. *Calluna vulgaris* (L.), *Sphagnum* spp) overlying peat with a depth >0.5m (JNCC, 2009). In total 27 plots were surveyed.

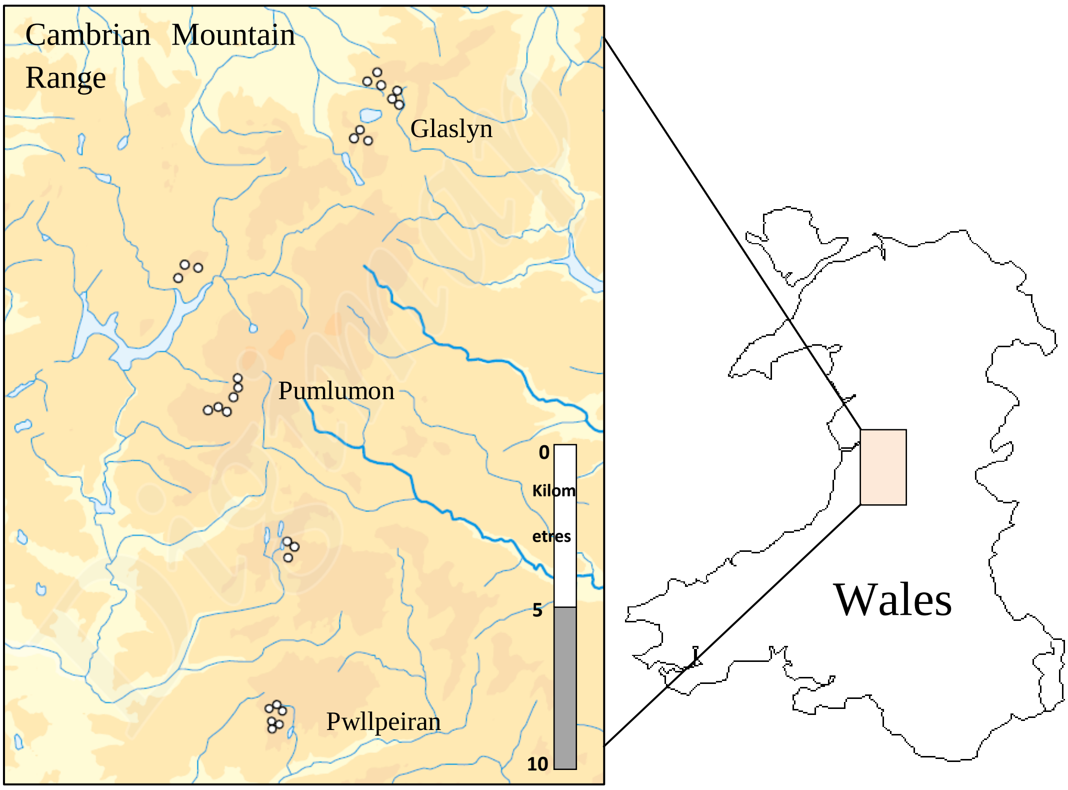

Figure 2.1. Location of study sites across the Cambrian Mountains, mid-Wales, UK. White circles indicate site locations. Map of Wales was created using ‘blighty’ R package (Lucy, 2010). Site maps from Ordnance Survey (OS data © Crown copyright/database right 2010) An Ordnance survey/ EDINA supplied Service.

Table 2.1 Climate, air pollution and grazing management details of the sites surveyed in the Cambrian Mountains.

Site name	OS grid ref	N deposition (kg N ha^{-1} yr^{-1})	Mean January temp (^{o}C)	Mean July temp (^{o}C)	Annual rainfall (mm)	Altitude range (m)	Stocking rate (LGU ha^{-1} yr^{-1})
Foel Fadian	SN 824 955	18.34	-0.34	16.38	2233.36	483 - 510	0.136154
Waun Y Dyffryn	SN 833 946	18.34	-0.34	16.38	2233.36	485 - 486	0.226923
Glaslyn Lake	SN 830 942	18.34	-0.34	16.38	2233.36	502 - 508	0.004453
Banc Llech' Mawr	SN 773 899	14.84	-0.09	16.37	1908.56	517 - 530	0.084286
Pumlumon	SN 789 868	14.84	-0.09	16.37	1908.56	624 - 675	0.0028
Y garn	SN 775 851	13.02	-0.09	16.55	1659.16	664 - 670	0.048684
Llynoedd Ieuan	SN 802 812	14.98	-0.3	17.46	2122.58	523 - 535	0
Pwllpeiran 1	SN 796 764	12.6	0.06	17.38	1512.2	542 - 544	0.057
Pwllpeiran 2	SN 798 760	12.6	0.06	17.38	1512.2	557 - 566	0.057

Meteorological data were obtained from the Met Office long-term average data at 5 km resolution. N deposition data at 5 km resolution was provided by the Centre for Ecology and Hydrology, Edinburgh and accessed through the Air Pollution Information System (APIS 2014).

2.3.2 DUNG COUNT

The relative stocking rate of livestock at the three sample sites was estimated from two methods of dung survey during March 2014. Two surveys were carried out at each study site: presence/absence of dung was recorded for 15 randomly placed 0.25 m^{-2} quadrats within each 400 m^{-2} study plot and averaged to a single figure; dung presence/absence was also recorded for 45 quadrats of 0.25 m^{-2} on a site-wide ‘Z’ transect, with a quadrat taken every 20 m. The two estimates were averaged to reduce the effects of localised variation in dung aggregation between the stratified sample plots. The estimate of relative stocking rate based on the dung survey was then used to verify the documented stocking densities recorded for each site (tested with linear regression).

2.3.3 VEGETATION SURVEY

For each 20 x 20 m study plot, vegetation was recorded from three transects, established from one plot edge. Point quadrats were taken at three points along transects 1 and 3 (at 5, 10 and 15m) and at two points for transect 2 (at 7 and 13m). A total of 8 quadrats, each consisting of 8 pins (64 pins in total), were recorded per study plot. Height, species and canopy structure (vegetation layers and plant part) were recorded at each pin, with presence logged if any live part of a specimen touched the descending pin. Multiple hits of the same species/individual were also recorded to give insight into canopy structure. For cover results, only the first hit per specimen/species was counted. All plants and bryophytes were identified to species level (see appendix 1 for vegetation data). Vegetation height was taken to be the average of all first contact heights for pins in each study plot. Vegetation biomass was harvested once in summer 2013; two 0.25 m^{-2} quadrats were randomly placed in each study plot before all vegetation was removed to surface level. Vegetation material was divided into live and dead (litter) components, dried for 24 hrs at 105 ^{o}C, then weighed. To determine the impact of environmental change on the plant community, C-S-R functional signatures were calculated for each study plot (Hunt *et al.*, 2004). These scores for competitiveness, stress-tolerance and ruderality offer insight into changes within the vegetation community to levels of competition, stress and environmental disturbance (Grime, 1974).

2.3.4 SOIL SURVEY

Soil samples were collected in August 2013 from five locations within each survey plot, using a 20mm diameter soil corer up to a depth of 30cm, and bulked together. The samples were air dried and passed through a 2 mm sieve. Soil pH was measured with a Hydrus 400 meter (Fisherbrand, Leicestershire, UK) in a slurry of 10 g fresh soil in 25 mL water. Total N and C were established by the Dumas combustion method using an elemental analyser (Vario MAX Cube – Elementar Analysensysteme, Hanau, Germany). Total P was determined using the Kjeldahl method on oven-dry soil (105 ^{o}C). Soil samples were digested using 1 mL H_2SO_4 + Se catalyst per 0.1 g sample at 400 ^{o}C for 2.5 hrs with a Buchi block digester (Buchi digest system K-437, Buchi labortechnik, Switzerland), before being analysed colorimetrically using the molybdenum blue method for P and the indophenol blue method for N, on a continuous flow auto analyser (AutoAnalyzer III, Bran+Luebbe GmbH, Germany).

2.3.5 CRANEFLY (TIPULOIDEA) SURVEY

Craneflies and allied species (Diptera: Nemotocera: Tipuloidea) were surveyed through the adult emergence period for 60 days in spring commencing in April 2013 and again in 2014. Cranefly emergence trap design followed Carroll *et al.*, (2011): traps consisted of a plastic basket (L41cm x W28cm x H17 cm) placed open end down, with yellow sticky traps on the inner surfaces. In total 54 emergence traps were used (two per survey plot) and each was *in situ* for 60 days to cover peak adult emergence times. Sampling points for the traps were identified at random within each survey plot. Emergence traps were recovered after 60 days and craneflies counted and identified to species level. The amount recorded is the sum of individuals per trap. Results for 2013/2014 were combined to give a single abundance figure per trap. Tipuloidea have a large range of body sizes; to allow for the relative ecosystem energy contribution of different species, biomass was calculated as the median average of the wing length range for each species, specified by Coe *et al.*, (1950). This approach was ultimately discarded because the dominance of one smaller species (*Molophilous ater* Meigen) resulted in significant similarity between biomass and abundance results. Instead, results for large bodied cranefly species (referred to henceforth as LBCS) were also considered independently, to ascertain factors affecting this group, which contain a keystone species for blanket bog (Coulson, 1962) essential for the productivity of higher trophic level species.

2.3.6 CLIMATE AND DEPOSITION DATA

The climate information for mean annual rainfall, average January temperature minimum and average July temperature maximum, were derived from long-term average data using a modelled UK 5x5 km gridded data set (UK Meteorological Office, no date). Modelled estimates of N deposition (N_{dep}) were obtained from the APIS database, as the total of reduced (NH_x) and oxidised (NO_y) N_{dep}, with a 5x5 km resolution, averaged over three years (2010 – 2012) (APIS, 2014).

2.3.7 STATISTICAL ANALYSES

Relationships between parameters that characterised ecosystem processes (vegetation structure and functional type cover, soil chemistry, cranefly abundance) and with livestock stocking rate and N_{dep} rates were tested using generalized linear mixed models (GLMMs), with habitat type included as a categorical variable and altitude entered as a random effect, to account for altitudinal variation both within and between stratified study sites and sample plots. The interaction between habitat and stocking rate was also included to allow for the effect of preferential selection by grazers between habitats. Non-normally distributed data were Log_{10} (n+1) transformed where appropriate prior to modelling, apart from cranefly count data which were modelled untransformed using error correction terms (Poisson error and log link). Relationships between ecosystem parameters and grazing intensity stocking rate and/or N_{dep} were investigated for individual habitats using the GLMM construct minus the habitat and habitat × grazing interaction as explanatory variables. Where there was significant curvature in the data, models were fitted with a quadratic function. Multiple regression analysis was used to further investigate relationships between cranefly abundance and associated environmental parameters (soil chemistry, vegetation community/structure), both for the total data set and the habitat subsets. The strongest single predictors were determined for each data set by multiple regression analysis, using all-possible-subsets model procedure in preference to the less reliable stepwise variable selection method (Whittingham *et al.*, 2006). All statistical analyses were conducted with SPSS version 21.0 (IBM, 2012).

2.4 RESULTS

2.4.1 VEGETATION COMMUNITY RESPONSES TO GRAZING AND N DEPOSITION

The estimate of stocking rate based on the dung survey verified the documented stocking rates recorded for each sample site (linear regression: a = 0.004; b = 0.233; $F_{1,26} = 30.90$; $P < 0.001$; $R^2 = 0.55$) and this relationship was used to convert the dung counts into annual stocking rates. Significant inverse relationships were recorded between vegetation height ($F_{1,20} = 19.034$, $P = <0.001$), vegetation biomass ($F_{1,20} = 10.558$, $P = 0.004$) and litter biomass ($F_{1,20} = 5.431$, $P = 0.03$) with greater grazing intensity (Figure 2.2). When considered by habitat, grazing reduced vegetation biomass across the grazing intensities on average by 14.3 % (161.1g m^{-2}) for blanket bog, 58.1 % (1037 g m^{-2}) for heath and 56.2 % (1102g m^{-2}) for calcifuge grassland, reduced litter biomass by 53.9 % (722.2 g m^{-2}) for blanket bog, 40.4 % (628.7 g m^{-2}) for heath and 63 % (1662.3 g m^{-2}) for calcifuge grassland, and reduced vegetation height on average by 9.6 % (1.79 cm), 50.8 % (11.45 cm) and 68.3 % (19.28 cm) for blanket bog, heath and calcifuge grassland respectively. No habitat × grazing interaction was observed suggesting consistency of effect across habitat types. There was a significant inverse relationship between vegetation biomass and N_{dep} ($F_{1,20} = 8.248$, $P = 0.009$), with a significant response observed for heath ($F_{1,6} = 12.815$, $P = 0.012$). Species richness increased with greater levels of N_{dep} ($F_{1,20} = 7.745$, $P = 0.011$) for the total data set, but not for individual habitats. A significant effect of habitat on species richness was observed however, with similar richness found in heath and blanket bog (means of 16.9 and 17.1 species per plot respectively) but notably less in calcifuge grassland (mean of 12.7 species). There was no significant effect of N_{dep} rate on vegetation height or litter biomass.

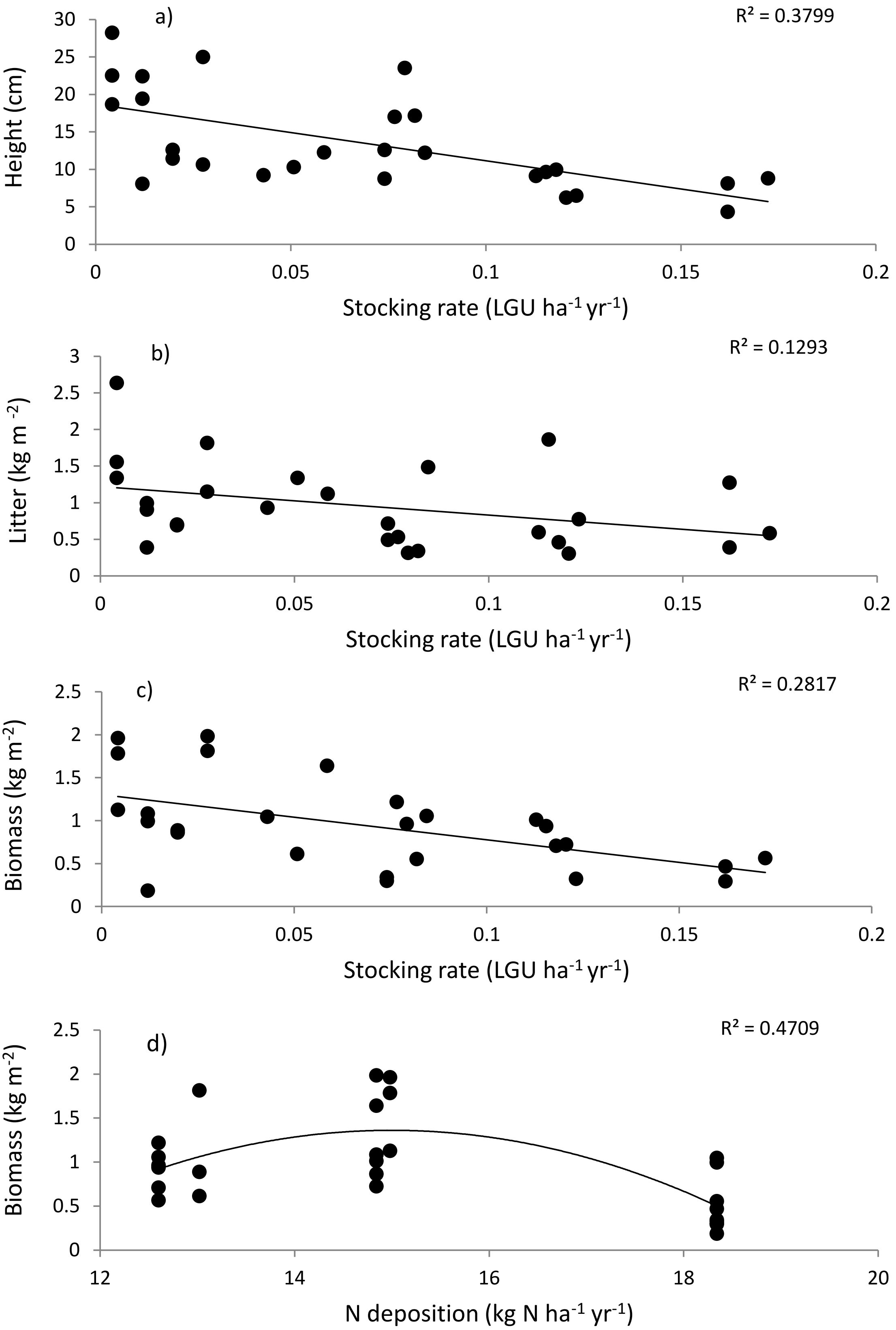

Figure 2.2. Relationship between stocking rate of sheep and a) vegetation height (b) litter biomass (c) vegetation biomass and d) N_{dep} with plant biomass.

The representation of different plant functional types (PFT) varied, with higher stocking rates associated with significantly higher ground cover of graminoid species ($F_{1,20}$ = 5.212, P = 0.034, Figure 2.3a) and significantly lower cover of dwarf shrub species ($F_{1,20}$ = 7.675, P = 0.012, Figure 2.3b). For individual habitats graminoid species cover was observed to be higher in heath ($F_{1,6}$ = 14.257, P = 0.009) and blanket bog ($F_{1,6}$ = 16.273, P = 0.007), and dwarf shrub species cover was observed to be lower in heath ($F_{1,6}$ = 9.079, P = 0.024) under higher grazing intensity. No significant change in cover for forb or bryophyte species groups was observed with changes in grazing intensity and no PFT was affected significantly by differences in N_{dep} rate. No interaction was recorded between habitat × grazing.

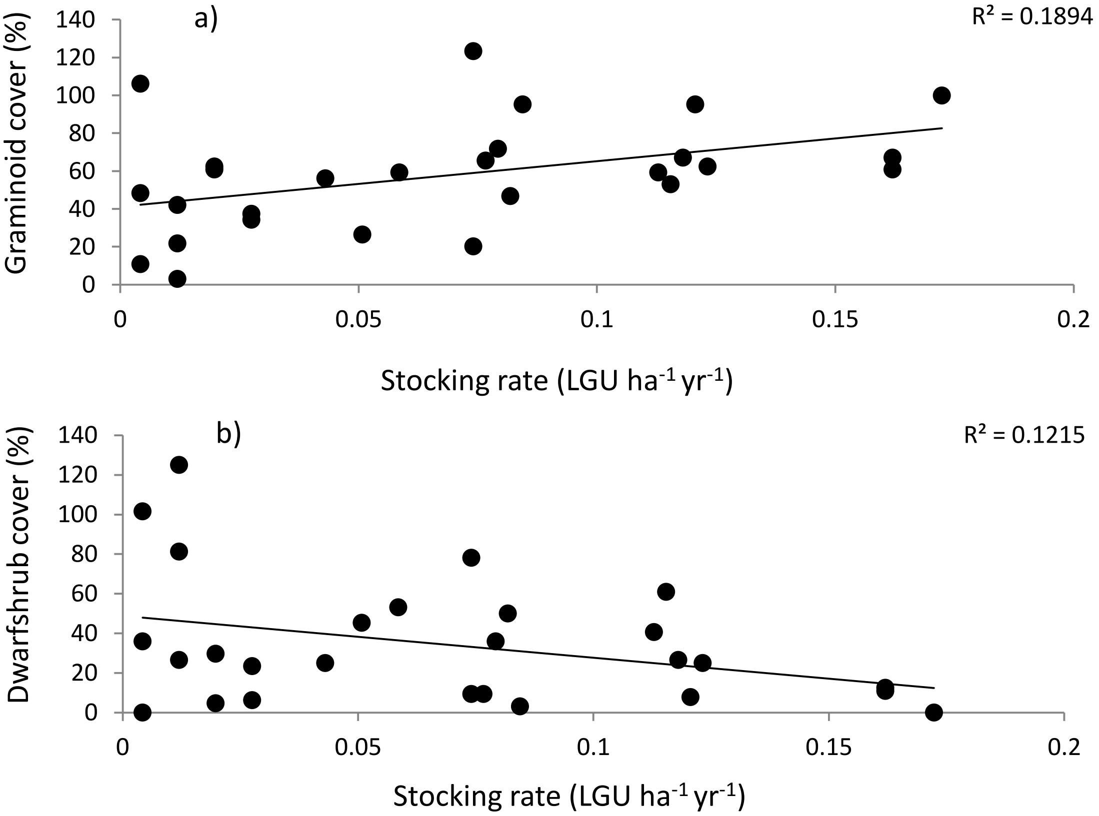

Figure 2.3. Responses of vegetation community to increases in stocking rate: (a) graminoid species and (b) dwarf shrub species cover change.

The C-S-R functional signature of vegetation in the study plots showed significant differences in the proportion of C-S-R (competitor – stress tolerator – ruderal) components. Grazing intensity was found to drive a reduction in the competitor component ($F_{1,20}$ = 17.229, P = <0.001), with corresponding increases in the ruderal component ($F_{1,20}$ = 5.425, P = <0.03). N_{dep} was also found to be inversely related to the competitor component ($F_{1,20}$ = 18.838, P = <0.001), however the corresponding increase in the ruderal component was not significant.

2.4.2 SOIL CHEMISTRY RESPONSES TO GRAZING AND N DEPOSITION

Significant inverse relationships were observed between total soil C and stocking rate ($F_{1,20}$ = 5.13, P = 0.035, Figure 2.4a), N_{dep} ($F_{1,20}$ = 13.067, P = 0.002, Figure 2.4b) and habitat ($F_{1,20}$ = 8.837, P = 0.002). Across all habitats there was an overall inverse trend between N_{dep} and soil C pools, with the largest effect observed for calcifuge grassland ($F_{1,6}$ = 12.396, P = 0.013), where an average increase of 1 kg N ha^{-1} yr^{-1} resulted in 1.45% reduction in C. Stocking rate was inversely related to soil C in both blanket bog ($F_{1,6}$ = 8.038, P = 0.030) and calcifuge grassland ($F_{1,6}$ = 7.737, P = 0.032), with no effect observed for heath but no significant habitat × grazing interaction suggesting consistency of effect across habitat types. Total soil N was not significantly influenced by stocking rate, but significantly decreased with greater N_{dep} ($F_{1,20}$ = 14.829, P = 0.001, Figure 2.4c), an overall effect principally driven by the strong negative response of the soil N pool to N_{dep} in calcifuge grassland ($F_{1,6}$ = 16.513, P = 0.007), which equates to a reduction of 0.09 % total soil N for every 1 kg N ha^{-1} yr^{-1} increase. Soil phosphorus (P) levels were unaffected by either stocking rate or N_{dep} rate, either overall or for any individual habitat. Soil N:P and C:P ratios were significantly lower in sites with higher stocking rate, but analysis of the separate habitats for each ratio showed variation in the direction of response between

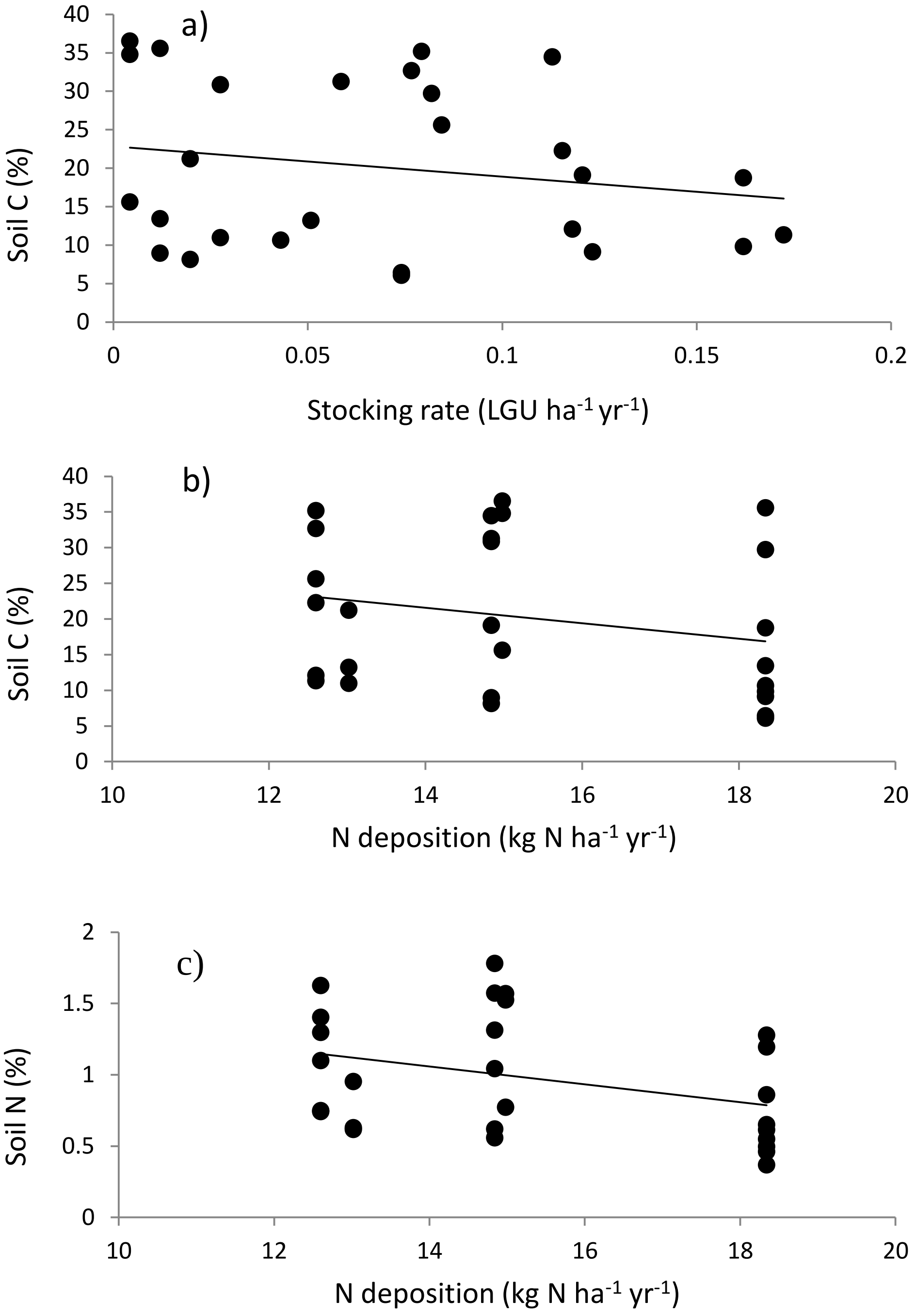

Figure 2.4. Relationships between a) stocking rate of sheep and total soil C, b) N deposition rate and total soil C and c) N deposition rate and total soil N.

habitats, with no significant relationships recorded for any one habitat or for any ratio, suggesting the overall trends may be driven by other factors. The N_{dep} rate had no effect on the C:N ratio, either overall or for any individual habitat. Soil C:P was lower at sites with higher N deposition ($F_{1,24} = 6.546$, $P = 0.017$), a trend which was consistent across habitats, but without significant effect for any one habitat. In contrast the N:P ratio showed a clear prevailing negative trend overall and for all habitats, with the strongest response observed for heath ($F_{1,6} = 6.861$, $P = 0.04$), where on average for every 1 kg N ha^{-1} yr^{-1} increase, the N:P ratio was reduced by 1.194, which follows the inverse relationship between N deposition and total soil N reported above. There was also a strong effect of habitat observed for all stoichiometric ratios.

2.4.3 CRANEFLY (TIPULOIDEA) ABUNDANCE

Cranefly population results were dominated by a single species (*M. ater*), which represented 93% (76% yr1, 96% yr2) of all individuals recorded, thus the models were also run for LBCS to ascertain effects on this group. Total cranefly abundance was significantly lower at sites with higher N_{dep} ($F_{1,47} = 32.082$, $P = <0.001$, Figure 2.6a); a relationship observed for heath ($F_{1,15} = 28.203$, $P = <0.001$) and calcifuge grassland ($F_{1,15} = 34.160$, $P = <0.001$). A significant effect of habitat type was found for cranefly abundance ($F_{2,47} = 3.408$, $P = <0.041$). Mean abundance of individuals per trap was similar for blanket bog (34) and calcifuge grassland (36.6), and slightly larger for heath (41.9), however these averages are augmented by singular high values, particularly for calcifuge grassland, therefore median average figures offer more realistic differentiation of abundance per habitat (blanket bog = 35.5, heath = 17.5, calcifuge grassland = 1, Figure 2.5). Although there was no significant relationship between stocking rate and cranefly abundance, there was a

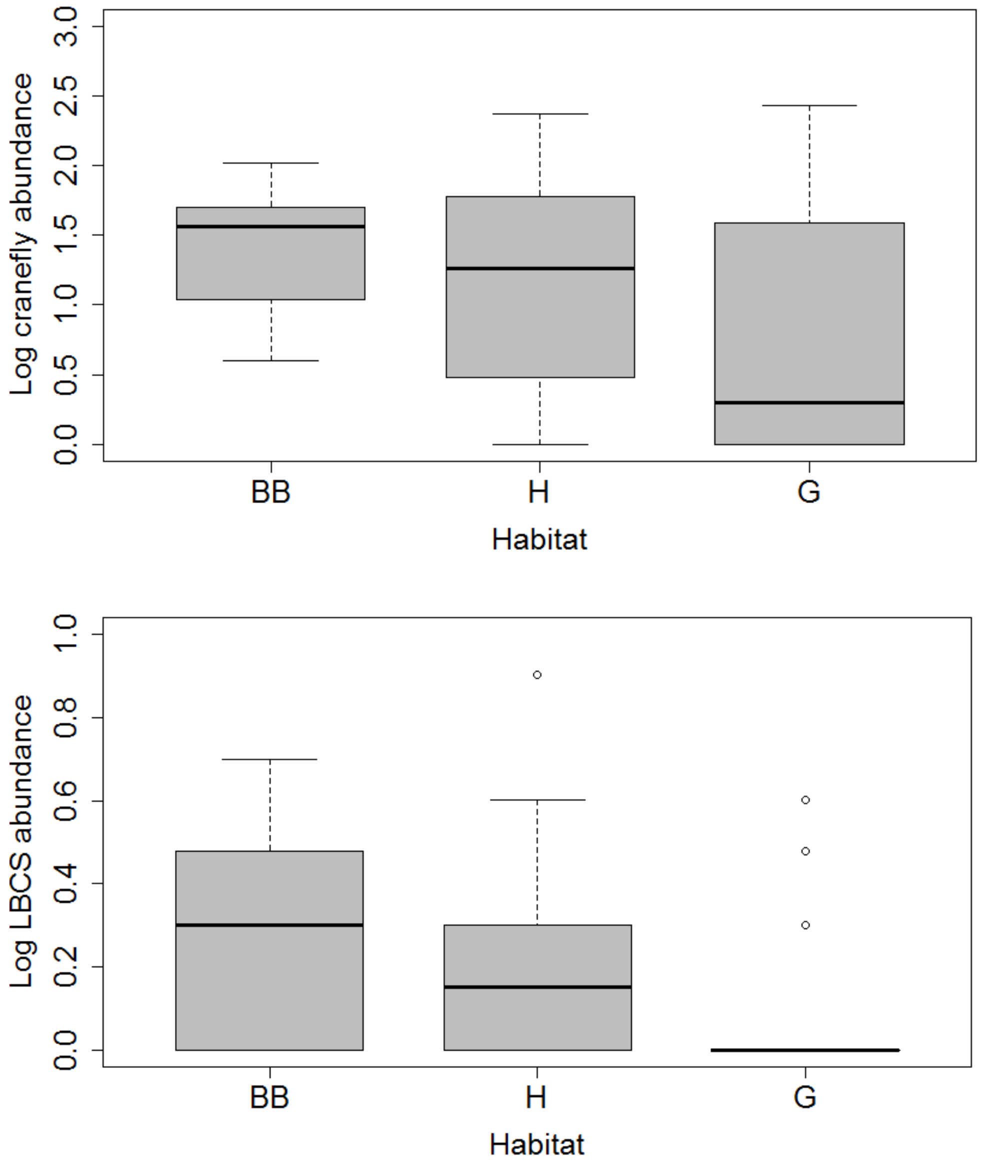

Figure 2.5. Comparison of total cranefly abundance, ($\log_{10}$ (x+1) individuals per trap) and LBCS (large body cranefly species) abundance ($\log_{10}$ (x+1) individuals per trap), between habitats. BB = blanket bog, G = calcifuge grassland, H = heath. Box midline indicates median, box edges indicate interquartile range, whiskers indicate range of data.

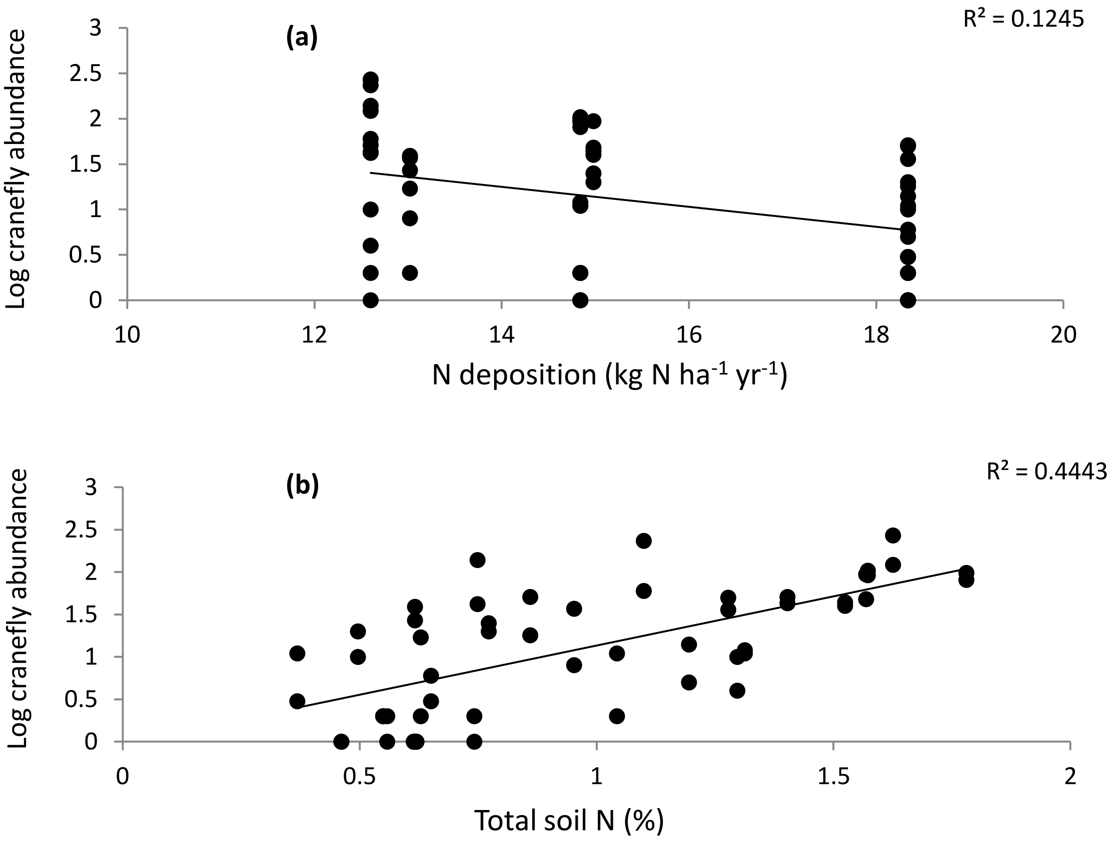

Figure 2.6. The effect on cranefly abundance ($\log_{10}$ (x+1) individuals per trap), of (a) increasing N deposition and (b) total soil N.

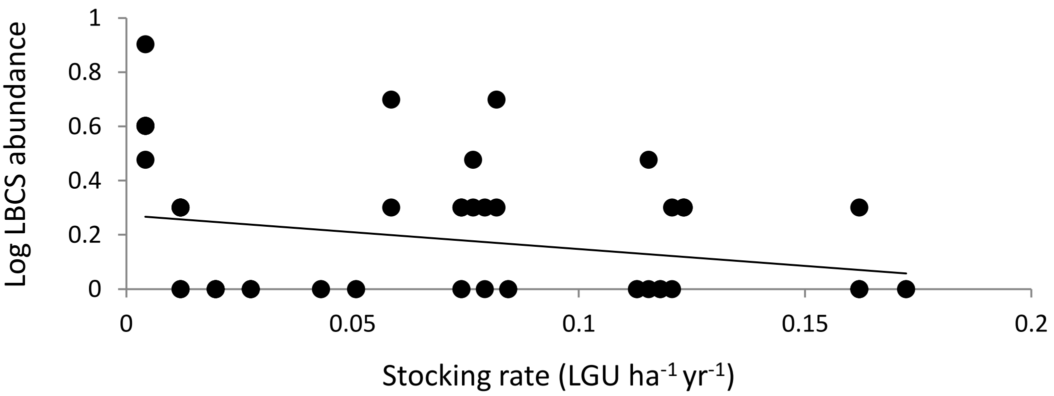

Figure 2.7. The relationship between stocking rate and abundance of LBCS (large body cranefly species) ($\log_{10}$ (x+1) individuals per trap).

significant (positive) effect of the grazing × habitat interaction ($F_{2,47}$ = 11.780, P = <0.001), indicating a potentially beneficial influence of stocking rate in certain habitats. For the LBCS subset, similar overall trends were observed, with a negative relationship observed for N_{dep} ($F_{1,47}$ = 9.620, P = 0.003), no direct effect for habitat type, but a significant positive effect of the grazing × habitat interaction ($F_{2,47}$ = 3.587, P = 0.035). In contrast to the total cranefly figures there was a significant negative relationship between stocking rate and LBCS abundance ($F_{1,47}$ = 18.304, P = <0.001, Figure 2.7). Multiple regression analysis considering all vegetation and soil variables, indicated that soil N was the largest single predictor of cranefly abundance for the total data set (b = 1.16; $F_{1,47}$ = 41.57; P <0.001; R^2 = 0.44, Figure 2.6b). Analysis between habitats found soil N was the strongest single predictor of cranefly abundance for calcifuge grassland (b = 1.619; $F_{1,16}$ = 31.86; P <0.001; R^2 = 0.67), the N:P ratio the strongest single predictor for heath (b = 0.13; $F_{1,16}$ = 17.71; P < 0.001; R^2 = 0.53) and the C:N ratio the strongest single predictor for blanket bog (b = -0.09; $F_{1,16}$ = 12.11; P < 0.003; R^2 = 0.43).

2.5 DISCUSSION

2.5.1 VEGETATION RESPONSE

Higher stocking rates of sheep and N_{dep} were associated with higher species richness, shorter vegetation, and a lower biomass of foliage and litter for three upland habitats. Vegetation height, aboveground- and litter-biomass were all lower in situations of higher grazing intensity for each habitat type, driven by increased herbivore activity, supporting our hypothesis that higher rates of grazing intensity result in lower vegetation height and biomass through offtake and reflecting findings from previous experiments (Holland *et al.,* 2008; Bai *et al.,* 2012; Evans *et al.,* 2015). The reduction in biomass as N_{dep} increases may be explained by a multi-step feedback loop suggested by Van der Wal *et al.,* (2003), where increases in graminoid abundance from grazing impact and fertilisation rate from N_{dep} serve to increase the palatability and attraction of vegetation to herbivores, with associated rises in grazed offtake and trampling of vegetation. Additionally as a concurrent effect, increases in soil acidity as a result of N enrichment (often together with ongoing effects of previous sulphur pollution) can reduce plant biomass by limiting base cation availability and increasing solubility of toxic metals (Roem & Berendse, 2000; Phoenix *et al.,* 2012) restricting growth.

The small increase in plant species richness associated with higher N_{dep} was contrary to the weight of existing evidence (i.e. Stevens *et al.,* 2004 & 2010). Most studies into the impact of N_{dep} on ecosystems however, have failed to consider the interaction with other factors such as stocking rate of grazing livestock. Livestock grazing, in instances of nutrient enrichment, can limit species loss by alleviating stress from restricted light availability (Borer *et al.,* 2014), by limiting the effect of shading from additional growth, a major mechanism regulating plant competition (Hautier *et al.,* 2009). Studies of N_{dep} effects undertaken at locations with active grazing, but in lowland grassland systems, corroborate this effect and report either no significant change in plant species richness under light grazing (Van den Berg *et al.,* 2011), or increases in species richness with higher stocking rate (Pasari *et al.,* 2014). No direct relationship was observed between grazing intensity and species richness in this survey; however the significant effect of habitat on species richness suggests that in the long term, as habitats are transformed by the activity and intensity of grazing livestock (i.e. from heath to grassland), biodiversity may well be negatively impacted. Greater stocking rate resulted in the homogenisation of upland habitats, through pressure on vegetation species composition and a shift in PFT cover (Bardgett *et al.,* 1995; Thompson *et al.,* 1995).

PFT cover was modified by grazing intensity, with higher cover observed for graminoid and lower cover for observed dwarf shrub species as hypothesised, which is consistent with previous research (Van der Wal *et al.,* 2003; Hartley & Mitchell, 2005). N_{dep} was not related to any differences in PFT cover, however the negative effect of grazing on dwarf shrub cover is reportedly amplified by higher N_{dep} rates (Hartley & Mitchell, 2005), which may have increased the recorded effect of grazing intensity. The C-S-R functional signature analysis showed a shift in prevailing plant life history strategy, characterised by a decrease in the competitor component and an increase in the ruderal component, across habitats, with both higher stocking rate and N_{dep}, which reinforces the observed PFT responses. Overall this is indicative of a shift from a stable to a more disturbed ecosystem; a transfer in the regulation of species composition by plant competition to one resilient to grazing. In addition, the significant contribution of N_{dep} towards the trend of disturbance tolerance rather than eutrophication (which would be recognised as a shift in the S component), also supports the multi-step feedback loop effect (Van der Wal *et al.,* 2003) between stocking density of domesticated herbivores and N_{dep} explained previously.

2.5.2 SOIL CHEMISTRY RESPONSE

Significant inverse relationships were observed between both the higher stocking rate of sheep and higher rates of N_{dep}, supporting the hypothesis that higher rates of N_{dep} and grazing would result in lower belowground C pool size. The rate of terrestrial C loss in upland soil is principally controlled by the decomposition rate of organic matter, which is influenced by changes to vegetation composition because this alters the quality and quantity of litter returned to the soil (Dorrepaal *et al.,*

2005) and the abundance of polyphenolic compound producing species (notably dwarf shrubs in this context), which inhibit microbial activity and therefore decomposition rate (Ward *et al.,* 2014); an effect corroborated by the largest response observed for calcifuge grassland. Increased disturbance from higher stocking rates of grazing livestock can also increase decomposition rates due to a reduction in plant root biomass that favours soil microbial populations observed to facilitate faster rates of decomposition (Klumpp *et al.*, 2009). In acid bog, the input of N increases litter decomposition directly through the stimulation of microbial function and indirectly via production inhibition of phenolic and polyphenolic compounds by plants in instances of high N availability; compounds which would otherwise exert a suppressive effect on microbial activity (Bragazza *et al.*, 2006).

Soil N was also inversely related to N_{dep}, but not grazing intensity. This result contrasts with surveys elsewhere on the effects of N_{dep}, which have shown increases in soil N as a result of N inputs (Pilkington *et al.*, 2005; Phoenix *et al.*, 2012), but echoes studies which have surveyed soil N in response to modelled values of N_{dep}, which have shown no relationship to soil N (Jones & Power, 2012; Southon *et al.*, 2013). N enrichment, particularly in association with livestock grazing, stimulates microbial activity and consequently decomposition. This response releases N from storage in soil organic matter and thus can reduce the N pool over time (Klumpp *et al.*, 2009). This increase in N cycling results in greater uptake of N by plants (Bai *et al.*, 2012); an effect which in the presence of herbivores could result in further N removal and export through livestock products. This suggests that livestock grazing could limit the impact of eutrophication in instances of high N_{dep} (McGovern *et al.*, 2014) or reduce soil fertility with low deposition rates. N_{dep} rate may alter Soil N content, however this is potentially influenced by other site specific variables, which are difficult to discriminate in this current study but may also have an effect. Soil P was not influenced by either livestock grazing or N_{dep}. The inverse relationships between C:P and N:P for both stocking rate and N_{dep} reflect the decreasing soil N and C along gradients of increasing stocking rate and N_{dep}. Neither stocking rate grazing nor N_{dep} appeared to account for variations in soil P between sample plots, and the C:N ratio, whilst highly variable between sites and study plots, was unaffected by either grazing or N_{dep}.

2.5.3 RESPONSE OF CRANEFLIES (TIPULOIDEA)

The population size of craneflies was smaller where soil N content was less, which supports our hypothesis. This appeared to be explained by N_{dep} rates, but not by variations in the stocking rate of domesticated herbivores. The abundance of soil invertebrates is affected by the enhancement of nutrient availability, a bottom-up process (Coulson & Butterfield, 1978; Van der Wal *et al.*, 2009) which for this invertebrate group relates to the quantity and availability of soil organic matter, particularly decomposing plant material; an essential food source for the larval stages (Pritchard, 1983). This effect is likely to be highly variable between habitat and soil types and is confirmed by the significant effect of habitat in the results. Cranefly abundance was also explained by an interaction between stocking rate and habitat, which suggests that for certain habitats grazing modification may improve habitat quality by increasing resource availability in the form of light to host plants in the understory (i.e. liverwort species). The principal factor predicting cranefly abundance differed for blanket bog and heath. C:N ratios were best predictors of abundance for blanket bog, which in organic soil such as peat, indicated an increased abundance of craneflies with greater values of tissue N in soil organic matter. For heath, the best predictors were N:P ratios, which when increasing, indicate greater soil N availability and thus greater resource availability (Coulson & Butterfield, 1978). Previous studies into the effects of N on soil invertebrates have described quite varied responses according to taxon (Cole *et al.,* 2008*;* Prendergast-Miller *et al.,* 2008; Payne *et al.,* 2012). Large differences in explanation were offered despite similar reported relationships with N availability to that reported here. Nitrogen addition simulations result in a spike in nutrient availability, which potentially drives a short term rise in productivity (Cole *et al.,* 2005). Our results demonstrate the ecosystem response to ongoing pressure from N_{dep} and stocking rate. Changes in vegetation species composition, driven by increases in grazing intensity or nutrient addition, have been previously linked to changes in the abundance, richness and composition of upland invertebrate communities (Hartley *et al.,* 2003). For soil macrofauna, the effects are analogous, but are likely to be driven by ensuing plant-soil feedbacks, which are potentially slow moving responses (Bardgett *et al.,* 1998). In addition, fecundity rates for craneflies are reportedly greater with higher N availability (Coulson & Butterfield, 1978), thus, there are likely to be cumulative effects for cranefly abundance over time.

In contrast to the total cranefly dataset, the model results for the LBCS subset show that grazing intensity is inversely related to large body species abundance. This could potentially relate to habitat degradation through fragmentation, where land management (in this context, draining to improve pasture quality for sheep) has resulted in the loss of suitable habitat, particularly blanket bog (Coulson & Butterfield, 1985). The almost total absence of *Tipula subnodicornis* Zett., a species which is recognised as a keystone invertebrate of blanket bog that constitutes 75% of the annual aboveground invertebrate biomass (Coulson, 1962; Coulson & Butterfield, 1985), is highly concerning. This suggests that habitat degradation, particularly for the blanket bog locations surveyed, is severe. This has significant implications for higher trophic level species reliant on the spring mass emergence of large bodied craneflies such as *T. subnodocornis* (Pearce-Higgins *et al.*, 2010).

2.6 CONCLUSION

This study has found significant association between ecosystem processes and livestock stocking rate and atmospheric N_{dep} across an upland ecosystem. The observations are consistent with scientific hypotheses that have proposed ecosystem processes that are altered through vegetation change caused by nutrient enrichment and livestock grazing intensity, and may influence soil biology and chemistry. The interaction between stocking rate and N_{dep} is variable. Above ground, the effect of livestock grazing is predicted to offset the impact of N_{dep} on ecosystems (Borer *et al.*, 2014). This study corroborates this hypothesis, but demonstrates that inverse responses are still observable with increasing stocking rate and N_{dep} rates. Below ground, the combined effects are synergistic, potentially amplifying any negative effect, in particular decomposition rate which has significant implications for C storage in upland soil, and ultimately through the reduction of nutrient availability, on the soil invertebrate community. A consideration of the combined impact of N_{dep} and stocking rate was demonstrated to provide a more realistic assessment of ecosystem function. This study adds support to previous evidence (Van der Wal *et al.*, 2003; Hartley & Mitchell, 2005) which demonstrated that N_{dep} critical loads and prescribed grazing intensities require re-evaluation to establish a guidance framework for land managers which takes account of the demonstrated synergy. The effect of grazing on the upland ecosystem is potentially altered by the effects of N enrichment; for upland habitats exposed to N deposition rates higher than current critical load levels, reductions in livestock stocking rates may be essential to reduce impact to upland ecosystem processes, at least in the period until controls on atmospheric pollution and deposition of N take effect.

CHAPTER 3

LONG TERM NITROGEN AND PHOSPHORUS ENRICHMENT ALTERS VEGETATION SPECIES COMPOSITION AND REDUCES CARBON STORAGE IN UPLAND SOIL

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TARGET JOURNAL: ENVIRONMENTAL POLLUTION

3.1 ABSTRACT

Reactive nitrogen (N) deposition can affect ecosystem processes particularly in the oligotrophic upland ecosystem. Phosphorus (P) limitation may constrain ecosystem productivity, and P addition has been considered as a method to reduce the effects of chronic N enrichment on N leaching and acidification. Release from P limitation can increase vegetation biomass production enabling ecosystems to sequester greater reactive N inputs. However, biodiversity is often reduced in more productive ecosystems and P limitation may protect against this effect. Responses to P availability in instances of high N deposition are poorly understood. This study investigated ecosystem response to alleviation of P limitation using a long term nutrient addition experiment (1996 - 2012), three years after ceasing N inputs and 15 years after a single P application. Exposure to nutrient enrichment was responsible for substantial changes in the structure and composition of vegetation species, above-ground vegetation biomass and vegetation diversity. Treatment-driven shifts in vegetation species cover had cascading effects on nutrient cycling rates, with reductions observed in soil C (-14%) and N (-16%) pool sizes relative to control, suggesting P limitation may have a larger than expected effect on below-ground ecosystem processes. This study demonstrated the importance of P availability for upland ecosystem processes and highlights the long term modifying effects of P addition on vegetation species composition, ecosystem function and C storage. Thus the addition of P as a management tool to alleviate the impact of N deposition is not endorsed as a suitable course of action.

3.2 INTRODUCTION

Reactive nitrogen (N) enrichment from anthropogenic sources is a significant threat to ecosystem processes and function in oligotrophic upland ecosystems (Jones & Power, 2012; Southon *et al*., 2013). Oligotrophic habitats are highly sensitive to changes in nutrient availability, where increases in reactive N can alter plant competitive interactions and ultimately species composition (Stevens *et al.,* 2004 & 2010; Maskell *et al*., 2010). Nitrogen enrichment may also impact upon vegetation through direct toxicity, increased soil acidity and through enhancing plant susceptibility to other environmental stresses (Bobbink *et al*., 2010). Globally, reactive N inputs from anthropogenic sources are projected to double in size from current levels by 2050 (Galloway *et al.,* 2004; Phoenix *et al.,* 2006), whereas in western Europe inputs of reactive N are reported to be in decline (Fowler *et al.*, 2004). Nonetheless current estimations suggest that 90% of upland habitats are subject to N deposition rates in excess of established limits for critical loads, with some areas receiving threefold the recommended limit (RoTAP, 2012). Increases in reactive N availability in conventionally N limited environments can cause a shift from N limitation to phosphorus (P) limitation (Crowley *et al.*, 2012; Peñuelas *et al*., 2013; Rowe *et al.,* 2014). Environments which are P limited may be unable to sequester reactive N inputs if P limitation restricts vegetation biomass production, which could result in N saturation and therefore N leaching (Emmett *et al.,* 1995; Britton & Fisher, 2007). However, by constraining ecosystem productivity in this way, P availability may limit the impact to biodiversity of increasing N enrichment (Olde Venterink, 2011; Ceulemans *et al*., 2014).

Reactive N deposition can be categorised as either reduced (NH_x) or oxidised (NO_y) N. Reduced forms (e.g., NH_3, NH_4^+) predominantly arise from agricultural activities such as livestock production and fertiliser addition, whereas oxidized forms (i.e. NO_3^-, HNO_3, N_2O) are primarily emitted from transport and industrial sources (Bobbink *et al.,* 2010; Stevens *et al.,* 2011). In soil, NO_y binds weakly to soil particles and is readily leached (Sparks, 2003). Ammonium ions have a longer residence time than NO_y, since they bind more strongly to soil cation exchange sites (Rowell, 1994). Nitrogen retention is therefore dependent on the NO_y/NH_x ratio in soil and thus on factors governing nitrification such as aeration and pH. Nutrient pollution in the form of P deposition from anthropogenic activities receives less attention than N deposition, but is a potential risk from the spread of mineral aerosols of dust from P fertilizer usage onto natural ecosystems (Ceulemans *et al*., 2014; Tipping *et al.*, 2014). Phosphorus has a low mobility, dependent on the sorption capacity of the soil (Nye & Tinker, 1977), and is strongly adsorbed onto particle surfaces resulting in long term residence in soil (Rowell, 1994) and therefore long term influence on ecosystem function; a highly important point for management strategies considering P addition as a method to negate the effects of N enrichment (Armitage *et al.*, 2012; Blanes *et al*., 2012). The legacy effect of N and P enrichment in upland systems has been under-investigated. Where the effect of declining N enrichment has been considered there has been a consistent reduction of impacts over time (Edmondson *et al*., 2013) which suggests the potential for recovery.

The availability of both N and P can alter species composition (Avolio *et al*., 2014), with increases in bryophyte cover reported with P enrichment (Gordon *et al*., 2001) and increases in graminoid cover with N enrichment (Field *et al.*, 2014). This can impact the size and structure of the soil microbial pool (Fanin *et al*., 2015), affecting key soil processes such as carbon (C) turnover (Kaspari *et al*., 2008; Schimel & Schaeffer, 2012). Microbial biomass and activity are also governed by N and P availability with direct implications for N & C mineralisation rates (White & Reddy, 2000; Liu *et al*., 2012). Microbial abundance and activity are also regulated by the species composition of vegetation (Legay *et al*., 2014; Robroek *et al*., 2015) since species composition change modifies plant-soil interactions, which alters soil characteristics such as the size of C and N pools (Quin *et al.,* 2014; Ward *et al.,* 2014), directly impacting soil biota (Bardgett *et al.,* 1999; Hooper *et al.,* 2000). Changes to plant-soil feedbacks also affect soil organisms through alterations in root exudation patterns and C allocation, and via modification of plant litter input quality (Bardgett *et al.,* 1998).

The combined roles of N and P availability in modifying rates of plant litter decomposition are complex. Experimental trials into ecosystem responses to N and P enrichment have shown mixed responses with regard to decomposition rate and C storage. Mack *et al*., (2004) found nutrient enrichment in arctic tundra systems resulted in stimulation of both plant production and decomposition, but had a larger effect on decomposition, therefore resulting in an overall net loss of C. For blanket bog, increased *Sphagnum* spp. growth aided by improved N assimilation facilitated by P availability resulted in greater C sequestration (Limpens *et al*., 2004). For grasslands, soil C has been shown to decrease with N and P inputs (Scott *et al*., 2015), to be unaffected by multi-nutrient inputs (N, P, potassium and magnesium) but to increase with N alone (Fornara *et al*., 2013), and to increase with either N or P addition when released from limitation (He *et al*., 2013). Nitrogen and P were also shown to positively interact with P addition reducing N leaching through increased biomass assimilation (Scott *et al*., 2015).

The aim of this study was to assess the long term impact of N and P addition on upland vegetation structure and species composition, soil chemistry and the size of soil C and N pools, and to assess the role of P availability as a factor controlling productivity. This was done using a long term nutrient addition experiment (1996 - 2012) three years after ceasing N addition and 15 years after a single P application. P was originally added to plots to test the hypothesis that N impacts would be greater in non-P limited systems (Emmett *et al*., 2007) and this treatment offers an opportunity to further understanding of long-term ecosystem responses to increased P availability and concurrent effects on nutrient limitation. We hypothesised that increased nutrient availability would: 1) alter vegetation species composition, with greater graminoid species cover found in N addition treatments and greater cryptogam species cover with P addition; 2) reduced soil C content resulting from differences in vegetation species composition and nutrient availability; and 3) that these effects will be largest where P limitation has been reduced.

3.3 METHODS

3.3.1 EXPERIMENTAL DESIGN

The original experiment was established at Pen y Garn, Pwllpeiran (52^0 37' N, 3^0 76' W), mid-Wales, on a transition between NVC U4 *Festuca ovina - Agrostis capillaris* grassland and H18 *Vaccinium myrtillus - Deschampsia flexuosa* heath on shallow ferric stagnopodzol soil (Emmett *et al.*, 2007; Phoenix *et al*., 2012). The site is within an altitude range of 500 - 600 metres a.s.l., with an annual rainfall rate of 1512 mm (UK Meteorological Office, no date) and a background N deposition rate of 12.6 kg N ha^{-1} yr^{-1} (APIS, 2014). N was added fortnightly between 1996 and 2012 in a randomised block design, consisting of four 3 × 3 m treatments (Figure 3.2)

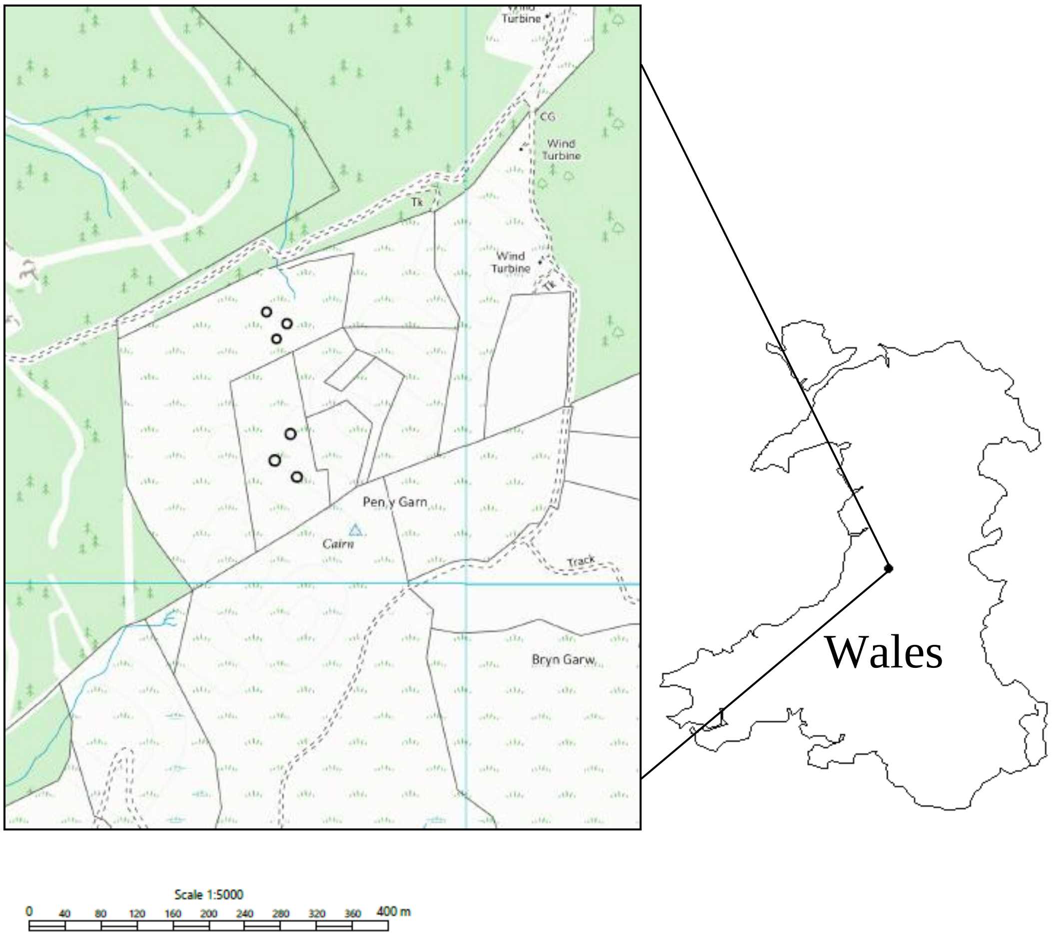

Figure 3.1. Location of study sites at Pen Y Garn, mid-Wales, UK. White circles indicate treatment block locations. Map of Wales was created using ‘blighty’ R package (Lucy, 2010). Site maps from Ordnance Survey (OS data © Crown copyright/database right 2010) An Ordnance survey/ EDINA supplied Service.

CONT	AS10 $(NH_4)_2SO_4$ + NaH_2PO_4	AS20 $(NH_4)_2SO_4$	SN20 $NaNO_3$

Figure 3.2. Treatment inputs: **CONT** = control (no addition); **AS10** = ammonium sulphate at 10 kg ha^{-1} yr^{-1} + phosphorus (sodium dihydrogen orthophosphate) at 20 kg ha^{-1} yr^{-1} (applied once); **AS20** = ammonium sulphate at 20 kg ha^{-1} yr^{-1}; **SN20** = sodium nitrate at 20 kg ha^{-1} yr^{-1}.

replicated six times. Phosphorus was added once in 2000 to the ammonium sulphate 10 kg N ha^{-1} yr^{-1} treatment (AS10) plots to test whether the impact of N would be greater in non-P limited systems. The original experiment incorporated sheep grazing as a factor; sheep were present between 1990 and 2007 at two levels (1.0 sheep ha^{-1} and 1.5. sheep ha^{-1}), with three replicate blocks in each paddock. Grazing effect is not considered as a factor in this current study as sufficient time was deemed to have lapsed to allow vegetation regrowth and limit any legacy effect.

3.3.2 VEGETATION ANALYSIS

All vegetation data were collected in June 2015. Vegetation covers were recorded visually with the Domin scale, subsequently transformed to percentage cover for statistical analysis (Currall, 1987). All plants and bryophytes were identified to species level. Vegetation height is the average of five measurements taken using a sward stick (Dennis *et al*., 2005). Plant strategy functional signatures were calculated for each study plot (Hunt *et al.*, 2004) to determine the effect of changes within the vegetation community to levels of competition, stress and environmental disturbance

(Grime 1974). Environmental indicator values (Ellenberg, 1992) adjusted for British vascular plants (Hill *et al.,* 2004) and bryophytes (Hill *et al.,* 2007) were also calculated from the vegetation survey data, without cover-weighting. Ellenberg scores indicate habitat characteristics by utilising known values of plant species preference/tolerance for nutrient level, acidity, moisture and light, which relate to plant niches (Hill *et al*., 1999). Vegetation species diversity was calculated for each treatment plot with the Shannon diversity index.

3.3.3 SOIL ANALYSIS

Soil samples were collected in June 2015 from five locations within each treatment plot, using a 20 mm diameter soil corer up to a depth of 20 cm, and bulked together. The samples were air dried and passed through a 2 mm sieve. Soil pH was measured with a Hydrus 400 meter (Fisherbrand, Leicestershire, UK) in a slurry of 10 g fresh soil in 25 mL water. Total N and C were measured by the Dumas combustion method using an elemental analyser (Vario MAX Cube – Elementar Analysensysteme, Hanau, Germany).

3.3.4 STATISTICAL ANALYSIS

Prior to analysis all data were tested for normality and homoscedasticity and Log (x+1) transformed where necessary. Analysis of variance was used to assess relationships between response variables for vegetation (height, species richness and diversity), soil variables (total C, total N, C:N and pH) and the four experimental treatments (Figure 3.2). When differences between treatments were detected ($P < 0.05$), post-hoc tests were conducted using LSD pairwise comparisons. For analysis of vegetation cover between treatments, species were grouped into functional types (graminoid, cryptogam (bryophyte and lichen), dwarf shrubs and forbs). These data did not conform to parametric test assumptions so a Kruskal–Wallis test was used for analysis. Differences in the composition and abundance of vegetation species between treatments were investigated using Principal Component Analysis (PCA). The first and second principal components were also compared using one-way ANOVA, with LSD pairwise comparisons used to further investigate individual relationships. All univariate statistical analyses were conducted with SPSS 21.0 (IBM SPSS Statistics for Windows, 2012), and PCA was undertaken using Multi-Variate Statistical Package (MVSP version 3.2 - Kovach Computing Services, Anglesey, Wales).

3.4 RESULTS

Vegetation was considerably taller in AS10 than either control or N-only treatments, but N form treatments (AS20 and SN20) did not increase height relative to the control. Species richness did not differ overall, but a response for forb richness (Table 3.1) was revealed by analysing plant functional types (PFT) independently, with fewer forb species in control plots (0.67 on average) compared to the two N form treatments, AS20 and SN20 (1.67 on average for both). Species diversity was different between treatments (Table 3.1, Figure 3.3) with lower vegetation species diversity recorded for the N + P (AS10) treatment than either control or N form treatments (AS20 and SN20). No difference in Shannon diversity was observed between control and N form treatments. PFT composition response varied between treatments. Graminoid species cover was higher in the two N form treatments, AS20 and SN20 (Table 3.1, Figure 3.4) compared to the control and N + P (AS10) treatments. Cryptogam species cover was higher (Table 3.1, Figure 3.4) in the N + P (AS10) treatment compared to control or either N form treatment (AS20 or SN20).

Table 3.1. Mean (± standard deviation) response of vegetation variables to experimental N and P addition treatments 19 years after commencement and three years after cessation. *F* values are displayed for one-way ANOVAs, *H* values for Kruskal-Wallis tests.

	CONT		**AS10**		**AS20**		**SN20**		***F*/*H***	***P***
Height (cm)	10.3	±2.9	14.3	±3.6	8.1	±1.7	7.5	±1.3	***F*=8.83**	**0.001**
Species diversity	2.4	±0.2	1.9	±0.3	2.3	±0.1	2.3	±0.2	***F*=6.94**	**0.002**
Species richness	17.7	±2.7	15.3	±1.5	17.8	±2.0	17.8	±1.5	NS	-
Graminoid richness	5.2	±1.0	5.0	±1.7	4.5	±1.1	4.8	±0.8	NS	-
Cryptogam richness	7.2	±2.5	6.2	±1.6	6.5	±1.6	7.2	±1.5	NS	-
Dwarf shrub richness	2.7	±1.4	1.8	±0.8	3.5	±1.1	2.8	±0.8	NS	-
Forb richness	0.7	±0.8	1.2	±0.4	1.7	±0.8	1.7	±0.5	***F*=3.11**	**0.049**
Graminoid cover (%)	45.9	±6.8	34.3	±23.3	73.2	±29.8	81.2	±17.7	***H*=12.31**	**0.006**
Cryptogam cover (%)	54.2	±17.3	79.6	±22.0	34.2	±17.5	35.6	±16.7	***H*=13.15**	**0.004**
Dwarf shrub cover (%)	14.9	±7.9	8.8	±4.0	17.5	±11.3	10.1	±5.6	NS	-
Forb cover (%)	2.9	±3.6	3.0	±1.8	3.8	±3.9	5.8	±2.4	NS	-
Leaf chlorophyll	18.7	±1.0	19.8	±3.7	17.6	±1.8	18.6	±2.6	NS	-
Ellenberg Fertility:										
All species	2.0	±0.2	2.2	±0.2	2.0	±0.2	2.0	±0.1	NS	-
Vascular plants	1.9	±0.2	2.3	±0.2	2.1	±0.1	2.0	±0.1	***F*=3.85**	**0.025**
Cryptogams	2.0	±0.2	2.1	±0.3	1.9	±0.3	2.0	±0.1	NS	-

CONT = control; AS10 = ammonium sulphate at 10kg ha^{-1} yr^{-1} + phosphorus at 20kg ha^{-1} yr^{-1} (applied once in 2000); AS20 = ammonium sulphate at 20kg ha^{-1} yr^{-1}; SN20 = sodium nitrate at 20kg ha^{-1} yr^{-1}. Bold highlights results significant at $P < 0.05$.

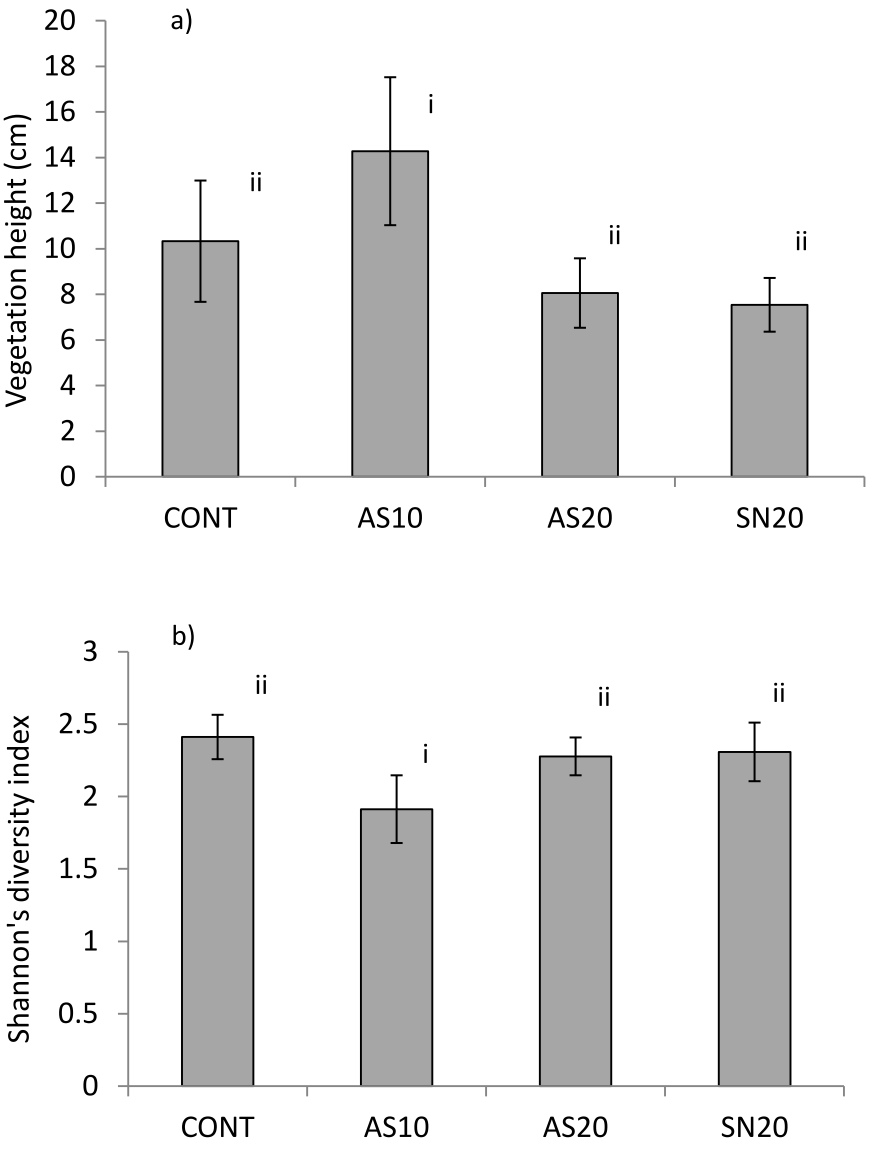

Figure 3.3. Vegetation height (a) and Shannon diversity (b) responses to nutrient additions. CONT = control; AS10 = ammonium sulphate at 10kg ha^{-1} yr^{-1} + phosphorus at 20kg ha^{-1} yr^{-1} (applied once in 2000); AS20 = ammonium sulphate at 20kg ha^{-1} yr^{-1}; SN20 = sodium nitrate at 20kg ha^{-1} yr^{-1}. Error bars denote standard deviation. Post hoc tests conducted with LSD pairwise comparisons.

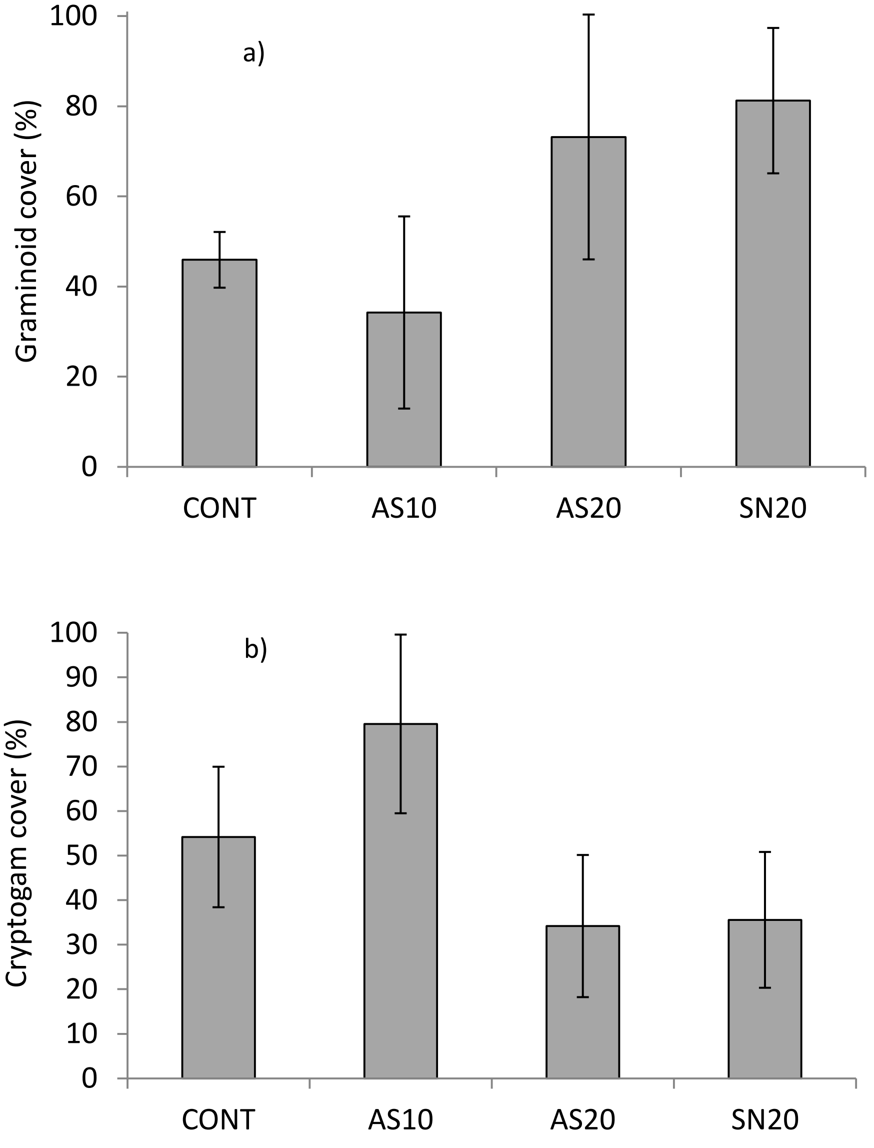

Figure 3.4. Cover of different plant functional types recorded between treatments; (a) graminoid, (b) cryptogam. CONT = control; AS10 = ammonium sulphate at 10kg ha^{-1} yr^{-1} + phosphorus at 20kg ha^{-1} yr^{-1} (applied once in 2000); AS20 = ammonium sulphate at 20kg ha^{-1} yr^{-1}; SN20 = sodium nitrate at 20kg ha^{-1} yr^{-1}. Error bars denote standard deviation.

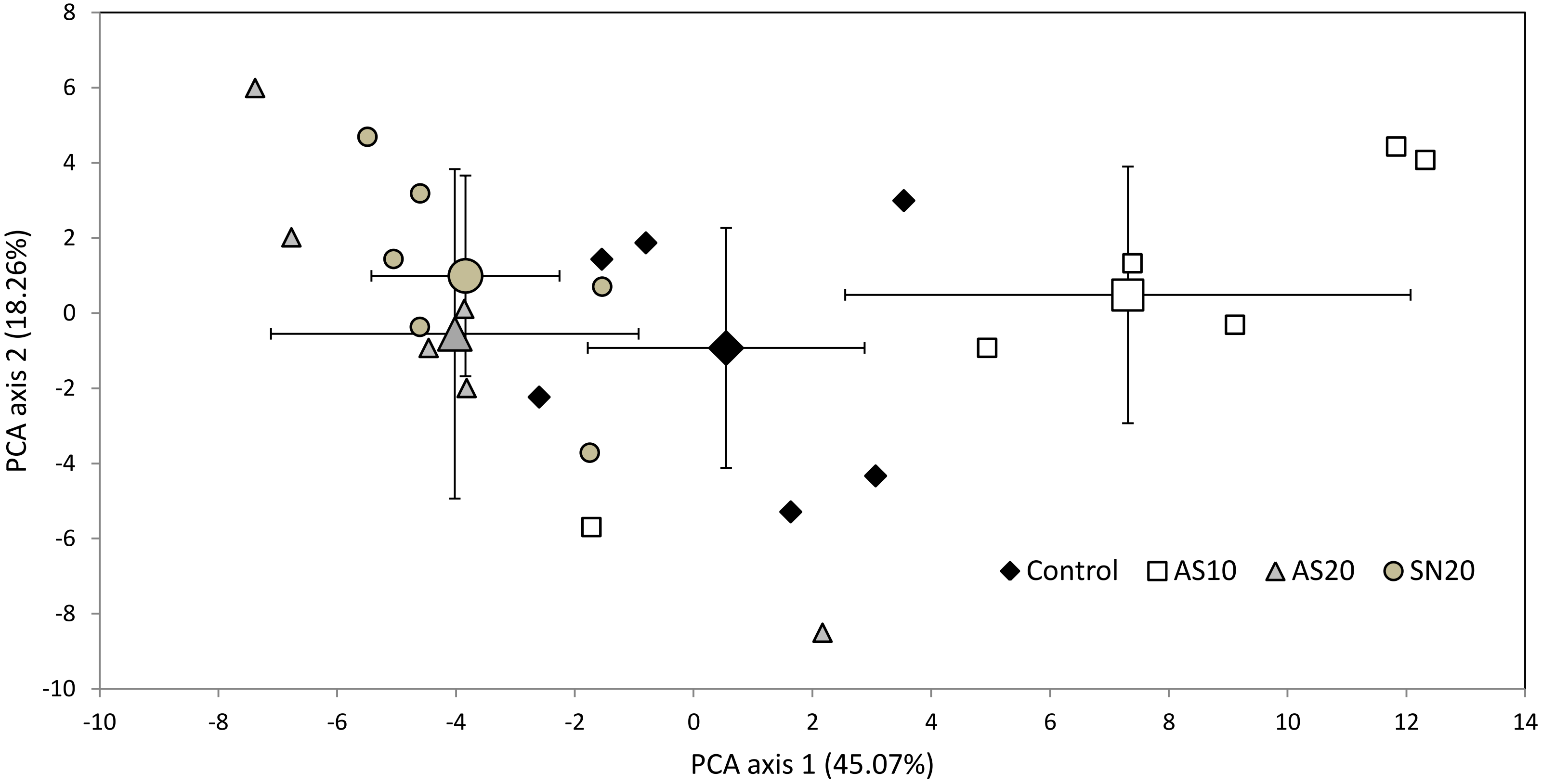

Figure 3.5. PCA of the vegetation species composition for the 24 treatment plots. CONT = control; AS10 = ammonium sulphate at 10kg ha^{-1} yr^{-1} + phosphorus at 20 kg ha^{-1} yr^{-1} (applied once in 2000); AS20 = ammonium sulphate at 20kg ha^{-1} yr^{-1}; SN20 = sodium nitrate at 20kg ha^{-1} yr^{-1}. Error bars denote standard deviation.

Table 3.2. Mean (± standard deviation) response of soil pH and total soil C and N to experimental N and P addition treatments 19 years after commencement and three years after cessation. $F_{3,20}$ and *P* values are derived from one-way ANOVAs

	CONT		**AS10**		**AS20**		**SN20**		***F***	***P***
pH	4.1	±0.12	4.0	±0.08	4.0	±0.12	4.0	±0.13	NS	-
Soil C (%)	9.4	±0.96	8.2	±1.14	10.5	±2.04	10.4	±1.13	**3.50**	**0.035**
Soil N (%)	0.6	±0.07	0.5	±0.06	0.6	±0.11	0.7	±0.09	**3.76**	**0.027**
C:N	16.2	±0.98	16.3	±0.70	16.4	±1.35	16.1	±0.97	NS	-

CONT = control; AS10 = ammonium sulphate at 10kg ha^{-1} yr^{-1} + phosphorus at 20kg ha^{-1} yr^{-1} (applied once in 2000); AS20 = ammonium sulphate at 20kg ha^{-1} yr^{-1}; SN20 = sodium nitrate at 20kg ha^{-1} yr^{-1}. Bold highlights results significant at $P < 0.05$.

No difference was recorded between treatments for dwarf shrub or forb species. PCA analysis revealed differences in vegetation species composition with plots clustering strongly between treatments. The first two axes explained 63.32% of the total variation of the species composition. Differences were observed between treatments for axis 1 ($F_{3,20} = 14.056$, $P = <0.001$, Figure 3.5), where the value of the first principal component for AS10 (N + P) was greater than either the control or N form treatments (AS20 or SN20). Control plots also had higher values for the first principal component than either AS20 or SN20 treatments. No difference was observed between AS20 and SN20, thus any effects from different N forms on vegetation species composition were not evident three years after the last application.

No response was observed in Ellenberg fertility score for total vegetation species data, but when considered as separate groups for cryptogams and vascular plants, differences were observed for vascular plant species (Table 3.1, Figure 3.6). LSD pairwise comparisons revealed the difference to lie between N + P (AS10), and control and high nitrate treatment (SN20). No difference was recorded between the high ammonium treatment (AS20) and the N + P treatment (AS10). Differences between treatments were recorded for both soil C and soil N pools (Table 3.2, Figure 3.7). For both pools, no treatment differed from control, however the addition of P resulted in smaller C and N pools in AS10 treatment compared with the N form treatments (AS20 and SN20). No differences were recorded between treatments for soil pH or the C:N ratio.

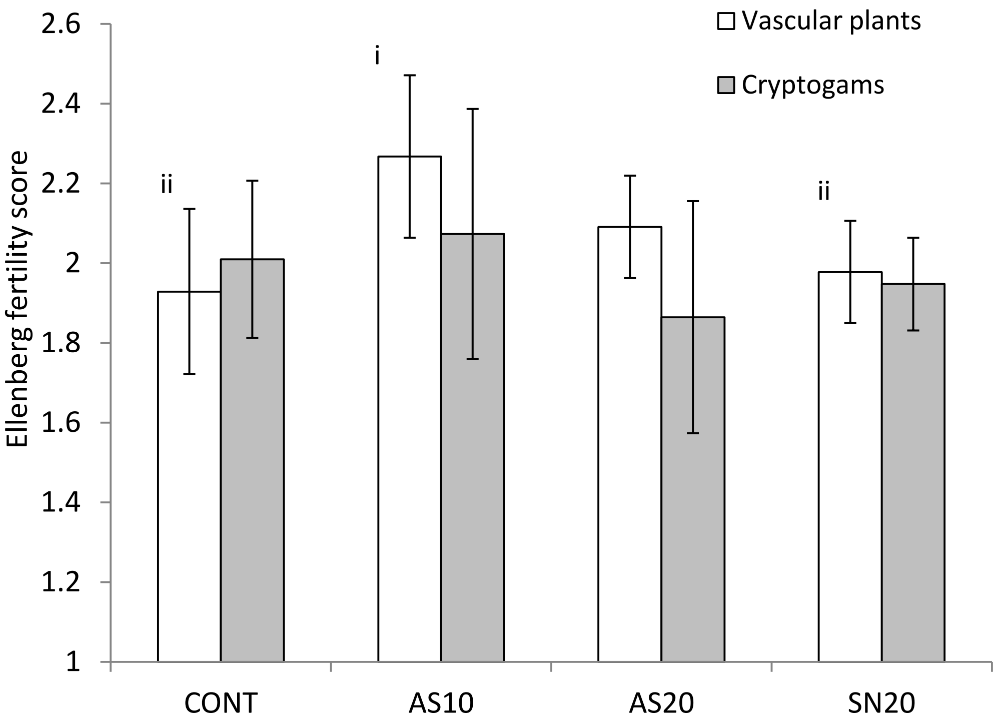

Figure 3.6. Ellenberg fertility levels between addition treatments, for both vascular plants and cryptogam species. CONT = control; AS10 = ammonium sulphate at 10kg ha^{-1} yr^{-1} + phosphorus at 20kg ha^{-1} yr^{-1} (applied once in 2000); AS20 = ammonium sulphate at 20kg ha^{-1} yr^{-1}; SN20 = sodium nitrate at 20kg ha^{-1} yr^{-1}. Error bars denote standard deviation. Post hoc tests conducted with LSD pairwise comparisons.

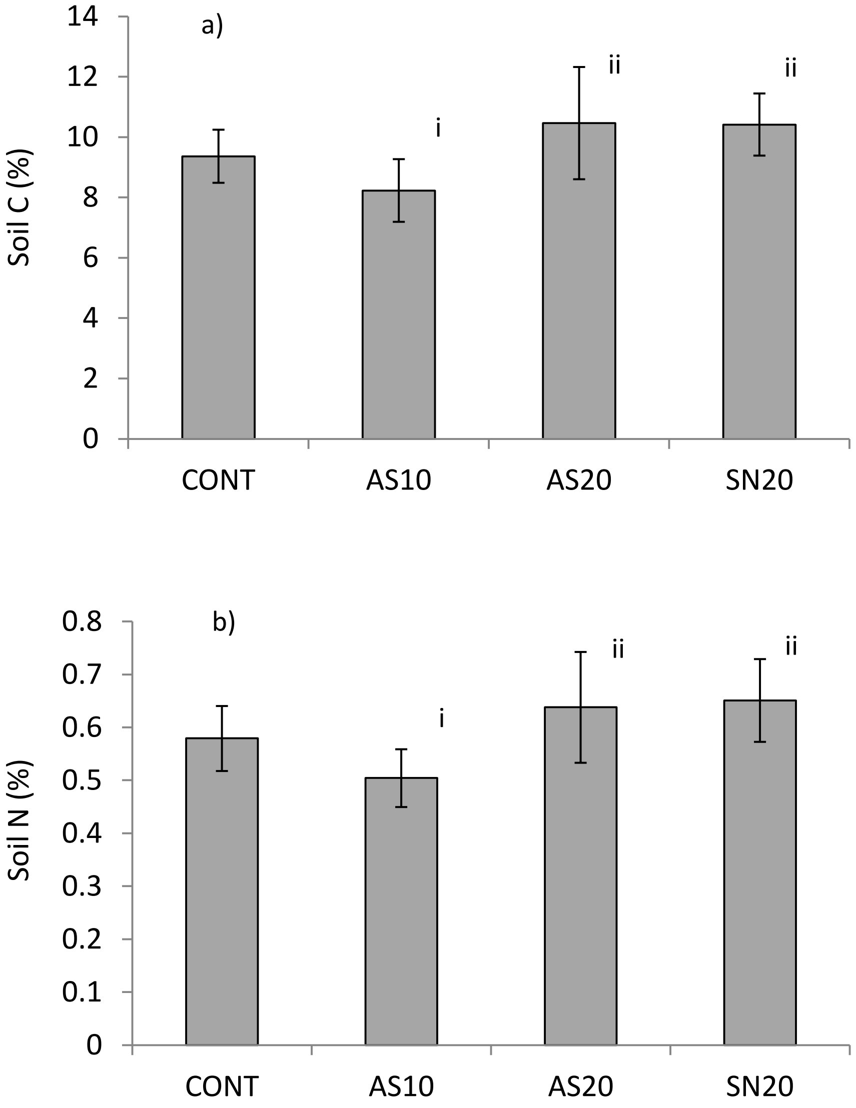

Figure 3.7. Total soil C (a) and N (b) levels between addition treatments. CONT = control; AS10 = ammonium sulphate at 10kg ha^{-1} yr^{-1} + phosphorus at 20kg ha^{-1} yr^{-1} (applied once in 2000); AS20 = ammonium sulphate at 20kg ha^{-1} yr^{-1}; SN20 = sodium nitrate at 20kg ha^{-1} yr^{-1}. Error bars denote standard deviation. Post hoc tests conducted with LSD pairwise comparisons.

3.5 DISCUSSION

3.5.1 VEGETATION RESPONSES TO NUTRIENT ADDITION TREATMENTS

Significant differences in vegetation characteristics were observed in this study when exposed to either N or N + P enrichment, which demonstrates the effect of P limitation in systems exposed to long term N enrichment as expected from the impact of N deposition (Crowley *et al.,* 2012; Peñuelas *et al.*, 2013). Shifts in vegetation species composition were observed between treatments, which is consistent with previous research into effects of N and P additions (Gordon *et al*., 2001; Pilkington *et al*., 2007; Avolio *et al*., 2014). The PFT covers recorded supported our first hypothesis regarding vegetation species composition change with greater graminoid species cover found in N treatments (AS20 and SN20) and greater cryptogam species cover with N + P (AS10) addition. N enrichment has been shown to both decrease cryptogam cover (Arróniz-Crespo *et al*., 2008) and to increase graminoid cover (Field *et al*., 2014). Cryptogam sensitivity to N deposition is a result of tissue N build-up from high surface absorption capacity (Arróniz-Crespo *et al*., 2008). P availability can alleviate this effect by increasing nitrate reductase activity via stimulation of photosynthesis and export of photosynthetic products from the chloroplast to the cytosol (Lambers *et al*., 1998; Gordon *et al*., 2001), which enhances N uptake allowing additional growth and reducing tissue N concentrations (Pilkington *et al*., 2007; Arróniz-Crespo *et al*., 2008). Thus available P enhances cryptogam productivity and abundance (Gordon *et al*., 2001; Limpens *et al*., 2004) and the response of cryptogams to N deposition will vary with soil P status (Gordon *et al*., 2001). Any beneficial influence of P enrichment on cryptogam cover however, is expected to be highly species-specific, with negative impacts from increased competition likely to affect slow growing species (Arróniz-Crespo *et al*., 2008). An effect observed in this study by the dominance of certain fast growing species such as *Rhytidiadelphus loreus* in AS10 plots. The long term impact on vegetation species composition from the addition of different N forms was shown to have little effect, which is contrary to previous studies (Stevens *et al*., 2011), presumably since soil mineral N was mainly in reduced form in the poorly aerated, low pH soils at the site. For both vegetation cover and composition, the control treatment was intermediate in response between N only and N + P treatments, significantly different from either of the N form treatments (AS20 and SN20) or the N + P treatment. (AS10). This demonstrates the polarity of response in vegetation species composition as a consequence of alleviation from P limitation.

The Shannon diversity of vegetation was lower in treatments in which N + P were added. Nitrogen enrichment is often considered detrimental for vegetation species diversity, but long term analysis has shown that the presence of other limiting factors such as P availability may have more effects than N alone (Hejcman *et al*., 2007). Our results support this and show the influences on species diversity of N addition alone are negligible, but with increasing P availability become more significant. The species richness of the vegetation was the same for all treatments but more forb species were found in both the N addition treatments compared to control. This is in contrast to a large UK wide survey (Stevens *et al*., 2006) and can potentially be attributed to species-specific responses to N which have been shown to be variable (Henrys *et al*., 2011; Stevens *et al*., 2012). The taller vegetation height recorded in the N + P treatments of the experiment is consistent with other studies (Limpens *et al*., 2004; Pilkington *et al*., 2007). This demonstrates the productivity potential of cryptogam species when released from P availability constraint in situations of high N enrichment (mechanism discussed above).

No difference was observed in Ellenberg fertility scores between treatments for the total vegetation community, however when considered separately for vascular plant and cryptogam species, differences were revealed for vascular plants, with Ellenberg fertility scores higher in the N + P (AS10) treatments than either control or nitrate (SN20), indicating higher levels of plant productivity (Wagner *et al.*, 2007); an effect which is attributed to the long term influence of P in soil. No difference was observed with AS20, which suggests that the fertility scores may also be sensitive to the effects of N form and the addition of NH_4. Grime's C-S-R functional signature analysis for vegetation composition showed no significant shift in prevailing plant strategy. Although treatments caused dramatic shifts in plant species composition, the prevailing strategy (C: competitor) remained stable (Grime 1977).

3.5.2 SOIL CHEMICAL RESPONSES TO NUTRIENT ADDITION TREATMENTS

The addition of N and N + P in combination resulted in differences in soil C and N pool sizes suggesting P limitation has a large effect on below-ground ecosystem process. The treatment with P added had smaller C and N pools compared to treatments with N alone, which is consistent with similar nutrient addition studies (Bradford *et al.*, 2008; Scott *et al.*, 2015) and supports the third hypothesis. The release from P limitation exerts a dual effect with regard to soil C cycling. The size of soil C pool is controlled by the rate of organic matter decomposition, which is influenced by changes to vegetation species composition through the alteration of litter quality and quantity returned to the soil (Dorrepaal *et al.*, 2005) and through the availability of nutrients, which stimulates soil microbial function (Bragazza *et al.*, 2006). The shifts in vegetation species composition towards greater cryptogam cover observed here also resulted in vegetation cover with reduced root systems, thus less direct input of organic matter into soil; a principal vector for organic C sequestration (Freschet *et al*., 2013). In addition, roots with higher P tissue content are also potentially more susceptible to decomposition, resulting in faster C cycling rates (Smith *et al*., 2014). By contrast, He *et al*., (2013) found that N and P addition increased soil C pools through greater input of below-ground plant organic matter, which highlights the potentially variable response of different environments subject to prevailing vegetation composition.

The size of the N pool was also reduced in the current experiment by the addition of N + P compared to N alone. Increases in the rate of decomposition augment the rate of N mineralisation by releasing N from storage in soil organic matter (Bragazza *et al*., 2013). Once modified from organic to inorganic form, N can be readily leached or assimilated into biomass through plant uptake (Rowell, 1994). Changes to vegetation species composition can affect the rates of N mineralisation, via cascading effects from above-ground to below-ground systems (Bragazza *et al*., 2012; Ward *et al*., 2014). Phosphorus availability enhances N uptake by increasing nitrate reductase activity, thus alleviating the negative effect of tissue N saturation by facilitating growth (Pilkington *et al*., 2007; Arróniz-Crespo *et al*., 2008). The smaller soil N pool observed in the N + P treatment may also be in part accredited to increased uptake and biomass assimilation, an effect corroborated by the largest vegetation biomass (measured as height) observed in the N + P addition treatment. N pool sizes were similar between N only (AS20 and SN20) and control treatments, contrasting with surveys which have shown rises in soil N as a result of N inputs

(Pilkington *et al.,* 2005; Edmondson *et al.,* 2010; Phoenix *et al.,* 2012). This is potentially a consequence of background N deposition, which for this location is 12.6 kg N ha^{-1} yr^{-1}. Critical loads for this habitat are between 10 - 15 kg N ha^{-1} yr^{-1} (APIS, 2014), hence the influence exerted from this enrichment may reduce the size of experimental response. Alternatively this indicates a rapid recovery from chronic N exposure three years post addition cessation; however previous studies on the latent effect on N enrichment have shown associated effects still occurring after three years (Edmondson *et al*., 2013), and therefore this would seem unlikely. In addition, no difference in total soil N was observed between N forms. NH_4 binds more strongly to soil cation exchange sites than NO_3 and has a longer residence time which can result in accumulation in soil (Stevens *et al*., 2011). No effect of build-up was observed in this study suggesting no inhibition of N transformation as predicted for NH_4 addition in low pH soil (Stevens *et al*., 2011). No difference was observed for soil pH between treatments. This was contrary to expectation as the addition of N was expected to increase soil acidity as has been reported previously (Bobbink *et al*., 2010; Phoenix *et al*., 2012). Furthermore, differences in pH were anticipated between the different N forms. Ammonium, through the process of nitrification, releases H+ ions with chemical transformation (Reuss & Johnson, 1986) thus over time acidifying the soil. However, no evidence of these effects was observed.

Difficulties which exist due to the original experimental design mean some caution must be employed in the interpretation of the above results. The different level of N added to the P treatment and the omission of a P only treatment make it impossible to draw an absolute comparison. Nonetheless the long term nature of this existing experiment, including the rare opportunity to observe the effects of P addition on ecosystem processes over decadal timescale, make this a necessary study and effectively demonstrate the differential effect between nutrient availabilities and release from nutrient limitiation.

3.6 CONCLUSION

Diversity, structure and composition of vegetation were significantly altered by the addition of P to ecosystems with enhanced N availability. Soil C and N pool sizes were reduced by the addition of N and P in combination, via changes to the direct input of organic matter to soil, from vegetation species change towards the dominance of species with limited root systems, and through increased rates of decomposition facilitated by greater nutrient availability and higher nutrient content in SOM tissue. This demonstrates that P enrichment has the capacity to modify C storage potential in upland soils.

The persistent nature of P in soil means the addition of P to upland ecosystems may result in changes to the above-ground and below-ground ecosystem characteristics over the long term. The results presented here demonstrate the nature and impact of P addition 15 years after addition, highlighting the gradual nature of change and thus providing essential insight for projects considering P addition as an amelioration measure. This is shown by these results to be an unsuitable management tool to alleviate the more severe impacts of N deposition.

CHAPTER 4

INTERACTION BETWEEN SOIL FAUNA, VEGETATION CHARACTERISTICS AND NUTRIENT AVAILABILITY IN THE UPLAND ECOSYSTEM

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4.1 ABSTRACT

Nutrient availability rates govern numerous ecosystem processes and regulate the abundance and diversity of organisms in the oligotrophic upland ecosystem. Much research to date has focussed on the impact of nutrient availability on vegetation communities, but relatively little research has focussed on the effects of changing nutrient rates on soil invertebrate fauna which represents a significant proportion of ecosystems biodiversity. Reactive nitrogen (N) enrichment from atmospheric deposition is recognised as a major threat to ecosystem function in upland habitats. Increases in N availability can result in N saturation and lead to a reduced capacity for N retention, which may be exacerbated by a shift in nutrient status, from N limitation to phosphorus (P) limitation, constraining ecosystem productivity and biomass production. This study investigated the role of nutrient availability in governing upland ecosystem processes, particularly the activity and abundance of soil invertebrate fauna (microarthropods & enchytraeids), as these groups influence plant productivity, and nutrient and carbon (C) cycling rates. This was undertaken using a nutrient addition experiment, in a fully randomised, replicated design with N and P added at three levels (0, 30 & 60 kg ha^{-1} yr^{-1}for N and 0, 20 & 40 kg ha^{-1} yr^{-1}for P) in all combinations. Nutrient availability had no significant effect on total soil fauna abundance, or on the abundance of any individual taxonomic group. The taxonomic group richness and soil biological quality index (QBS) scores however were significantly increased by the addition of P, suggesting the amount of different groups supported and their level of adaptation to life in soil, could be effective in discriminating between soils of different nutrient status. The addition of P had strong effects on vegetation growth and vegetation tissue N:P ratios. N had no significant effect on growth or tissue nutrient concentrations, potentially indicating N saturation.

Our results indicate P addition can increase taxonomic richness of soil faunal communities, with consequent increases in plant nutrient uptake, and can improve the rate of N assimilation in vegetation by enabling additional growth.

4.2 INTRODUCTION

Modification of nutrient availability rates, influenced by the effects of pollution from anthropogenic activities such as industry and food production (Stevens *et al*., 2011; Ceulemans *et al*., 2014), can impact numerous components and processes in the oligotrophic upland ecosystem (Pilkington *et al*., 2005; Jones & Power, 2012; Field *et al*., 2014). The effects of reactive nitrogen (N) deposition in ecosystems, particularly in traditionally N limited habitats, are well documented (i.e. Stevens *et al.*, 2004 & 2010; Maskell *et al.*, 2010; Bobbink *et al.* 2010). What is less well understood however is the relationship between N and phosphorus (P) in situations of high N deposition. Prolonged exposure to high levels of reactive N deposition in upland habitats can initiate a shift in fertility status from N-limited to P-limited (Britton & Fisher, 2007; Phuyal *et al*., 2008; Crowley *et al*., 2012). The implications of such nutrient limitation shifts are beginning to be understood (Olde Venterink, 2011). Environments which are limited by P availability may be unable to sequester additional reactive N inputs if P limitation restricts vegetation biomass production (Li *et al*., 2015), which could result in N saturation and therefore N leaching (Emmett *et al.,* 1995). However P limitation may act to constrain ecosystem productivity and limit the negative impact of N enrichment on biodiversity (Ceulemans *et al*., 2014). Understanding the role and influence of P availability in situations of increasing N enrichment could potentially improve critical load estimations (particularly on site specific bases) and may better inform potential management strategies considering P addition as an amelioration measure (Armitage *et al.* 2012).

Research into changes within ecosystem processes and biodiversity has predominantly focused on change within vegetation communities (i.e. Storkey *et al*., 2015). Soil fauna are major components of ecosystem biodiversity and often represent the largest proportion of species, the activities of which actively regulate various ecosystem processes (Reichle, 1977; Coulson & Butterfield, 1978; Wardle *et al*., 2004; Wagg *et al*., 2014). Soil organisms can be broadly categorised by body width as micro (<100 µm wide), meso (>100 µm, < 2 mm wide) and macro (> 2 mm, < 20 mm wide) fauna (Stork & Eggleton, 1992) and can be extremely abundant with estimations of populations of mesofauna alone in the region of 50 – 300k individuals per m^{-2} in UK soil (Bardgett & Cook, 1998). Soil organisms have the potential to offer considerable insight into environmental change because these groups are highly sensitive to environmental vicissitudes making them ideal indicators of shifts in prevailing ecosystem characteristics (Stork & Eggleton, 1992). The use and application of such organisms however is problematic as skill level and time required to identify individuals to species level is often prohibitive (Parisi *et al*., 2005). In addition, there remains much uncertainty over which soil fauna are the best indicators of soil quality and what soil fauna data should be used to assess soil quality (Yan *et al*., 2012). Therefore the use of soil fauna populations as indicators of environmental change is underutilised.

Nutrient availability influences soil faunal activity and abundance through bottom-up control (Scheu & Schaefer, 1988; Murray *et al*., 2006; Fountain *et al*., 2008). Soil invertebrate assemblages have been shown to be sensitive to changes in nutrient status (King & Hutchinson, 1980; Cole *et al*., 2005; Payne *et al*., 2012; Ochoa-

Hueso *et al*., 2014), but further research is necessary to fully understand the nature of this effect as it is likely to vary significantly between environments and soil types (Xu *et al*., 2007). The impact of N enrichment on soil fauna is complex. In one respect greater N availability relates to increased resource availability, particularly from increased plant inputs (Cole *et al*., 2005), which may support greater abundance and transfer energy into higher trophic levels (Fountain *et al*., 2008). However N deposition may also result in lower soil pH which can negatively impact numerous soil faunal groups (Ochoa-Hueso *et al*., 2014). Furthermore, where augmentation of nutrient availability alters the allometry, activity and abundance of soil organisms, knock on effects will be likely for numerous ecosystem process including nutrient and organic matter cycling rates (Bardgett & Chan, 1999; Mulder & Elser, 2009), which may have reciprocal or feedback effects, with implications for ecosystem function as a whole.

Phosphorus availability is potentially a limiting factor for both soil fauna and vegetation communities, where long term N enrichment has resulted in modification of nutrient limitation status, resulting in higher levels of competition between soil fauna and plants for available P (Parfitt *et al*., 2005). The aim of this study was to investigate the role of nutrient enrichment from N and P addition, in modifying ecosystem processes through the alteration of soil invertebrate community composition and abundance, and vegetation productivity, particularly when released from P limitation, which is expected to be a current factor controlling productivity. This was undertaken using a nutrient addition experiment, in a fully randomised, replicated design, with N and P added at three levels (0, 30 & 60 kg ha^{-1} $yr^{-1}$for N and 0, 20 & 40 kg ha^{-1} $yr^{-1}$for P). We hypothesised that release from P limitation would increase N utilisation and therefore ecosystem productivity (observed as greater plant shoot extension) and soil faunal abundance, as nutrient availability increases food resources (King & Hutchinson, 1980) and thus energy provision from the bottom-up. It is expected that through bottom-up regulation, increases in the abundance of soil fauna will be observed as increases in taxonomic richness with shifts in the ratio between predators and prey (zootroph vs phytotroph/saprophytotroph), as predator numbers increase in proportion with increases in prey species availability (Cole *et al*., 2005). We also hypothesized a positive relationship between soil invertebrate abundance and vegetation leaf tissue nutrient concentrations because soil fauna can increase nutrient mineralisation and uptake in plants (Bardgett & Chan, 1999; Bender *et al*., 2015).

4.3 METHODS

4.3.1 EXPERIMENTAL DESIGN

This study was conducted at Pwllpeiran Research Farm in the Cambrian Mountain range, mid-Wales (52^0 37’ N, 3^0 77’ W). A nutrient addition experiment was established, consisting of six replicate blocks of nine, 3x3 m plots, in a fully randomised design (Figure 4.2). The blocks were spread across three separate paddocks, within an altitude range of 500 - 600 metres a.s.l. on a transition between NVC U4 *Festuca ovina Agrostis capillaris* calcifuge grassland and H18 *Vaccinium myrtillus Deschampsia flexuosa* heath, overlying mixed soils ranging from shallow ferric stagnopodzol to deep peat (Emmett *et al.*, 2007). The mean annual rainfall rate for this location was 1512.2mm (UK Meteorological Office, no date) and the background N deposition rate was 12.6 kg N ha^{-1} yr^{-1} (APIS, 2014). Sheep grazing was active on site during this study, at a rate of 1.0 sheep/ha.

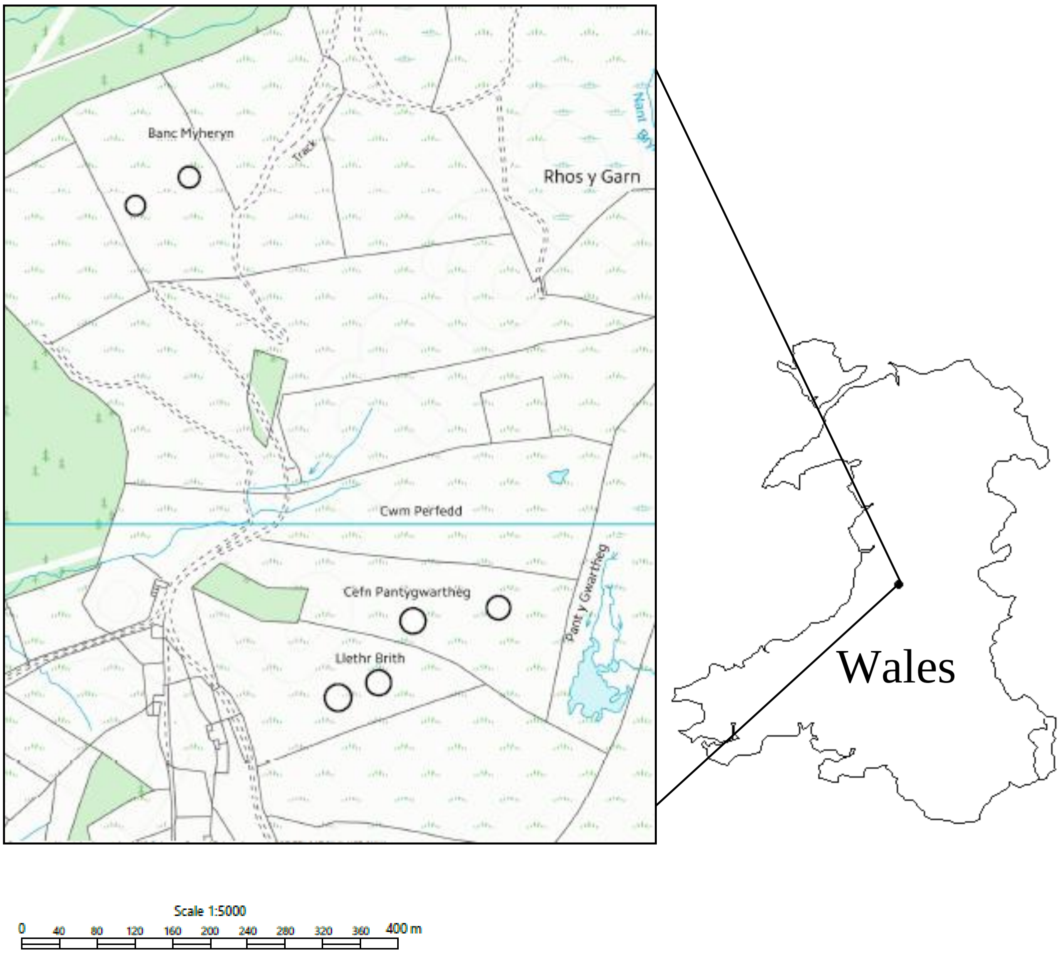

Figure 4.1. Location of study sites at Pwllpeiran, Cwmystwyth, mid-Wales, UK. White circles indicate treatment block locations. Map of Wales was created using ‘blighty’ R package (Lucy, 2010). Site maps from Ordnance Survey (OS data © Crown copyright/database right 2010) An Ordnance survey/ EDINA supplied Service.

CONT (no addition)	**LN** (low N)	**HN** (high N)
HNLP (high N, low P)	**LP** (low P)	**HP** (high P)
LNHP (low N, high P)	**LNLP** (low N, low P)	**HNHP** (high N, high P)

Figure 4.2. Experimental treatments: CONT = control (no addition); **LN** = NH_4NO_3 at 30kg ha^{-1} yr^{-1}, **HN** = NH_4NO_3 at 60kg ha^{-1} yr^{-1}; **LP** = NaH_2PO_4 at 20kg ha^{-1} yr^{-1}; **HP** = NaH_2PO_4 at 40kg ha^{-1} yr^{-1}.

Nitrogen was added every two weeks between the months of May – September 2014. Phosphorus was added once in May 2014. Both N & P were dissolved in 2 l of de-ionised water and applied evenly with a knapsack sprayer. Each control plot received 2 l de-ionised water without nutrients with every application.

4.3.2 SOIL ANALYSIS

Soil samples were collected in October 2014. Soil cores were collected from five locations within each survey plot using a 20mm diameter soil corer up to a depth of 20cm, and bulked together. Samples were air dried and passed through a 2 mm sieve. Soil pH was measured with a Hydrus 400 meter (Fisherbrand, Leicestershire, UK) in a slurry of 10 g fresh soil in 25 mL water. Soil organic matter was determined by loss-on-ignition (375°C). Total N and carbon (C) were established by the Dumas combustion method using an elemental analyser (Vario MAX Cube – Elementar Analysensysteme, Hanau, Germany). Total P was determined using the Kjeldahl method on oven-dry soil (105 ^{o}C). Briefly, soil samples were digested using 1 mL H_2SO_4 + Se catalyst per 0.1g sample at 400 ^{o}C for 2.5 hrs with a Buchi block digester (Buchi digest system K-437, Buchi labortechnik, Switzerland), before being analysed colorimetrically using the molybdenum blue method for P on a continuous flow auto analyser (AutoAnalyzer III, Bran+Luebbe GmbH, Germany).

4.3.3 VEGETATION ANALYSIS

All vegetation coverage data were collected in June 2014 and were recorded visually with the Domin scale, subsequently transformed to percentage cover for statistical analysis (Currall, 1987). All plants and cryptogams were identified to species level, but were analysed by functional type (graminoid, cryptogam (bryophyte and lichen), dwarf shrub and forb). For the shoot extension trial, grazing exclosures were erected in each treatment plot covering an area of ~ 1 m^2, inside of which, three separate *Vaccinium myrtillus* plants were selected and a single shoot in growth phase was measured from the tip to the main stem and marked. Growth was measured between June – October 2014. Shoots which had been subject to herbivory (small mammal or invertebrate) were omitted from subsequent analysis. Leaf tissue samples from *V. myrtillus* were collected in late September 2014. Samples were oven dried for 24hrs at 105 ^{o}C, Before being analysed for N & P using the Kjeldahl method and for total C by the Dumas combustion technique, using an elemental analyser (see soil methods above for detail).

4.3.4 SOIL INVERTEBRATE ANALYSIS

Soil cores were taken in July of 2014 and 2015. Each core was ~ 200ml (50mm diameter x 100mm depth). Soil fauna were then extracted from cores using Berlese-Tullgren funnels and preserved in 70% ethylic alcohol. Macro and mesofauna were identified to order and family level using a stereoscopic microscope. The abundance of all collected soil fauna (microarthropods & enchytraeids) populations was determined. To describe soil faunal activity, the ratio between Collembola : Acari (C:A) abundance (Bachelier, 1986) was calculated and results for each plot were applied to the Biological Soil Quality Index (QBS; Qualità Biologica del Suolo. Also referred to as BSQ elsewhere) (Parisi *et al.*, 2005). Soil quality is classified with QBS on the basis of microarthropod biodiversity and adaptation to life in soil. Scores from 1 – 20 on an eco-morphological index (EMI) are awarded for each group recorded (with higher scores indicating a higher level of adaptation), and then summed for a single figure for each plot. This approach allows a rapid characterisation of differences in soil habitat quality between sites, without the need for species level identification (Parisi *et al.*, 2005). To identify differences in the abundance of zootrophic species, a ratio for predator vs prey species was also calculated (Pred:Prey). This was determined as the ratio between Mesostigmata : Collembola. Mesostigmata alone were considered as predators as the majority of species in this order are predatory (Koehler, 1999). Other Acari groups are less consistent. Trombidiformes, which include Prostigmata, have a variety of feeding preferences across taxa, including predators, parasites, phytophages, saprotrophs and omnivores (Krantz & Walter, 2009). Sarcoptiformes, which include Oribatida, are thought to be predominantly detritivores and fungivores (Schneider *et al.*, 2005), however these were not considered as prey as this group has been shown to suffer little from predation in the field (Peschel *et al.*, 2006).

4.3.5 STATISTICAL ANALYSIS

Prior to analysis all data were tested for normality and homoscedasticity and Log (x+1) transformed where data did not conform to these assumptions. Soil faunal abundances for 2014 and 2015 were summed to a single figure for each plot to avoid temporal pseudoreplication (Hurlbert 1984). General linear models (GLM) were used to test the effect on soil faunal abundance of the nutrient addition treatments, and the interaction between nutrients when added in combination. Nitrogen and P (three levels: no, low and high) were considered as fixed factors with an identifier for each replicate block as a random factor, to allow for differences in inter-site characteristics (altitude, soil type, exposure). When significant differences between treatments were detected ($p < 0.05$), post-hoc tests were conducted using LSD pairwise comparisons. Pre-existing environmental characteristics of the study site were likely to influence soil faunal abundance between blocks. Linear models were used to investigate the effect of soil chemical characteristics (soil N, P, C and organic matter content (OM), and soil stoichiometric ratios: C:N, C:P and N:P), vegetation diversity and plant functional type coverages on all soil faunal groups which accounted for >1% of the total soil faunal abundance recorded. A 'best subsets' model selection procedure was specified due to accepted weakness in stepwise model selection methods (Whittingham *et al*., 2006). Variable terms were removed from full models systematically until optimal models were achieved as indicated by Akaike's information criterion (AIC). Relationships between leaf tissue P and N content and between soil fauna abundances and leaf tissue chemistry were also investigated with simple linear regression analysis. All statistical analyses were conducted with SPSS 21.0 (IBM SPSS Statistics for Windows, 2012).

4.4 RESULTS

4.4.1 SOIL FAUNA

A total of 8762 soil invertebrates, across 16 taxonomic orders (including Araneae, Acari, Enchytraeidae, Collembola, Coleoptera, Hemiptera, Hymenoptera, Thysanoptera, Diptera, and Psocoptera – see Table 4.1) were extracted from soil samples. Acari and Collembola were the dominant soil invertebrate groups, accounting for 59% and 32% of individuals recorded respectively. Soil faunal abundance was larger in treatments with added nutrients, particularly with the addition of P; however these differences were shown to be non-significant (Table 4.2), as higher figures were the result of biota aggregations. The addition of N and P had limited effect on the abundance of individual faunal groups, with the only significant effect observed for Araneae, which were only recorded in high P addition treatments ($F_{2,53} = 3.33$, $p = 0.046$). Phosphorus addition was however shown to have a significant effect on the number of different taxa recorded (*T-richness*), with significantly more taxa present ($F_{2,53} = 3.56$, $p = 0.038$) in plots receiving high P addition (40kg ha^{-1} yr^{-1}), compared to control plots (no nutrient addition), which had the lowest *T-richness* overall (Figure 4.3b). No difference in *T-richness* was recorded after N addition at either high or low rate. Biological soil quality (QBS) scores were also significantly higher in plots where P was added ($F_{2,53} = 3.91$, $p = 0.028$, Figure 4.3a), particularly at the higher addition rate. Nitrogen addition had no direct effect on QBS score, however there was a significant interaction between N x P ($F_{4,53} = 2.78$, $p = 0.039$). The ratio of Collembola to Acari (C:A) was also significantly influenced by the addition of N x P in combination ($F_{4,53} = 3.11$, $p = 0.025$), but not by the addition of either nutrient on its own.

Table 4.1 Taxa distribution and abundance across nutrient addition treatments.

Class (subclass)	**Order**	**Control**	**LN**	**LP**	**HN**	**HP**	**LNLP**	**LNHP**	**HNLP**	**HNHP**	**Total**
Arachnida	Mesostigmata	41	33	41	47	33	61	41	38	53	**388**
(Acari)	Sarcoptiformes	339	187	200	266	334	399	436	280	251	**2692**
	Trombidiformes	150	123	205	380	400	350	141	123	241	**2113**
Chilopoda	Geophilomorpha	4	0	0	1	1	2	1	0	1	**10**
Clitellata (Oligochaeta)	Enchytraeidae	7	2	5	4	8	4	5	6	15	**56**
Entognatha	Entomobryomorpha	147	217	283	523	138	193	239	191	139	**2070**
(Collembola)	Poduromorpha	41	55	144	70	75	63	66	40	129	**683**
	Symphypleona	0	2	0	4	3	1	2	3	4	**19**
	Neelipleona	2	5	8	9	1	8	10	2	1	**46**
Insecta	Coleoptera	30	36	40	20	61	51	43	57	50	**388**
	Thysanoptera	8	8	21	10	8	10	4	5	6	**80**
	Diptera	14	4	23	21	23	23	21	24	38	**191**
T-richness		7.7(±06)	8.3(±0.8)	8.2(±0.3)	9.2(±0.6)	10.2(±0.5)	9.8(±0.5)	9.2(±0.7)	9.2(±0.4)	9.8(±0.7)	
Total		**784**	**675**	**971**	**1355**	**1089**	**1174**	**1012**	**771**	**931**	**8762**

T-richness mean average (± S.E.) number of taxonomic orders represented per plot.

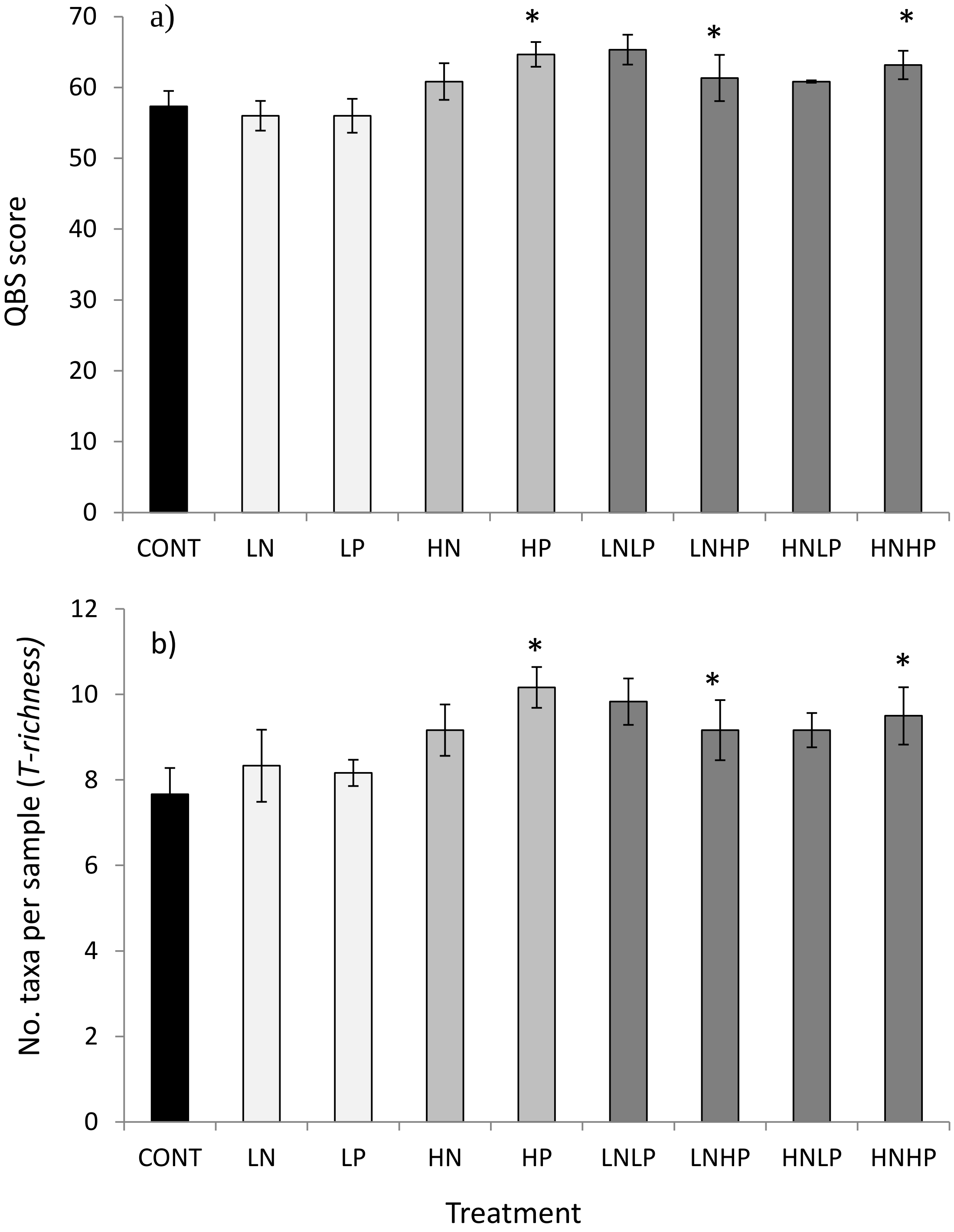

Figure 4.3 Comparison of a) biological soil quality index score (QBS) and b) number of taxonomical groups (*T-richness*) between nutrient addition treatments. Error bars denote standard error (n = 6). * represents a significant difference from control ($p < 0.05$).

Table 4.2 General linear models describing soil fauna characteristics, soil chemistry and vegetation biomass and tissue chemistry between nutrient addition treatments.

Variable	**Factor**			
	N	**P**	**N x P**	**Block**
QBS	0.85	**3.91***	**2.78***	1.11
Pred:Prey	1.81	0.12	0.11	**2.91***
C:A	1.02	0.30	**3.11***	**4.75****
H'	0.23	1.89	0.51	**2.46***
T-richness	0.95	**3.56***	1.95	2.05
Total abundance	0.25	0.09	0.88	0.41
pH	0.77	0.27	0.80	**11.69*****
OM	0.50	0.92	0.52	**6.62*****
Soil C	0.29	0.11	1.04	**7.11*****
Soil N	0.35	0.41	0.71	**12.20*****
Soil P	1.00	**3.12***	1.32	**3.68****
Shoot extension	0.44	**4.45***	0.34	0.70
Leaf N	1.27	0.64	0.66	**18.44*****
Leaf P	1.63	2.01	2.44	**5.61*****
Leaf C:N	1.08	0.59	0.86	**3.35***
Leaf C:P	1.02	**6.15****	1.26	**8.10*****
Leaf N:P	0.20	**5.38****	0.40	**8.02*****

Bold indicates significant results, *p < 0.05; **p < 0.01; ***p < 0.001. QBS biological soil quality index score, C:A Collembola : Acari ratio, Pred:Prey Mesostigmata : Collembola ratio, *T-richness* number of taxonomical groups per plot, *H'* Shannon diversity index, OM soil organic matter content.

Linear regression analysis revealed total abundance of soil fauna was not adequately described by any environmental factor ($p = 0.11$), whereas the abundance of each order was explained by at least one environmental variable (Table 4.3). Mesostigmata abundance was inversely related to soil C:P ($p = 0.006$). Sarcoptiformes were positively related to soil N ($p = <0.001$), and inversely to soil C:P ($p = 0.006$) and forb coverage ($p = 0.009$). Trombidiformes were positively related to soil C ($p = 0.003$), and inversely related to soil N ($p = 0.003$) and the C:N ratio ($p = 0.005$). Entomobryomorpha abundance was positively related to soil pH ($p = <0.001$) and soil C:N ($p = <0.001$). Poduromorpha abundance was inversely related to cryptogam coverage ($p = 0.002$) and positively to vegetation diversity ($p = 0.014$). Coleoptera abundance was inversely related to dwarf shrub coverage ($p = 0.012$) and positively to soil C:N ($p = 0.013$), Diptera abundance was positively related to soil C ($p = 0.002$).

Linear models also described factors influencing soil faunal diversity, QBS index and the C:A and Pred:Prey ratios. Differences in biological soil quality as indicated by QBS were positively related to soil C ($p = 0.007$). The C:A ratio was positively related to soil pH ($p = 0.001$), inversely to soil N ($p = 0.011$) and positively to OM ($p = 0.021$). The ratio between predator and prey numbers was inversely related to soil pH ($p = 0.001$) and soil C:N ($p = 0.001$). The diversity of soil fauna was inversely related to soil pH ($p = 0.02$), positively related to soil C:N ($p = 0.024$) and inversely to soil N:P ($p = 0.032$).

Table 4.3 Multiple linear regressions between soil fauna data and environmental variables (soil characteristics and vegetation coverage)

		Linear Models					
Class (subclass)	**Order**	**Variable**	**Coefficient**	**Variable *p***	**df**	**Model *F***	**Model *p***
(Acari)	Mesostigmata	Soil C:P	-0.68	0.006	2,51	4.24	0.02
	Sarcoptiformes	Soil N	3.27	<0.001	3,50	6.14	0.001
		Soil C:P	-0.80	0.006			
		Forb	-0.02	0.009			
	Trombidiformes	Soil C	10.53	0.003	5,48	3.58	0.008
		Soil C:N	-10.60	0.003			
		Soil N	-20.19	0.005			
Entognatha (Collembola)	Entomobryomorpha	pH	0.93	<0.001	2,51	10.53	<0.001
		Soil C:N	3.18	<0.001			
	Poduromorpha	Cryptogam	-0.01	0.002	2,51	5.22	0.003
		Vegetation *H’*	0.84	0.014			
Insecta	Coleoptera	Dwarf Shrub	-0.01	0.012	4,49	3.47	0.014
		Soil C:N	10.03	0.013			
	Diptera	Soil C	0.74	0.002	1,52	10.58	0.002
Response variables							
QBS		Soil C	12.30	0.007	1,52	7.81	0.007
C:A		pH	1.35	0.001	4,49	6.36	<0.001
		Soil N	-5.44	0.011			
		OM	0.02	0.021			
Pred:Prey		pH	-0.50	0.001	3,50	6.96	0.001
		Soil C:N	-1.83	0.001			
Fauna *H’*		pH	-0.42	0.02	3,50	4.42	0.008
		Soil C:N	1.07	0.024			
		Soil N:P	-0.49	0.032			
Total abundance		NS					

NS no significant model. QBS biological soil quality index score, C:A Collembola : Acari ratio, Pred:Prey predator : prey ratio, OM soil organic matter content. *H’* indicates Shannon diversity index for soil fauna and vegetation.

4.4.2 VEGETATION AND SOIL CHARACTERISTICS

Vaccinium myrtillus growth was significantly increased by the addition of P, both for the high and low application rate ($F_{2,53} = 4.45$, $p = 0.018$, Table 4.2, Figure 4.4), whereas the addition of N for either level had no significant effect on shoot growth ($F_{2,53} = 0.44$, p = NS). The N x P interaction was also non-significant ($F_{4,53} = 0.34$, p = NS), indicating the sole influence of P on growth, thus the additional growth observed was enabled through release from P limitation. Leaf nutrient concentrations did not differ significantly between treatments for tissue N or P, either at high or low application rates, however significant differences were observed in leaf tissue stoichiometric ratios, C:P and N:P. Both ratios were significantly lower in high P treatments ($F_{2,53} = 6.15$, $p = 0.005$, and $F_{2,53} = 5.38$, $p = 0.009$ respectively, Table 4.2, Figure 4.4), which relates to a rise in the proportion of P in leaf tissue relative to the C or N content. Phosphorus availability was also expected to increase the uptake and assimilation of N into plant biomass. Simple linear regression analysis demonstrated a positive relationship between leaf tissue P and N ($R^2 = 0.17$ $p = <0.002$ Figure 4.5).

Soil fauna activity rates were expected to influence nutrient uptake through increases in the nutrient mineralisation rate (Bardgett & Chan 1999). Simple linear regression revealed an inverse relationship between leaf tissue C:N and soil fauna total abundance ($R^2 = 0.098$, $p = 0.021$), which was primarily driven by a significant positive relationship between Entomobryomorpha (Collembola) and leaf tissue C:N ($R^2 = 0.16$, $p = 0.02$).

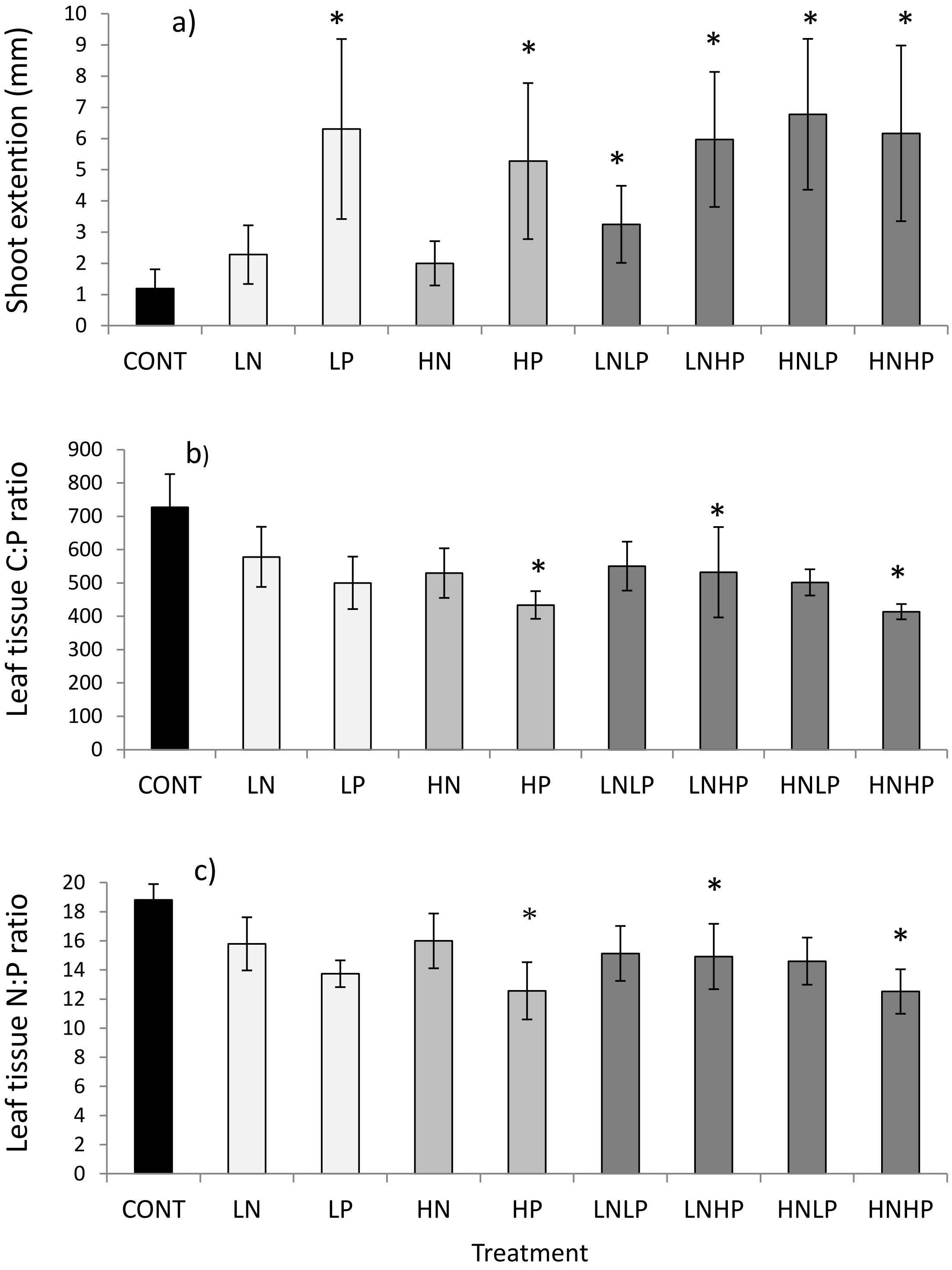

Figure 4.4 Mean averages for *Vaccinium myrtillus* a) shoot extension and b) leaf tissue C:P ratios and c) leaf tissue N:P ratios between nutrient addition treatments. Error bars denote standard error (n = 6). * represents a significant difference from control ($p < 0.05$).

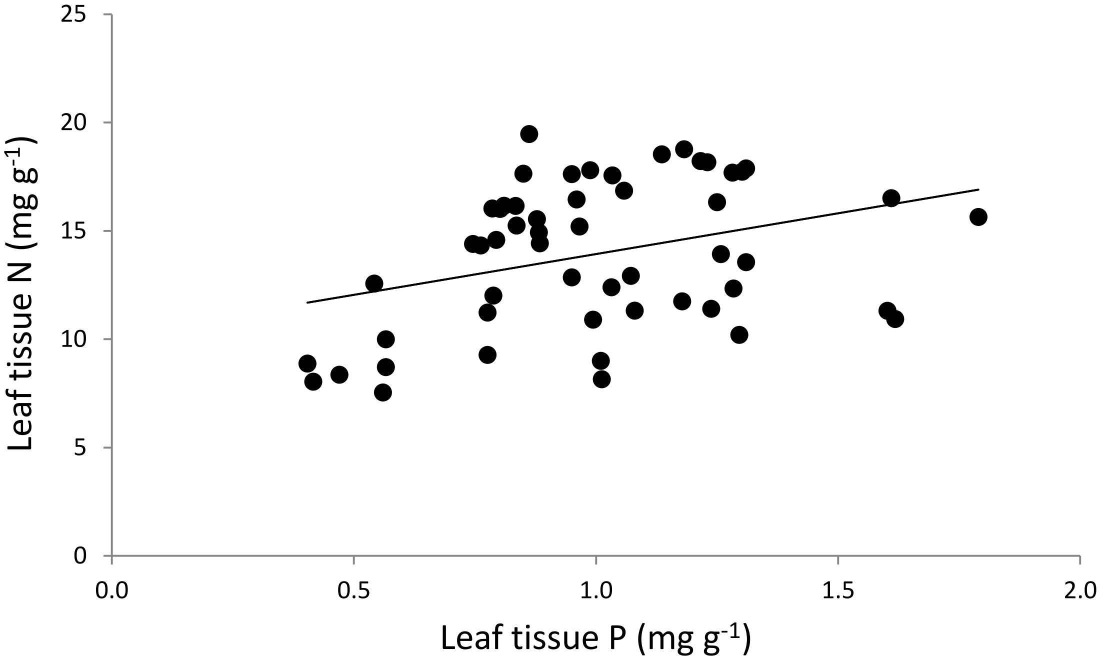

Figure 4.5 The relationship between *V. myrtillus* leaf tissue P and N across nutrient addition treatments ($R^2 = 0.17$).

4.5 DISCUSSION

4.5.1 ECOSYSTEM PRODUCTIVITY AND NUTRIENT STATUS

The growth of *V. myrtillus* was significantly higher in plots where P was added either with or without N, which supported our hypothesis for P enrichment effects on vegetation growth, but the lack of influence on vegetation from N addition was contrary to expectation. This is likely the result of high levels of N availability currently in this environment. The background N deposition rate for the site is 12.6 kg N ha^{-1} yr^{-1}. Critical loads for this particular habitat type are between 10 - 15 kg N ha^{-1} yr^{-1} (APIS, 2014) therefore it is reasonable to assume that the requirement for N is reduced across all treatments, thus limiting the differences between treatments. Nitrogen saturation has been demonstrated in similar environments with high levels of N deposition (Emmett *et al*., 1995; Curtis *et al*., 2005). The limited response to N addition observed in this study potentially indicates a system at capacity for N utilisation (Blanes *et al*., 2012). This is also indicated by the significant influence on vegetation growth from P addition which suggests this system is currently P limited (Blanes *et al*., 2012; Li *et al.,* 2015). The effect on N saturation could also explain the limited response of soil faunal communities to nutrient additions. Soil faunal abundance has been shown to increase under moderate N enrichment but to decrease at higher levels of input (reported to be between 50 – 100 kg ha^{-1}) (Xu *et al*., 2007; Ochoa-Hueso *et al*., 2014), suggesting a 'bell-curve' like response to increasing levels of N availability and therefore a critical load level above which populations of soil fauna will begin to decline.

Plant tissue stoichiometric ratios also indicate a shift in nutrient limitation status towards P limitation. Leaf N:P ratios ranged from 6.8 (observed in high P treatment) to 23.2 (observed in control). No difference was observed between control and either

N addition rate, indicating the additional availability of N did not result in increased uptake of N by *V. myrtillus*. Phosphorus addition had a strong effect on leaf tissue N:P, resulting in significantly lower ratios. Vegetation tissue N:P has been used as an indicator of nutrient limitation status (Güsewell, 2004; Britton & Fisher, 2007) with values <10 relating to N limitation and values >20 to P limitation. Intermediary values indicate co-limitation (Güsewell, 2004). Current levels of N deposition for this environment have increased N:P ratios to the upper level for co-limitation on average with several treatment plots over the P limitation threshold (N:P > 20). Interestingly, plots receiving additional N had lower N:P ratios on average than control, which could potentially be the result of increased P acquisition in situations of greater N availability as demonstrated by Rowe *et al*., (2008). Comparison of leaf nutrient N and P concentrations confirmed a positive relationship between the availability and uptake of P and the uptake of N. Therefore release from P limitation is demonstrated to increase the ability of vegetation to incorporate N into biomass which is enabled through additional growth (Li *et al*., 2015).

4.5.2 SOIL FAUNA

Plant nutrient uptake rates were also anticipated to be affected by the abundance of soil fauna. Bardgett & Chan, (1999) demonstrated an increased rate of mineralisation and uptake of N with greater abundance of Nematodes and Collembola. A significant inverse relationship between total faunal abundance and the C:N ratio of *V. myrtillus* leaf tissue was observed, which further analysis determined was driven by the abundance and activity of a Collembolan group (Entomobryomorpha). This suggests a significant influence on plant uptake of nutrients, particularly N, by these organisms. Entomobryomorpha were the third most abundant taxon in the soil fauna recorded (23.6% of individuals – see table 4.1), the abundance and activity of this group therefore could influence nutrient mineralisation rate and thus vegetation productivity, which supports our hypothesis and reflects the findings from previous experiments (Lussenhop, 1992; Partsch *et al*., 2006; Bender *et al*., 2015).

The number of taxonomic groups was higher in treatment plots where P was added, supporting our hypothesis as taxonomic richness is expected to relate to available resources through the amount of different organisms which can be sustained (Kuperman, 1996; Van der Wal *et al.,* 2009). However, this was not translated into shifts in the proportion of predator to prey organisms in the community which was predicted. A greater proportion of higher trophic level species would indicate greater energy transference through trophic levels from the bottom-up as nutrient availability increases (Cole *et al*., 2005). Thus, whilst in this study greater nutrient availability supports a greater number of taxonomic groups, these additional resources were not observed to directly influence higher trophic levels; although, this is potentially a consequence of the limited duration of this study. In contrast, Mesostigmata abundance was inversely related to soil C:P which suggests that even with no direct relationship between Mesostigmata abundance and P addition, the abundance of this group may increase with higher P availability tentatively suggesting bottom-up energy transference to higher trophic levels (Scheu & Schaefer, 1988; Cole *et al*., 2005).

No direct effect of N addition was observed for the abundance of soil faunal groups between treatments, although the abundance of Sarcoptiforms was positively related to soil N which is consistent with other studies (Xu *et al*., 2007; Van der Wal *et al*., 2009). Sarcoptiformes are predominantly detritivores and fungivores (Schneider *et al*., 2005). The abundance of soil bacteria and fungi has been shown to increase with greater N availability, therefore this population increased concomitantly with available food resources. Nitrogen input rates can also influence soil faunal communities through changes to soil pH status. Soil pH was positively related to Entomobryomorpha abundance and negatively to soil faunal diversity. This difference in response suggests that diversity increases with reduced competition from dominant groups such as Entomobryomorpha, which is likely to be a consequence of increasing soil acidity altering microbial activity and/or reducing the quality and quantity of vegetation inputs (Silvertown *et al.*, 2006; Ochoa-Hueso *et al.*, 2014). Populations of detritivores may also be indirectly affected by N enrichment. Trombidiformes and Diptera were positively related to soil C content, which is related to available food resources in the form of soil organic matter (Cole *et al.*, 2005). Soil C content may be affected in the longer term by nutrient enrichment, particularly N, as this may stimulate microbial activity resulting in C loss (Mack *et al.*, 2004; Bragazza *et al.*, 2006).

Scores for QBS were similar to the amount of taxonomic orders represented (*T-richness*) and demonstrated that microarthropod biodiversity and the prevalence of traits across groups which indicate adaptation to life in soil increases with nutrient enrichment, particularly with the addition of P. Soil microarthropods respond to changes in land management (Stork & Eggleton, 1992) and to changes in nutrient status (Xu *et al.*, 2007; Ochoa-Hueso *et al.*, 2014). The QBS approach enables a rapid characterisation of edaphic populations (Parisi *et al.*, 2005) which this study indicates could be used to identify changes in soil nutrient status, particularly changes to P availability. However this approach should be applied cautiously, particularly with regard to interpretation, as higher scores for QBS such as those observed here with greater nutrient availability, typically relate to reduced pressure on land use (Gardi *et al*., 2003; Parisi *et al*., 2005) which for oligotrophic environments is potentially misleading.

Lastly, the limited duration of the study means long term influence of nutrient enrichment on the ecosystem processes recorded here is impossible to determine. Short term effects have been previously recorded for soil invertebrate populations (Cole *et al*., 2005) as these respond rapidly to changes in nutrient, and therefore resource, availability. In this context population increases are likely the result of greater belowground productivity from augmented soil fertility (Gastine *et al*., 2003), rather than through increases in plant litter input which would be a longer term effect. This potentially explains the limited effect of treatment and the large effect of block location, as existing differences in soil characteristics effectively govern soil fauna population sizes. In addition, data for the two years were summed to a single figure to avoid temporal pseudoreplication (Hurlbert 1984). Differences in soil fauna populations year on year are therefore impossible to discriminate in the current analysis, however inspection of the data confirmed similar patterns for both years, which was concluded to be the result of differences in treatment.

Livestock stocking rate was also constant across the experiment, however where nutrient enrichment has increased vegetation growth (and possibly other visual cues) attraction to herbivores may have increased localised grazing rates between treatment plots (Van der Wal *et al*., 2003). Therefore the level of background grazing at site could potentially have influenced soil faunal abundance as herbivory can affect soil organisms through changes to root exudates and C allocation and by changes to the relative nutrient content of vegetation tissues and therefore quality of plant litter input (Bardgett *et al*., 1998; Bardgett & Wardle 2003).

4.6 CONCLUSION

Vegetation growth and tissue nutrient concentrations were significantly influenced by the addition of P which indicates an ecosystem currently limited by P availability. Plant tissue concentrations of N and P were positively related, indicating that greater P availability could facilitate N uptake by plants and act to sequester greater N in plant biomass, reducing the potential for N leaching.

Upland soil faunal communities were influenced by increases in the availability of P through bottom-up processes, which in turn influenced the mineralisation rate and uptake of nutrients by vegetation through increased activity. The taxonomic group richness and the soil biological quality index (QBS) scores were also significantly increased by the addition of P, suggesting the amount of different groups supported and their level of adaptation to life in soil, could offer a method to discriminate between soils of varying nutrient status, which may offer a rapid and simple method to determine soil biological quality. Nitrogen addition had no direct effect on soil fauna communities, but was related to soil fauna groups via soil chemical characteristics. Nitrogen availability has been shown to positively influence soil faunal abundance but with an upper limit beyond which detrimental effects occur (Xu *et al.*, 2007). Further work is necessary to determine critical load levels of N enrichment for soil invertebrate fauna as these constitute a significant proportion of biodiversity and may better inform existing critical load levels prescribed for these upland habitats.

CHAPTER 5

NITROGEN AND PHOSPHORUS ENRICHMENT AND CARBON FLUXES OF THE UPLAND ECOSYSTEM

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TARGET JOURNAL: SCIENCE OF THE TOTAL ENVIRONMENT

5.1 ABSTRACT

Soils of high organic matter content in the upland ecosystem represent significant terrestrial reservoirs of carbon (C), which have the potential to be transformed from sink to source with current environmental change. Reactive nitrogen (N) deposition can affect ecosystem processes, particularly in the oligotrophic upland ecosystem, and is expected to impact soil C storage potential by increased microbial decomposition rate due to release from N limitation. Phosphorus (P) limitation may also constrain ecosystem productivity yet ecosystem responses to P availability in instances of high N deposition are poorly understood. This study investigated CO_2 and CH_4 flux responses to N and P enrichment using both short term (1 year) and long term (1996 - 2012) nutrient addition experiments. We hypothesised that the addition of N and P will increase CO_2 and CH_4 fluxes, with the largest response expected where P is added, since microbial activity is likely to increase with alleviation from nutrient limitation. The data presented here demonstrate the modification of C fluxes from N and P enrichment, with differing results subject to the duration of nutrient addition. On average, relative to control, N addition inhibited CO_2 flux in the short term (-9% / -33.93 mg C CO_2 m^{-2} h^{-1}) but considerably increased CO_2 emissions in the long term (+35% / 69.66 mg C CO_2 m^{-2} h^{-1} for NH_4), reduced CH_4 uptake in the short term (-90% / -9.63 µg C CH_4 m^{-2} h^{-1}) and reduced CH_4 emission in the long term (-94% / -25.65 µg C CH_4 m^{-2} h^{-1} for NH_4). Phosphorus addition increased CO_2 and CH_4 emission in the short term (+20% / 77.73 mg C CO_2 m^{-2} h^{-1} and +184% / 19.68 µg C CH_4 m^{-2} h^{-1} respectively), with diminishing effect into the long term, suggesting microbial communities at these sites are P limited. Whilst a full C exchange budget was not examined in the experiment, the potential for soil C storage loss with long term nutrient enrichment is

demonstrated. This highlighted the limitation of use of short term nutrient addition experiments in the estimation of trajectories of long term environmental change.

5.2 INTRODUCTION

Global climate change is expected to have profound impacts on natural systems which could threaten biodiversity and ecosystem processes (Walther *et al*., 2002; Thomas *et al*., 2004; Grim *et al*., 2013; Carroll *et al*., 2015). The contribution of carbon (C) to current global climate change is well documented (i.e. Cox *et al*., 2000; Friedlingstein *et al*., 2006). Increases in atmospheric concentrations of C in the form of two greenhouse gases (GHGs), carbon dioxide (CO_2) and methane (CH_4), is the principal driver of global climate change. This increase is predominantly caused by anthropogenic emissions from the use of fossil fuel and from land use and land use changes, in particular agriculture (IPCC, 2013), which can release C from long term storage in soils. Carbon has been accumulating in terrestrial reservoirs since the end of the last ice age, sequestered mainly as organic matter in soils with low rates of decomposition such as peat (Yu *et al*., 2010). Maintaining these C stores is highly important in order to avoid the transformation from current status of C sink to potential C source (House *et al*., 2010). The effect of climate change on soil C is controversial (Worrall *et al*., 2004; Davidson & Janssens, 2006; Worral & Burt, 2007; Clark *et al*., 2010), but as temperatures rise the rate of organic matter decomposition is expected to increase resulting in positive feedback (Knorr *et al*., 2005). This effect is also likely to be exacerbated by current land use practices including drainage, grazing and burning (Wallage *et al*., 2006; Ward *et al*., 2007 & 2013) and by the effects of nutrient enrichment from nitrogen (N) deposition (Bragazza *et al*., 2006).

Carbon stored in soil is lost to the atmosphere in the form of CO_2 from soil organic matter mineralisation (Dawson & Smith, 2007) and CH_4 from the anaerobic decomposition of organic matter by methanogenic microbes (Bubier & Moore, 1994; Cooper *et al.*, 2014). Emissions of CO_2 are considerably larger than CH_4 (current (2011) atmospheric content mass for CO_2 = 828 Pg C and CH_4 = 3.7 Pg C), but the global warming potential of CH_4 is 28 times greater than CO_2, making it an important GHG (IPCC, 2013). Rates of soil C flux are affected by numerous environmental factors and ecosystem processes. Soil moisture, temperature and pH have strong controlling effects on soil C flux by influencing soil microbial and plant root activity and the diffusion of gases through soil pores (Smith *et al.*, 2003; Chen *et al.*, 2015). Vegetation composition also affects rates of both CO_2 and CH_4 emission (Raich & Tufekciogul, 2000; Robroek *et al.*, 2015). For CH_4, the effect is direct via the transport of gas through the aerenchymatous tissue of some vascular plants (notably sedges) allowing gaseous exchange with the atmosphere (Joabsson *et al.*, 1999; McEwing *et al.*, 2015). For CO_2 it is indirect, through changes to decomposition resistance rates of vegetation species, rates of root respiration and through the influence on soil microclimate and structure (Raich & Tufekciogul, 2000). This has significant implications for soil C flux in the context of current environmental change (Berendse *et al.*, 2001; Ward *et al.*, 2013; Xu *et al.*, 2015) and potentially makes prediction of future scenarios for soil C flux highly complex, as all biotic and abiotic factors not only act directly, but also indirectly through the modification of vegetation composition by soil characteristics, and vice versa (McEwing *et al.*, 2015).

Nitrogen enrichment from pollutant deposition affects ecosystem processes (Magnani *et al.*, 2007; Jones & Power, 2012; Southron *et al.*, 2013) and can affect rates of C flux through changes to vegetation and microbial activity (Basiliko *et al*., 2006; Juutinen *et al*., 2010; Wu *et al*., 2015). The effect of N on soil CO_2 flux is controversial, with studies demonstrating an inhibitory (Phillips & Fahey, 2007; Janssens *et al*., 2010; Ramirez *et al*., 2010) or a stimulatory (Bragazza *et al*., 2006; Cleveland & Townsend, 2006; Zhang *et al*., 2013) effect. In oligotrophic environments with soils of high organic matter content, nutrient enrichment from N deposition is expected to impact soil C storage potential by increasing microbial driven decomposition rates when released from N limitation (Bragazza *et al*., 2006). It is also expected that the increase in N availability will shift ecosystems towards limitation of other nutrients such as phosphorus (P) (Crowley *et al*., 2012; Peñuelas *et al*., 2013). Understanding the role of nutrient availability on C flux and C storage potential is of considerable importance. The availability of reactive N in upland habitats composed of peaty soils has been increased by anthropogenic sources and is projected to double in size globally from current levels by 2050 (Galloway *et al.*, 2004; Phoenix *et al.*, 2006). The rate of reactive N input is reportedly in decline in Western Europe (Fowler *et al.*, 2004), however current rates remain higher than the estimated critical load for many upland habitats (RoTAP 2012).

To date, research in this area has focussed on the environmental factors which can promote C flux such as vegetation species, temperature and soil moisture. Few studies have considered the effect of increasing N availability on C flux within upland habitats exposed to N deposition rates near or above the critical load limit, with none considering the impact of P limitation in this context. This study aimed to establish the effect of N and P enrichment on soil C flux by measuring CO_2 and CH_4 emissions across two randomised block experiments, which have been run in both the short (one year) and longer term (1996 - 2012, with sampling undertaken three years after ceasing N inputs and 15 years after a single P application). We hypothesised that the addition of nutrients (N or P) will increase CO_2 and CH_4 flux at these upland sites, and that this response will be greatest in treatments where P is added, as microbial activity is expected to increase with alleviation from nutrient limitation.

5.3 METHODS

5.3.1 EXPERIMENTAL DESIGN

This study was conducted at two sites: Pen y Garn (PEN - 52^0 37’ N, 3^0 76’ W) and Pwllpeiran (PWL - 52^0 37’ N, 3^0 77’ W) in the Cambrian Mountain range mid-Wales. The sites are located within 1 km of one another and within an altitude range of 500 - 600 metres a.s.l., on a transition between NVC U4 *Festuca ovina Agrostis capillaris* grassland and H18 *Vaccinium myrtillus Deschampsia flexuosa* heath overlying mixed soils ranging from shallow ferric stagnopodzol to deep peat (Emmett *et al.*, 2007; Phoenix *et al.*, 2012). The mean annual rainfall rate for this location was 1512.2mm (UK Meteorological Office, no date) and the background N deposition rate was 12.6 kg N ha^{-1} yr^{-1} (APIS, 2014). At both locations a nutrient addition experiment was established, consisting of six replicate blocks of four, 3x3 m plots, in a fully randomised design. At PWL (Figure 5.1a) N was added every two weeks between the months of April – October 2014. P was added once in April 2014. At PEN (Figure 5.1b) N was added fortnightly between 1996 and 2012. P was added once in 2000 to the ammonium sulphate 10 kg N ha^{-1} yr^{-1} treatment (AS10) plots. Sheep grazing was active at PWL during this study, at a rate of 1.0 sheep/ha. Sheep grazing was not active at PEN, during this study, but the site is historically grazed; sheep were present between 1990 and 2007 at two levels (1.0 sheep/ha and 1.5. sheep/ha), with three replicate blocks in each paddock.

a) **CONT**	**N** NH_4NO_3	**P** NaH_2PO_4	**N + P** NH_4NO_3 + NaH_2PO_4

b) **CONT**	**AS10** $(NH_4)_2SO_4$ + NaH_2PO_4	**AS20** $(NH_4)_2SO_4$	**SN20** $NaNO_3$

Figure 5.1. Experimental treatments: **a) PWL** treatments: **CONT** = control (no addition); **N** = ammonium nitrate at 60kg ha^{-1} yr^{-1}; **P** = phosphorus (sodium dihydrogen orthophosphate) at 40kg ha^{-1} yr^{-1}; **N + P** = ammonium nitrate at 60kg ha^{-1} yr^{-1} + sodium dihydrogen orthophosphate at 40kg ha^{-1} yr^{-1}. **b) PEN** treatments: **CONT** = control (no addition); **AS10** = ammonium sulphate at 10kg ha^{-1} yr^{-1} + phosphorus (sodium dihydrogen orthophosphate) at 20kg ha^{-1} yr^{-1}; **AS20** = ammonium sulphate at 20kg ha^{-1} yr^{-1}; **SN20** = sodium nitrate at 20kg ha^{-1} yr^{-1}.

5.3.2 VEGETATION ANALYSIS

Vegetation data were collected for PWL in June 2014 and June 2015 and for PEN in June 2015. Vegetation coverages were recorded visually within each 3x3 m plot using the Domin scale, subsequently transformed to percentage cover for statistical analysis (Currall, 1987). All plants and bryophytes were identified to species level. Vegetation height is the average of five measurements taken using a metric sward stick, of ca. 1 cm diameter, marked at 0.5-cm intervals (Dennis *et al.*, 2005). At PEN heights were taken from the centre of each plot and then from the mid-point between the centre and each corner and at PWL heights were recorded from within grazing exclosure (~1 m^{-2}).

5.3.3 SOIL ANALYSIS

Soil samples for both sites were collected in June 2015 from five locations within each treatment plot, using a 20mm diameter soil corer up to a depth of 20cm, and bulked together. The samples were air dried and passed through a 2 mm sieve. Soil pH was measured with a Hydrus 400 meter (Fisherbrand, Leicestershire, UK) in a slurry of 10 g fresh soil in 25 mL water. Total C was established by the Dumas combustion method using an elemental analyser (Vario MAX Cube – Elementar Analysensysteme, Hanau, Germany).

5.3.4 SOIL FLUX

CO_2 and CH_4 flux was measured for the different nutrient addition treatments at both PWL and PEN from September 2014 to August 2015; initially every two months until March 2015, then every month during the growing season (May to August 2015), under dark conditions using a non-steady state, static chamber approach (Livingston & Hutchinson, 1995; Parkin & Venterea, 2010) and represents both soil and plant respiration. Soil collars, 25cm in diameter, were installed on each treatment in July 2014, two months prior to the first gas flux measurements to reduce disturbance. A soil knife was used to cut through the initial vegetation and topsoil layer, before the collar was inserted to a depth of 7cm, leaving 5cm aboveground for chamber attachment. The location of the collar was selected randomly for each treatment square and once installed, vegetation was trimmed from inside the collar and maintained trimmed throughout sampling. Chamber design followed the description by Parkin & Venterea, (2010). Each chamber measured 14cm in height (with the addition to the 5cm aboveground soil collar: 19cm in total) with an internal volume of 9.33 l. A modified pressure vent tube (Xu *et al.*, 2006) was also installed to allow for internal and ambient air pressure equilibration. Before each measurement, chambers were carefully placed on collars, ensuring a gas tight seal. The soil CO_2 and CH_4 fluxes were calculated on the basis of a linear increase in chamber concentrations over 30 minutes. Measurements were taken for each chamber at 0, 15 and 30 minutes from chamber attachment (Parkin & Venterea, 2010). The duration of measurement at each sampling point was 60 seconds. CO_2 and CH_4 concentrations were measured using an LGR™, Ultra- Portable Greenhouse Gas Analyser (Model 915–0011, Los Gatos Research, Palo Alto, CA, USA) with a 1 Hz sampling rate. Chambers were attached to the analyser via inlet and outlet tubing (2m by 4mm internal diameter). Chamber internal temperature, ambient air temperature and soil temperature at a depth of 10cm were also recorded. The rate of change in gas concentration inside the chambers was established with linear regression. Gas flux (CO_2 and CH_4) was then calculated from the rate of gas concentration change using the following equation (McEwing *et al*., 2015):

$$F_0 = S\,\frac{V\,M\,273.16}{A\,Vm(273.16 + T)}60$$

Where:

F_0 = Flux (µg C CH_4/CO_2 m^{-2} hr^{-1})

S = Rate of change in CH_4 and CO_2 concentration (ppm min^{-1})

V = Chamber volume (m^3)

A = Chamber area (m^2)

M = Molecular mass of CH_4/CO_2 (g mol^{-1})

V_m = Ideal gas mole volume (0.0224 m^3 mol^{-1})

Each regression plot was individually assessed using R^2 as an indicator of accuracy, with R^2 values >0.7 accepted for analysis. However low fluxes for CH_4 (particularly those close to zero), generally have a low R^2, and should be included to avoid an over-estimate of mean fluxes (Alm *et al*. 2007). Therefore CH_4 fluxes with R^2 <0.7 were retained as a zero measurement provided that the measurement range did not exceed 0.3ppm. In addition, two collars at PWL suffered from excessive water pooling, which visibly affected the vegetation and soil. These were removed from dataset before analysis.

5.3.5 STATISTICAL ANALYSIS

All variables were tested for normality and homoscedasticity prior to statistical analysis. Data for CO_2 and CH_4 fluxes were Log (x+1) transformed. Differences in CO_2 and CH_4 flux between treatments were analysed with linear mixed models (LMMs) specifying repeated measures, with treatment as a fixed factor and air temperature as covariate to allow for diurnal/seasonal variance. When significant differences between treatments were detected ($P < 0.05$), post-hoc tests were conducted using LSD pairwise comparisons. The relationship between GHG flux and air temperature was initially tested for significance with simple linear regression before inclusion in the model. Air temperature was used for model analysis as data for soil temperature was only captured from January onwards, but the two were shown to have significant correlation (PWL: $R^2 = 0.52$, $P = <0.001$; PEN: $R^2 = 0.81$, $P = <0.001$). Differences in mean annual soil emission of CO_2 and CH_4 between experimental treatments were also investigated with one-way analysis of variance (ANOVAs). LSD pairwise comparisons were used to further investigate individual relationships. Multiple regression analysis with all-possible-subsets model procedure was used to investigate the relationships between GHG fluxes (modelled as the annual average GHG flux for each treatment plot) and associated environmental parameters (soil chemistry, vegetation structure and functional type coverages). All statistical analyses were conducted with SPSS 21.0 (IBM SPSS Statistics for Windows, 2012).

5.4 RESULTS

5.4.1 ENVIRONMENTAL CONTROLS ON CO_2 AND CH_4 FLUX

Over the sampling period sizeable fluctuations were observed for air and soil temperatures in line with seasonal variation (Figure 5.2). The highest air temperatures were recorded in June for PWL (22.33^{o}C) and in August for PEN (19.42^{o}C). Air temperatures were lowest at both locations in January (PWL = 4.99^{o}c, PEN = 6.22^{o}C). Soil temperatures were similar with the highest values recorded in August for PWL (13.17^{o}C) and in July for PEN (12.85^{o}C) and lowest vales for both in January (PWL = 4.62^{o}C, PEN = 3.46^{o}C). Over the twelve months of sampling, the

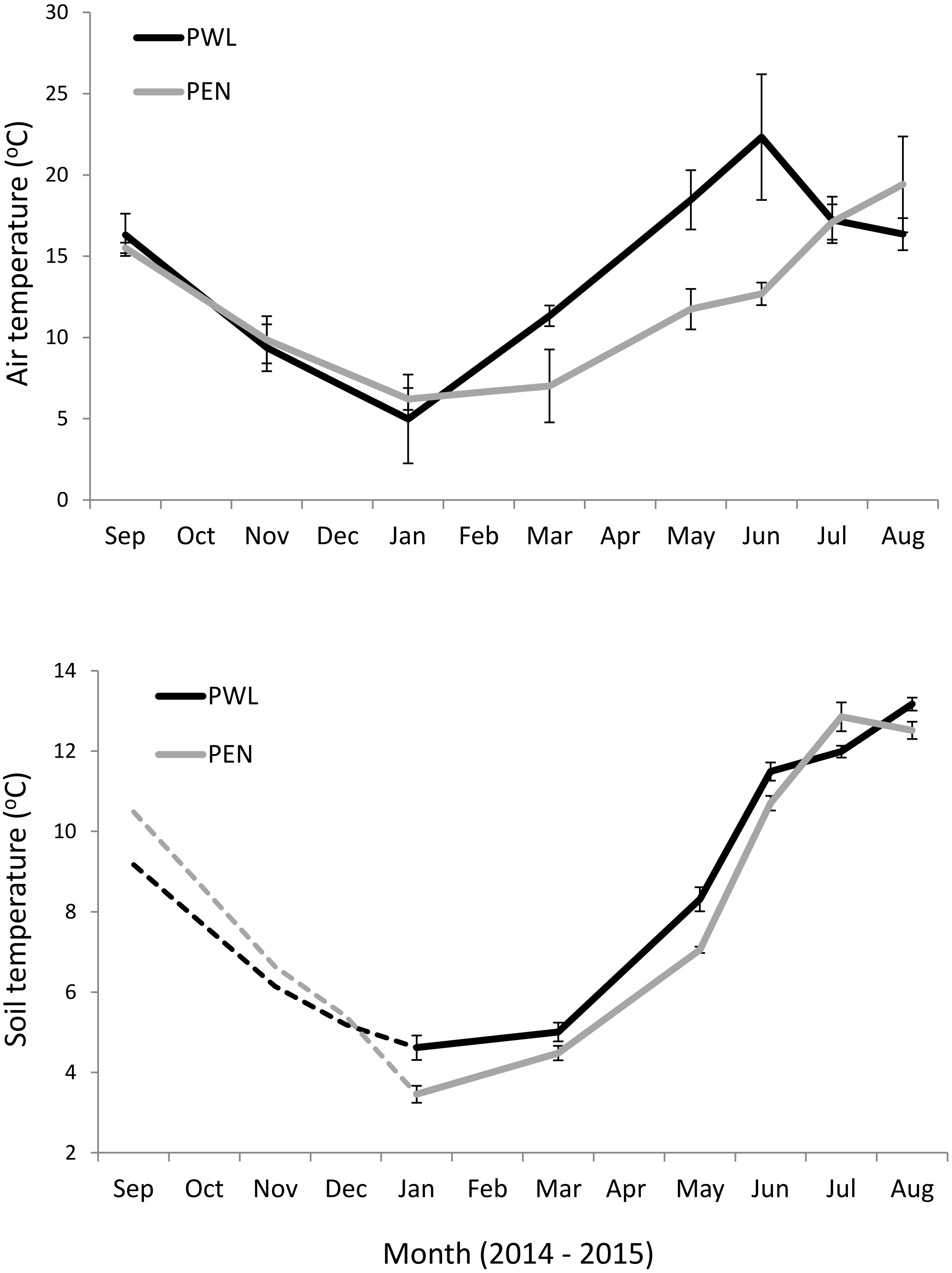


Figure 5.2. Air and soil temperatures across duration of analysis. Soil temperatures are backwards forecast from January (dashed lines) following linear model prediction (PWL: $R^2 = 0.52$; PEN: $R^2 = 0.83$).

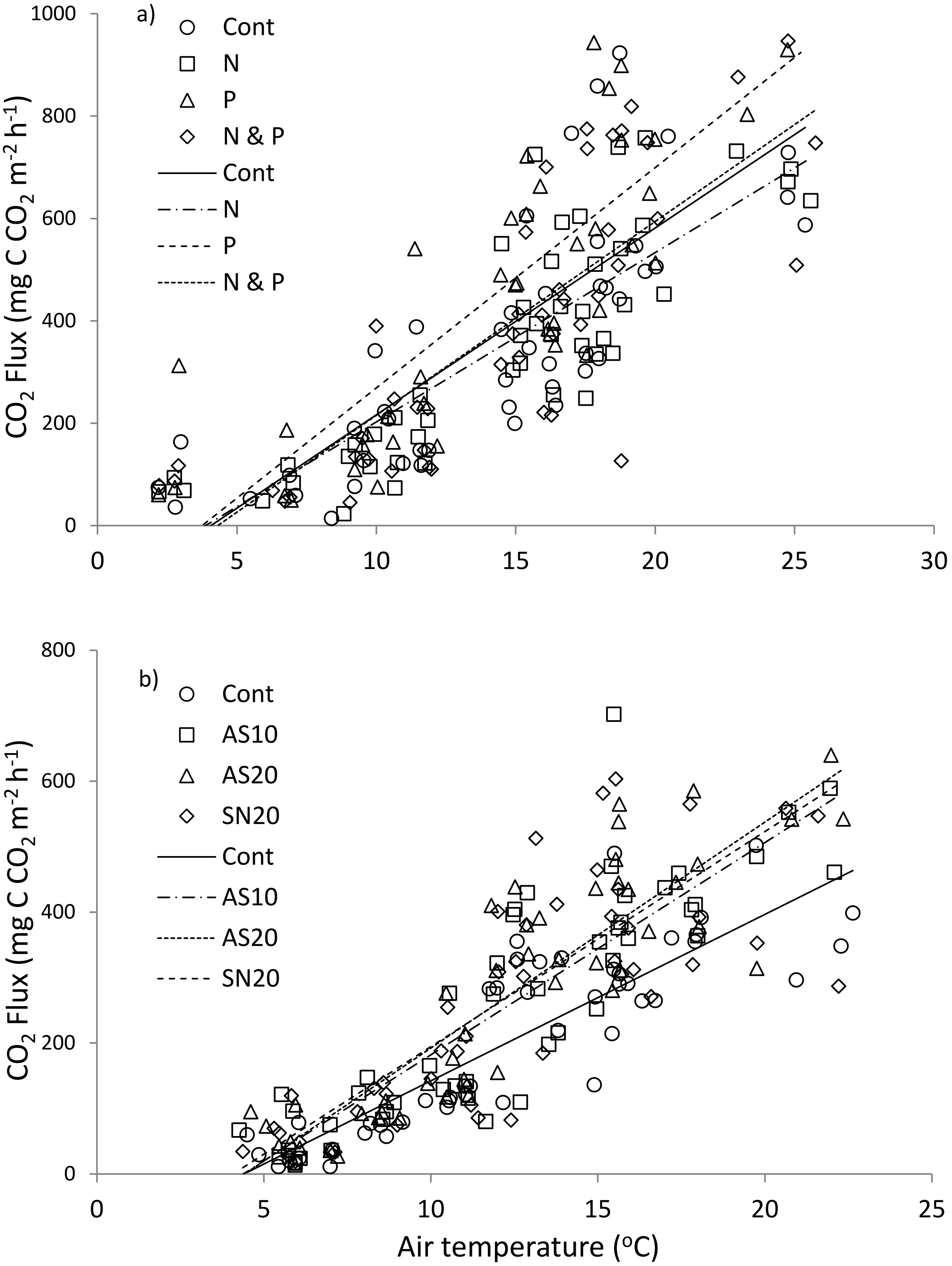


Figure 5.3. The relationship between CO_2 flux and air temperature for a) PWL and for b) PEN.

Table 5.1. Relationships between gas flux and environmental variables with potential influence on C emission.

Site	Variable	Regression model	Degrees of freedom	R^2	*P*
PWL	**CO_2**	**pH**	**1,21**	**0.52**	**<0.001**
	CH_4	**pH**	**1,21**	**0.25**	**0.019**
PEN	CO_2	-	1,23	-	ns
	CH_4	-	1,23	-	ns

P-values refer to a test of the coefficient being 0; bold highlights results significant at *P* <0.05. The best fitting regression model using all-possible-subsets model procedure regression analysis is shown for each parameter. Variables included in analysis were: plant functional type coverage (graminoid, cryptogam, dwarf shrub, forbs), vegetation height and soil pH.

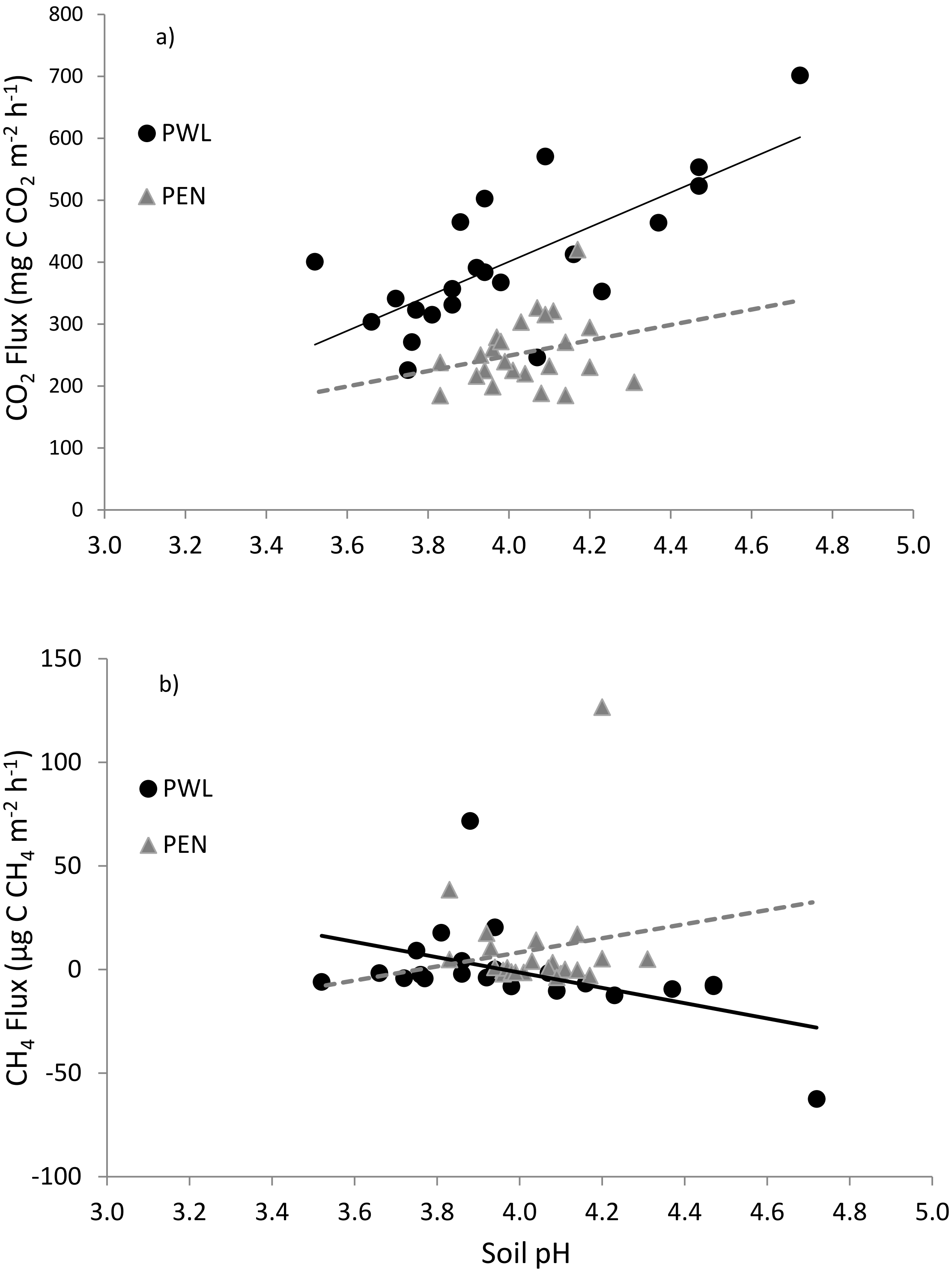
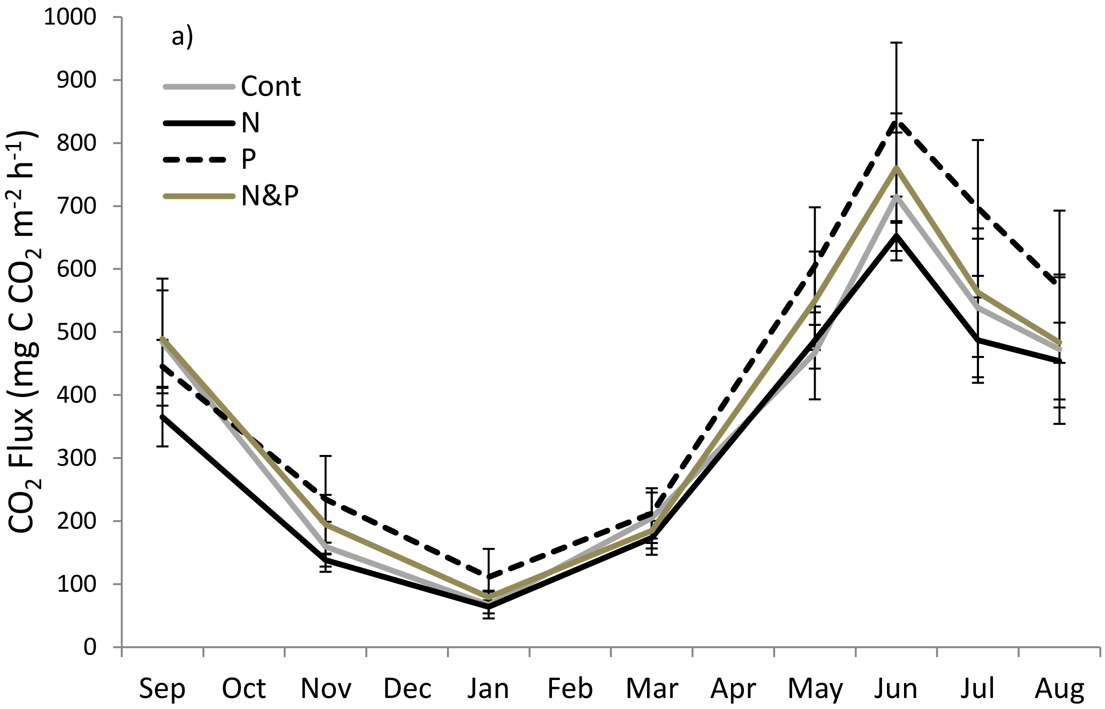


Figure 5.4. The relationship between soil pH and a) CO$_2$ flux and b) CH$_4$ flux. Solid line indicates significant relationship, dashed line non-significant.

effect of changing air temperature on CO_2 flux was significant for both sites, with positive relationships observed between increasing temperature and CO_2 emission (PWL: $R^2 = 0.68$, $P = <0.001$; PEN: $R^2 = 0.70$, $P = <0.001$, Figure 5.3). The effect of temperature on CH_4 flux was less consistent; no relationship was observed at PWL ($R^2 = 0.004$, $P = 0.37$), but a significant positive relationship was recorded at PEN ($R^2 = 0.033$, $P = 0.012$). The effect of plant functional type coverage (graminoids, cryptogams, dwarf shrubs and forbs), vegetation height and soil pH were also tested against CO_2 and CH_4 fluxes to determine additional environmental influence (Table 5.1). Multiple regression analysis revealed significant relationships between soil pH and fluxes for both CO_2 and CH_4 at PWL (Table 5.1, Figure 5.4), but other factors were shown to have no effect. At PEN no environmental factor was shown to have an effect on either CO_2 or CH_4 flux.

5.4.2 CO_2 FLUXES BETWEEN NUTRIENT ADDITION TREATMENTS

Differences were observed in the CO_2 flux between nutrient addition treatments at both sites, but with differences in the rate of response observed between sites. For PWL, the between treatment differences (Table 5.2, Figure 5.5a) were shown by post hoc analysis to be driven by the largest mean annual flux recorded for the P addition treatment (464.20 mg C CO_2 m^{-2} h^{-1}). The P treatment had consistently higher CO_2 flux in all but the first month sampled (September). The N addition treatment had the lowest mean annual flux (352.53 mg C CO_2 m^{-2} h^{-1}) and was consistently the lowest flux recorded across the sampling period, in all months except May. CO_2 fluxes in the control (no addition) and N + P treatments had similar mean annual fluxes (386.47 and 412.97 mg C CO_2 m^{-2} h^{-1} respectively) but were variable relative to one another over the sampling period. For PEN, post hoc analysis revealed the significant



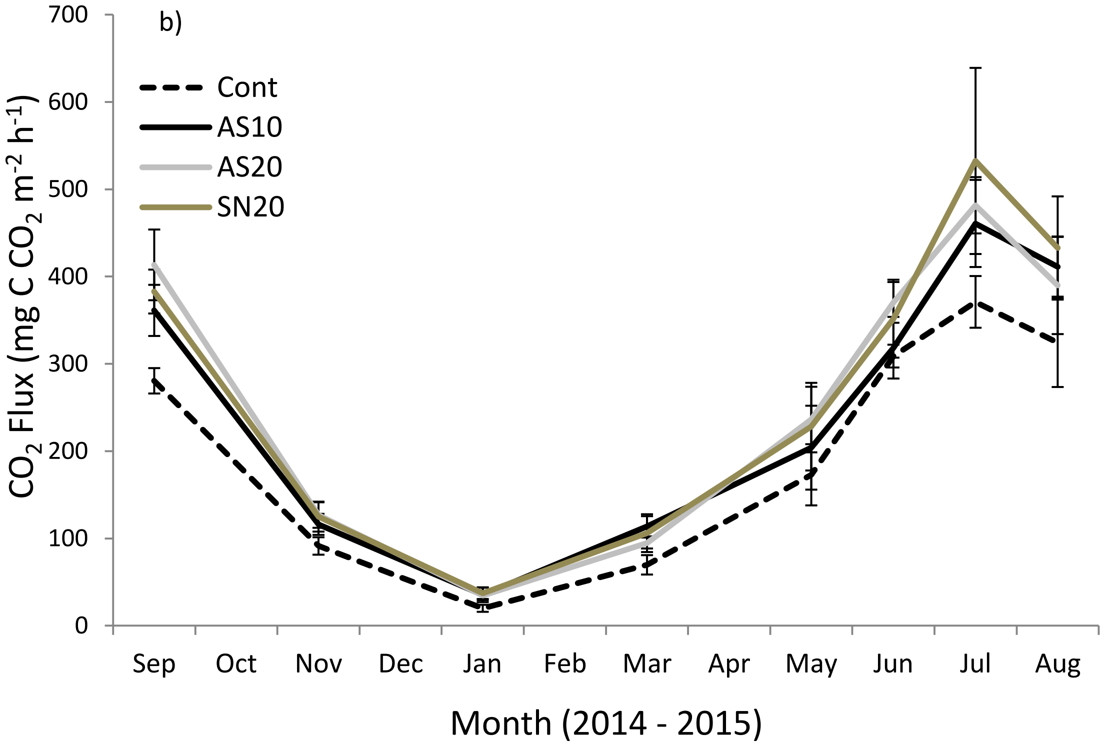
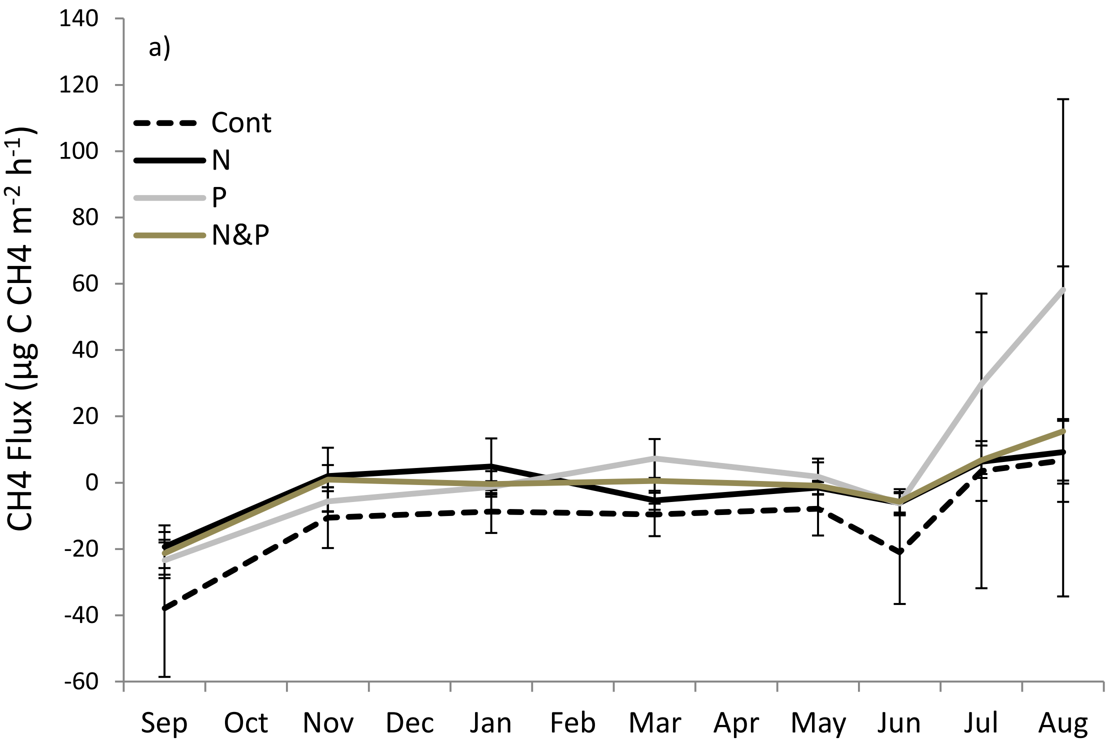


Figure 5.5. CO$_2$ fluxes for a) PWL and b) PEN. Error bars denote standard error. Dashed line indicates treatment with significant difference as determined by post hoc pairwise comparison (LSD).



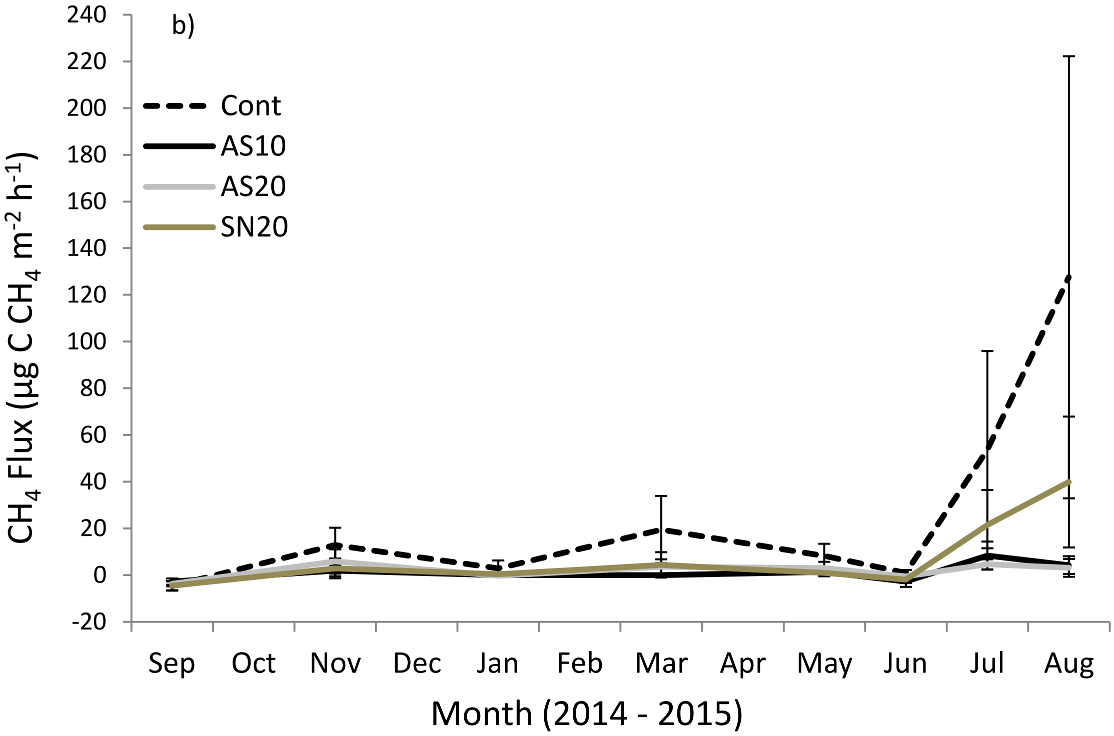


Figure 5.6. Methane flux for a) PWL and b) PEN. Error bars denote standard error. Dashed line indicates treatment with significant difference as determined by post hoc pairwise comparison (LSD).

Table 5.2. Summary of results from LMMs describing soil CO_2 and CH_4 flux responses to nutrient addition treatments, with treatment as fixed factor and air temperature as covariate.

Site	Variable	Factor	Degrees of freedom	*F*	*P*
PWL	CO_2	**Treatment**	3,171	**3.69**	**0.013**
		Air temperature	1,171	**422.32**	**<0.001**
	CH_4	**Treatment**	3,171	**2.86**	**0.041**
		Air temperature	1,171	0.60	0.439
PEN	CO_2	**Treatment**	3,187	**4.63**	**0.004**
		Air temperature	1,187	**461.23**	**<0.001**
	CH_4	**Treatment**	3,187	**4.22**	**0.006**
		Air temperature	1,187	**6.75**	**0.01**

P-values refer to a test of the coefficient being 0, bold highlights results significant at P <0.05.

differences between treatments (Table 5.2, Figure 5.5b) to be driven by the lowest mean annual flux recorded, which was the control (no addition) (204.78 mg C CO_2 m^{-2} h^{-1}). AS10, AS20 and SN20 had highly similar mean annual fluxes (261.17, 276.54 and 274.44 mg C CO_2 m^{-2} h^{-1} respectively), but showed a high degree of variability relative to one another over the sampling period. The differences observed between treatments at PEN suggest that nutrient enrichment in the long term significantly increases CO_2 flux relative to control. However, the limited difference between nutrient addition treatments potentially indicates a limited influence of P on CO_2 flux (added to the AS10 treatment) 15 years after application. Overall, the difference in response between the two experimental durations at PWL and PEN shows significant variability in CO_2 flux response subject to duration of nutrient enrichment.

5.4.3 CH_4 FLUXES BETWEEN NUTRIENT ADDITION TREATMENTS

Considerable variation was observed between sites for CH_4 flux. At PWL there were significant differences between treatments (Table 5.2, Figure 5.6a), independent of seasonal variations in temperature (Table 5.2), which post hoc tests revealed to be driven by the difference in CH_4 emissions between the control (no addition) and nutrient addition treatments (N, P and N + P). CH_4 uptake was greater in control plots, with this treatment behaving as a small sink (on average -10.68 µg C CH_4 m^{-2} h^{-1}), only emitting CH_4 during the last two months (June and August), at rates just above zero µg C CH_4 m^{-2} h^{-1}. The N, P and N + P treatments were highly variable in emission rate, with fluctuations recorded across the sampling range. The P addition treatment had the largest mean annual flux (9.00 µg C CH_4 m^{-2} h^{-1}), which can chiefly be attributed to an increase in emission rate during the last two months. N and N + P both remained near zero across the sampling range, with slightly negative

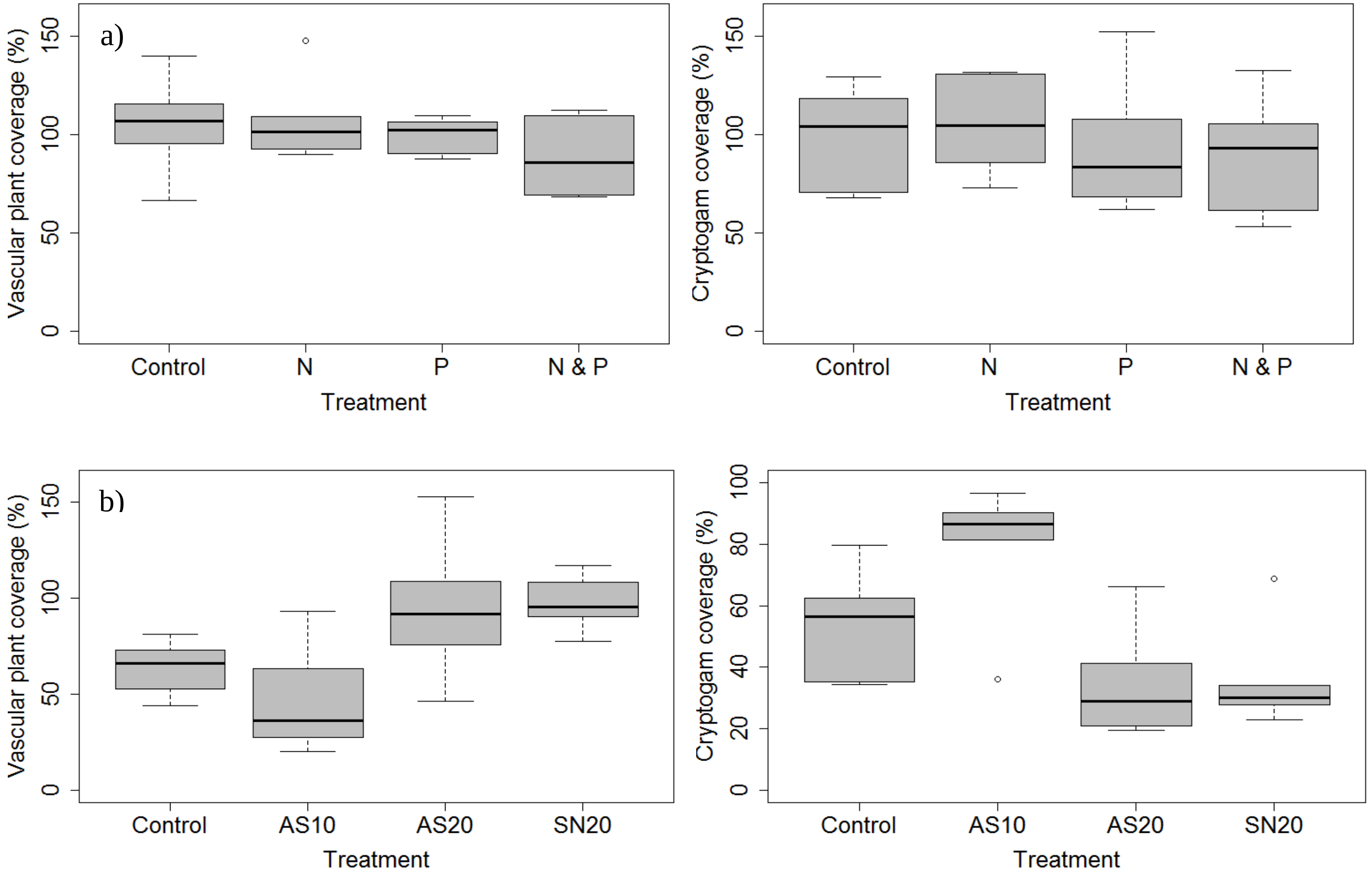


Figure 5.7. Vascular plant and cryptogam coverages recorded for nutrient addition treatments at a) PWL and b) PEN.

fluxes recorded for both N (-1.05 µg C CH_4 m^{-2} h^{-1}) and N + P (-0.77 µg C CH_4 m^{-2} h^{-1}) on a mean annual basis. At PEN there were also significant differences observed between treatments (Table 5.2, Figure 5.6b), which post hoc analysis revealed to be likewise driven by the difference between control and all nutrient addition treatments. However, at this site the CH_4 flux was significantly higher in control plots than nutrient added treatments, with mean annual emissions of 27.64 µg C CH_4 m^{-2} h^{-1}. All of the nutrient addition treatments at PEN otherwise had similar flux rates for the full sampling range, until the last two months, when the sodium nitrate treatment (SN20) increased markedly (Figure 5.6b).

5.5 DISCUSSION

5.5.1 NUTRIENT ADDITION EFFECTS ON CO_2 FLUXES

The addition of N and P influenced fluxes for CO_2, however there were substantial differences in effect between treatments and also differences in response between short term (PWL = 1 year for N + P) and long term nutrient additions (PEN = additions between 1996 – 2012, sampling undertaken three years after the cessation of N addition and 15 years after a single P application). At PWL, the addition of P increased CO_2 flux which supported the hypothesis and is in line with findings from similar studies in other environments (Cleveland & Townsend, 2006; Liu *et al.*, 2013). Phosphorus limitation is a significant mechanism constraining ecosystem processes, particularly in systems suffering from the effects of chronic N deposition (Cleveland *et al.*, 2011; Crowley *et al.*, 2012). The addition of P stimulates decomposition with observed rises in CO_2 flux associated with increases in heterotrophic (bacterial and fungi) biomass and activity and thus respiration (Liu *et al.*, 2012), rather than through increased fine-root biomass (Cleveland & Townsend, 2006). Phosphorus addition results in the modification of microbial community structure and reduces the ratio between Gram-positive and Gram-negative bacteria resulting in more copiotrophic communities (Fanin *et al.*, 2015). At PEN, the role of P was less clear. The CO_2 flux in the N + P treatment (AS10) was larger than control (no addition), however was mostly lower across the sampling period (bar January and February) than the two high N addition treatments (AS20 and SN20). This response is therefore likely driven by augmented N availability (at a lower application rate) rather than release from P limitation. This was contrary to our hypothesis as P was anticipated to have a strong effect even after 15 years, as P is known to be highly persistent in soil (Nye & Tinker, 1977) and was responsible for driving shifts observed in vegetation species composition (Figure 5.7). Liu *et al.*, (2013) observed a diminishing effect of P on microbial biomass after four years which was attributed to C limitation where the exhaustion of available soil C had a constraining effect on productivity (Fanin *et al.*, 2015). Soil C was lower in the N + P treatment at PEN (see Chapter 3, Figure 3.6), however this was not significant when compared to control. The shifts in bacterial composition to more copiotrophic communities as suggested by Fanin *et al.*, (2015) may also potentially facilitate this effect as time elapsed since P application at PEN is potentially sufficient to allow the predicted bacterial community modification, which would be expected to expedite C loss.

The addition of N had an inhibitory effect on soil CO_2 flux at PWL, which is consistent with other studies (Ramirez *et al.*, 2010; Chen *et al.*, 2015), but refuted the hypothesis. In soils where N is not a limiting factor for microbial growth and activity the addition of N can constrain organic matter decomposition (Janssens *et al.*, 2010). The background N deposition for these sites is 12.6 kg N ha^{-1} yr^{-1}. Critical loads for this habitat are between 10 - 15 kg N ha^{-1} yr^{-1} (APIS, 2014) which would suggest these soils are unlikely to be N limited. In such instances, reductions in CO_2 flux are driven by shifts in C allocation from belowground to aboveground biomass (Litton *et al*., 2007), which reduces rhizosphere and microbial respiration (Phillips & Fahey, 2007; Bae *et al*., 2015). In addition, associated reductions in soil pH also have a potential limiting effect (Chen *et al.,* 2015). This contrasts with the effects observed at PEN where N addition treatments had higher CO_2 flux in comparison to control (no addition) treatments, which does support the hypothesis. PEN was sampled three years post N addition cessation which could potentially indicate that any inhibitory effect is reduced with N losses from leaching in the intervening three years. This would seem unlikely however, as the size and activity of microbial populations exposed to N enrichment can remain elevated 6–8 years after addition cessation, suggesting a prolonged effect on the rate of nutrient cycling (Power *et al*., 2006). In this case, N availability is likely to increase respiration through greater soil organic matter decomposition from increased microbial abundance and activity (Mack *et al*., 2004; Bragazza *et al*., 2006) and by reduced production of more decomposition resistant species (Bragazza *et al*., 2012). This influence was not translated to a reduction in soil C levels in N treatment plots however, as these had the highest soil C content (see Chapter 3, Figure 3.6) albeit these were not significantly greater than control, suggesting a potential balance between increased rates of plant production and decomposition with greater N availability and thus productivity (Mack *et al.,* 2004). The impact of declines in fine root biomass from a shift in C allocation is also potentially offset by augmented concentrations of root tissue N in instances of increased N availability and can result in higher respiration per unit mass of root (Burton *et al*., 2002), which may also account for some of the difference.

The variability observed in CO_2 flux between sites with N enrichment could be explained by differences in the addition rate. PWL received a single year dose of N (60 kg ha^{-1} yr^{-1}) three times the size of the maximum annual application rate at PEN (20 kg ha^{-1} yr^{-1}). Janssens *et al.*, (2010) demonstrated that the inhibitory effect of N on soil CO_2 flux was larger in sites receiving N at dose rates greater than 50 kg ha^{-1} yr^{-1} which may account for some of the between-site disparity. The inhibitory effect of N was also observed at PWL to offset the stimulatory effect of P. The CO_2 flux response for the N + P treatment at PWL was intermediate in response between the P addition treatment and N addition treatment and had a similar flux rate to control (no addition) treatments, which was different to expectation. This suggests an equalising effect of N and P when added together in this situation.

5.5.2 NUTRIENT ADDITION EFFECTS ON CH_4 FLUXES

The addition of N and P altered CH_4 flux between treatments and also between short term (PWL) and long term (PEN) enrichment. CH_4 flux results are the net emissions of CH_4 recorded, therefore the results of this study are unable to partition effects between CH_4 production or oxidation or both. At PWL all nutrient addition treatments had lower rates of CH_4 uptake than the control, and supported our hypothesis. The reductions in uptake with N addition were attributed to reductions in CH_4 oxidation rate (Aerts & Toet, 1997; Aerts & Caluwe, 1999). This is primarily caused by competition for the CH_4 mono-oxygenase enzyme, which is affected when N addition increases rates of nitrification inhibiting CH_4 oxidisation rate and reducing the amount of methane consumed by methanotrophic bacteria (Bodelier, 2011). Phosphorus addition also increased the rate of emission for CH_4 which supports our hypothesis but is contrary to similar research conducted in other environments (Zhang *et al.*, 2011; Song *et al.*, 2012). These studies attributed the effects observed to the inhibition of methanogenesis, the stimulation of methanotrophic potential, and the elevation of plant root growth which increased water uptake reducing soil water content and increasing oxidation through aeration. This last effect would seem unlikely to operate in the PWL and PEN experiments because the vegetation community would not be expected to alter soil water availability significantly between treatments, even with the potentially augmented growth associated with P enrichment. The stimulation of methanogenesis is likely to be the principal mechanism and has been previously observed for some peat types (Keller *et al.*, 2006), which suggests the methanogen community at PWL is P Limited. Further tests would be necessary however to rule out any inhibitory effect of P on methane oxidation, but this is beyond the scope of this current study. At PEN the highest flux recorded was in the control and was larger than CH_4 fluxes in the nutrient addition treatments which was contrary to the hypothesis. The nutrient addition treatments remained near zero for the majority of the sampling period until the last two months (July and August), when the sodium nitrate treatment (SN20) increased substantially. Nitrate inhibits CH_4 emission but only in very high concentrations (Bodelier & Laanbroek, 2004). The negative effects of N are more severe with ammonia addition (Crill *et al.*, 1994). Thus here the ammonia treatments (AS10 and AS20) may have a stronger inhibitory effect explaining the ultimate spike in emission for the nitrate treatment. Overall, N addition inhibited CH_4 emission which is attributed to N induced increases in population size and activity of methane-oxidising bacteria (Bodelier & Laanbroek, 2004). The addition of P (AS10) had no obvious effect at PEN 15 years post application. As with the CO_2 flux results this was unexpected. Whilst the exact mechanisms remain unclear, the composition of the microbial community may change over the intervening years (as with the diminishing effect of P over time on CO_2 emission, discussed above) which may reduce the size of any initial effect. This may also account for the substantial differences observed in the results of the two experiments.

5.5.3 ENVIRONMENTAL CONTROLS ON CARBON FLUXES

Temperature influenced the rate of CO_2 flux at both PWL and PEN which is consistent with other studies (Dorrepaal *et al*., 2009; Briones *et al*., 2010; Imer *et al.*, 2013). Higher temperatures allow increased rates of soil decomposition and root respiration which are temperature dependent chemical and biochemical reactions (Knorr *et al*., 2005; Davidson & Janssens, 2006). The role of temperature in controlling CH_4 emission is less definite and was variable between sites. At PEN there was a positive effect of temperature on CH_4 flux, whereas at PWL no effect was observed. The effect of temperature on CH_4 production is reported to be variable and driven by the influence of site specific factors such as differences in soil type (Chin *et al*., 1999; Van Winden *et al*., 2012). The effect of temperature is likely to be closely linked with that of soil moisture and aeration (Smith *et al*., 2003). If high temperatures relate to drought and therefore dry soil, it would be expected that CH_4 emission would be reduced but CO_2 emission would increase (Sundh *et al*., 2000). The influence of vegetation species coverage and above ground biomass quantity was not related to C flux which is contrary to previous studies (McNamara *et al*., 2008; Green & Baird, 2012; Cooper *et al*., 2014; McEwing *et al*., 2015). This was also contrary to expectation, as differences in vegetation composition exist between treatments, particularly at PEN, where long term nutrient additions have resulted in divergent vegetation composition (Figure 5.7). Nitrogen addition (AS20 and SN20 treatments) has resulted in increases in graminoid species coverage, whereas N + P addition (AS10) has driven increases in cryptogam species coverage. Vegetation impacts on C flux are particularly associated with *Eriophorum* spp. presence and coverage (Green & Baird, 2012), as these sedges have aerenchymatous tissue which can facilitate the transport of CH_4 from the anaerobic zone to the atmosphere, and actively produces substrates which encourage methanogenesis (Cooper *et al*., 2014). The relative scarcity of these species at either site could potentially account for the limited effect of vegetation coverage on CH_4 flux between treatments.

The positive relationship for CO_2, and negative relationship for CH_4, observed between fluxes and soil pH at PWL, can be explained by changes in microbial activity. Soil acidity may be increased by N enrichment (Bobbink *et al*., 2010; Phoenix *et al*., 2012), an effect which is reported to have a stronger controlling effect on C flux than stimulation from N availability (Chen *et al*., 2015). Soil pH can affect the activity rate and composition of microbial communities, with decreases of 1.5 pH units shown to reduce activity by 50% (Fernández-Calviño & Bååth, 2010). Recruitment of bacterial species more tolerant of lower pH ultimately modifies the community, but with an intervening lag in activity (Rousk *et al.*, 2010). This hysteresis may explain the observed reduction in the CO_2 emission. Low soil pH may affect CH_4 flux inversely through inhibition of methanotrophic bacterial communities (Dedysh, 2002) with consequent reductions in oxidation rate, resulting in increases in CH_4 flux. These effects potentially explain some of the difference observed between PWL and PEN, where initial changes to pH driven by nutrient addition result in short-term fluctuations in microbial activity; an effect which would be expected to reduce in time as the system shifts to a new equilibrium, such as at PEN. The absence of any association with general environmental variables at PEN indicates that, for this study at least, the availability of nutrients over an extended period (even three years post applications for N and 15 years post application for P) has a greater influence on C flux rate than other background environmental factors. This conclusion should be treated with some caution however, as the environmental controls considered are likely to be highly site and ecosystem specific.

Lastly, as a cautionary note on experimental design; despite the differences in nutrient input rate between short and long term experiments, these were considered as comparable. The larger addition rate was applied to the short term experiment to maximise potential for response over a limited duration. Whether this would lead to differences in the long term is unclear, however this would only be expected to influence the size of the responses observed, which should therefore be interpreted accordingly. Furthermore, net ecosystem exchange was not considered as part of this study, so whilst the results demonstrate differences in C flux, primary productivity was not measured therefore the full influence of N and P availability on C budget is impossible to determine.

5.6 CONCLUSION

The addition of N and P had a significant effect on the rate of CO_2 and CH_4 fluxes but varied dependent upon the duration of the nutrient addition. Nitrogen addition inhibited CO_2 flux in the short term, but in the long term considerably increased emissions and could be expected, in conjunction with other environmental controls, to modify upland C storage potential which overall supports the hypothesis. The long term effect of P is less clear. In the short term P stimulates CO_2 flux via release from nutrient limitation, but this effect diminishes with time as the soil C stock is depleted. Further work would be required to completely determine this effect, but this also indicates a negative association with soil C storage. The emission rate of CH_4 for treatments with nutrient addition was of similar size between sites but with different factors controlling response relative to either control. This demonstrated the significance of modification by nutrient addition; however, the differences between emission rates for either site control (no addition) treatments indicates the importance and influence of other site specific characteristics at these locations.

The impact of nutrient availability on C flux is demonstrated here to be highly variable depending on the duration of nutrient enrichment. The inconsistent results from the two experiments demonstrate the differences in ecosystem response after sufficient time for alteration in regulating ecosystem processes to establish. Perturbation in ecosystems often drives gradual change requiring temporal scales larger than most experimental durations (Bubier *et al.*, 2007; Vitousek *et al.*, 2008). In this study the response difference between experimental additions in the short and long term are considerable, which has important implications for future environmental modification and C storage potential, with continuing N enrichment from deposition. The disparity between exposure duration also has implications for current understanding of ecosystem effects as many studies investigating these impacts are short term and, as demonstrated here, may potentially misrepresent ecosystem responses to nutrient enrichment in the long term as system modification may require transformation times measured in decades.

CHAPTER 6

GENERAL DISCUSSION

6.1 OVERVIEW OF RESEARCH

As detailed in section 1.4.1, the aim of this research was to further understand the effect of land management intensity and nitrogen (N) enrichment from atmospheric pollutant deposition on upland ecosystem processes, and the potential for ecosystem service delivery in the form of soil carbon (C) storage. The research specifically had four broad aims:

1) Evaluate the impact of reactive N deposition and livestock grazing intensity, and their interaction, on upland ecosystem processes and biodiversity.
2) Investigate the long term impact of N and phosphorus (P) availability on upland vegetation structure and species composition.
3) Assess the influence of N and P availability on upland soil chemistry and biology.
4) Quantify the impact of N and P availability on soil GHG emissions and C storage potential.

This thesis has considered the effects of current anthropogenically driven environmental change in sensitive upland habitats through a landscape scale survey and experiments in which nutrient availability was manipulated. It has explored the interactive effects of livestock stocking rate and N deposition (Chapter 2), and the role of N and P availability in influencing upland ecosystem processes (Chapters 3, 4, 5) such as could be expected in upland habitats exposed to chronic N enrichment, where P availability is predicted to be the primary mechanism constraining productivity (Li *et al*., 2015).

In Chapter 2, field surveys were conducted across the Cambrian Mountain range to investigate the combined influence of sheep stocking rate and N enrichment from deposition on upland ecosystem processes and the abundance of a keystone invertebrate. Our results indicate synergy between these two drivers of environmental change and demonstrate a need to reconsider critical load limits which account for this combined effect. In Chapter 3, vegetation and soil was sampled from an existing long term N and P addition experiment (1996 - 2012), three years after ceasing N inputs, to investigate the influence on ecosystem processes of release from P limitation. Phosphorus addition resulted in significant change in vegetation species composition, reductions in vegetation diversity, and lower soil carbon (C) in treatment plots, 15 years after a single P application. In Chapter 4, a factorial experiment was established where N and P were added over one year to explore nutrient availability effects on soil fauna and plant productivity in the short term. Nitrogen addition had no effect on vegetation growth and soil fauna populations, suggesting N is no longer a limiting factor for the study location. Phosphorus limitation was demonstrated however, as P addition had a positive influence on vegetation growth, the N:P ratio of vegetation tissue, and the taxonomic richness of soil fauna. Chapter 5 investigated the influence of N and P availability on soil C flux, in both the short and long term. Nitrogen addition had an inhibitory effect on C flux in the short term, but increased emission in the long term. Phosphorus addition significantly increased C flux in the short term, an effect which reduced over time. Differences in response to nutrient addition treatments and durations were the result of alteration in regulating ecosystem processes, which require sufficient time to re-establish after perturbation. Both N and P addition were negatively associated with soil C content by increased turnover and mineralisation from increased microbial activity.

The hypotheses detailed in section 1.4.2 were tested and are discussed in greater detail in the individual Chapters. Here we present synthesis to consider the implications of those individual investigations.

6.2 ENVIRONMENTAL CHANGE IN THE UPLANDS

Of the three key drivers of environmental change initiated by anthropogenic activities, this thesis focussed on the impact of two: Livestock grazing intensity and N enrichment from the deposition of atmospheric pollutants.

The combined influence of livestock stocking rate and N deposition has been shown in this thesis to have a detrimental effect on numerous upland ecosystem processes, both above and below ground (Chapter 2), which builds upon the model proposed by Van der Wal *et al*., (2003). The presence of grazing livestock has been shown to offset some of the effects of N enrichment by removing additional biomass from augmented growth and consequently reducing competitive exclusion (Borer *et al*., 2014). This effect, demonstrated for lowland grassland, may be more complex in the upland ecosystem as reductions in pressure from herbivory can result in greater coverage of dwarf shrub species and deciduous tree regeneration (Welch, 1984; Hartley & Mitchell, 2005), potentially enhancing the provision of ecosystem services (Quin *et al*., 2015). Grazing can either increase or reduce spatial heterogeneity of vegetation, an effect which is dependent on the initial spatial composition of vegetation (Adler *et al.,* 2001). The upland ecosystem is comprised of a patchwork of habitat types and transitional zones, however recent re-visitation studies have demonstrated biodiversity loss through biotic homogenisation of upland vegetation, attributed to acidification and temperature increases, and to the presence of livestock (McGovern *et al.,* 2011; Ross *et al*., 2012). This has significance for the future of the upland ecosystem as increasing habitat patch fragmentation, or loss through homogenisation, reduces habitat connectivity and the potential for exchange of individuals between patches. This could threaten long term viability and potentially drive localised extinctions, as indicated by the established theories of island biogeography (MacArthur & Wilson 1967) and metapopulation dynamics (Hanski 1998).

The current state of upland wildlife is of particular concern (Burns *et al*., 2013). The populations of numerous bird species have declined across upland habitats, an effect primarily attributed to reductions in prey species availability (Pearce-Higgins & Yalden, 2004; Pearce-Higgins *et al*., 2010). Craneflies are an essential invertebrate group, with one species, *Tipula subnodicornis* Zett., recognised as a keystone invertebrate of blanket bog (Coulson, 1962). Total cranefly populations surveyed were inversely related to N deposition rates and large bodied cranefly species specifically, which includes *T. subnodicornis*, were inversely related to grazing intensity (Chapter 2). Cranefly populations are known to be influenced by soil moisture levels (Carroll *et al*., 2011); the current study also demonstrates an association with total soil N, which relates to food resource quality (Pritchard, 1983) and to land management intensity through the loss of suitable habitat, particularly blanket bog (Coulson & Butterfield, 1985), from degradation and fragmentation. Hence, this demonstrates a link between current environmental change from anthropogenic activities and negative responses in the upland food web which could account for certain observed upland species declines (Burns *et al*., 2013). In addition, reductions in the activities of this group in the larval phase, which play a key role in various soil processes such as litter decomposition (Coulson & Butterfield, 1978) and hydrology by aiding soil water transport (Holden & Gell, 2009), can adversely affect ecosystem process and function.

Despite reductions since the 1980s in the input of N from pollution N deposition remains a significant problem for the upland ecosystem, with deposition rates in certain sensitive habitats still far in exceedance of established critical loads (Fowler *et al.*, 2004; RoTAP, 2012), potentially altering their nutrient status. The addition of N in the short term manipulation experiment (Chapters 4 and 5), did not significantly increase the growth of vegetation or the density of soil organisms, which was expected with release from N limitation, and may indicate a system unconstrained by N availability due to high rates of input. High levels of N addition were also observed to have a suppressive effect on the activity rates of soil organisms, particularly in the experiment of one year duration (Chapter 5). Prior research has shown increased soil fauna densities with N enrichment (Cole *et al.*, 2005), however addition rates greater than 50 kg N ha^{-1} yr^{-1}, may reduce soil faunal abundance (Xu *et al.*, 2007; Janssens *et al.*, 2010; Ochoa-Hueso *et al.*, 2014). This may facilitate increased C sequestration with high N enrichment by limiting saprotrophic activity and thus the rate of organic matter decomposition. This effect has been demonstrated previously in the boreal zone of northern Sweden, at a site with similar soil and comparable vegetation to those used in this thesis. Nitrogen enrichment was found to enhance soil C sequestration through reductions in soil microbial activity with the addition of 50 kg N ha^{-1} yr^{-1} (Maaroufi *et al.*, 2015). Here, the results support this and show an increase in soil C with N enrichment over the long term (Chapter 3), however not via the mechanism of inhibition as CO_2 flux was observed to be highest in the experimental treatments where N was added for ~16 years (Chapter 5). The highest addition rate of N in Chapter 3 was 20 kg N ha^{-1} yr^{-1} which therefore could indicate a potential for C gains in podzolic soils with N enrichment, not from the inhibition of soil organisms from N saturation, but probably as a result of increased organic matter input in the long term. This effect should be considered with some caution however in the context of the wider upland ecosystem, as N deposition rates at similar levels have also been shown to increase C loss in acid bogs (Bragazza *et al.*, 2006). Thus, there is great variability between different soil types, which are a feature of the heterogeneous upland ecosystem, in response to the effects of N enrichment with regard to soil C content change. Furthermore, the influence of soil moisture was not considered within this study. Soil moisture level is a key determinant of soil organic matter decomposition rate and of mortality rate in cranefly larvae. It was not considered as part of the nutrient addition trials due to the close proximity of study sites, which were exposed to comparable rainfall rates, therefore to what extent this may have affected these experimental results is unclear, however this is potentially a governing factor, particularly with regard to soil C flux as level of moisture (or conversely, aeration) would influence both CO_2 and CH_4 emission.

6.3 PHOSPHORUS AVAILABILITY

A consistent theme within this thesis is the consideration of nutrient limitation status change from N to P limitation as is expected with chronic N enrichment in oligotrophic upland habitats (Phuyal *et al.*, 2008; Crowley *et al.*, 2012). Results in this thesis have revealed significant environmental change initiated with P enrichment, and therefore conversely which could be constrained by P availability. Phosphorus availability influences numerous ecosystem processes, both above and below ground. The data presented here from Chapters 3, 4 and 5 indicate that the study locations are, in fact, P limited, with a consequence that the addition of P had significant effects on organisms after release from nutrient limitation. Thus, these effects could be assumed to be less pronounced in more eutrophic habitats.

The addition of P modified vegetation species composition (Chapter 3) with significant increases in the ground cover occupied by bryophyte species consistent with previous studies (Gordon *et al*., 2001; Limpens *et al*., 2004; Pilkington *et al*., 2007) and increased taxonomic richness of soil fauna with consequent increases in plant nutrient uptake (Chapter 4). Augmented vegetation growth in *Vaccinium myrtillus* plants was also demonstrated after P addition (Chapter 4), indicating that both vascular plants and bryophytes can benefit from an increase in P availability. This suggests that the differences observed between the vegetation species composition in the experimental treatment plots, driven by an increase in the availability of P is the result of changes to vegetation competitive interactions, specifically an increase in the competitive ability of bryophytes (notably in this study *Rhytidiadelphus loreus* and *Polytrichum commune*) over vascular plant species. This effect has been attributed previously to an increase in bryophyte potential for the utilisation of available N and to more comprehensive capture of available nutrients (Pilkington *et al*., 2007) particularly phosphate, through greater tissue absorption capacity (Chapin *et al*., 1987), and to an increase in the proportion of bryophyte shoots which were green (and therefore able to photosynthesise) early in the year, enhancing bryophyte productivity by effectively prolonging growing season duration (Gordon *et al*., 2001). In addition, nutrient stores may also be retained in decay-resistant litter limiting mineralisation and reducing N and P availability for vascular plants (Malmer *et al*., 2003; Street *et al*., 2015). This ability to hold on to nutrient resources suggests long term influence from P addition and is demonstrated in

Chapter 3 by the modified vegetation composition 15 years after a single P application.

Changes in the availability of nutrients can alter inter-specific competition within plant communities (Aerts, 1999) which can drive ecosystem simplification (Hautier *et al.,* 2009). An effect demonstrated widely as a result of N enrichment with ensuing losses of vegetation species richness and diversity (Stevens *et al*., 2006; Maskell *et al*., 2010). This effect is typically associated with greater growth and therefore competition for resources, primarily light (Borer *et al*., 2014). Phosphorus addition also resulted in reduced plant diversity (Chapter 3) as a consequence of competitive exclusion (Grime, 1973), but via the dominance of bryophyte species as opposed to graminoid species which are typically favoured by N enrichment (Field *et al.,* 2014). This has potentially significant implications for long term ecosystem stability with increasing P availability (Tilman *et al*., 2006; Hautier *et al*., 2015) and demonstrates P limitation is a major mechanism constraining ecosystem productivity in N enriched upland habitats and therefore limits potential losses in vegetation species diversity.

Microbial respiration also increased with the addition of P as the microbial population was released from P limitation (Liu *et al*., 2012), an effect which was shown to diminish with time (Chapter 5). Microbial priming may augment the population in the short term resulting in a more copiotrophic community (Fanin *et al*., 2015), however as C resources begin to decline with the increased activity, the availability of C may also become a limiting factor (Liu *et al*., 2013). What effect, if any, nutrient enrichment had on microbial species composition is unknown but if species loss or reductions in the size functional groups could be expected with increasing enrichment, as observed for vegetation species, then this is a particular area of concern. Ecosystem respiration remained higher than control with P addition in the long term experiment, but was similar in rate to the N only treatments. The similar CO_2 flux rates between nutrient addition treatments was surprising as soil analysis demonstrated that N only treatments had greater soil C than control, whereas soil C content was less where P was added (Chapter 3), which suggests higher rates of C flux with P enrichment. This difference was attributed to the sharp rise in microbial activity with P addition (Chapter 5), which Liu *et al*., (2013) found to last as little as three years but which can have a significant effect on soil C resource. In addition, change in vegetation species composition may alter organic matter inputs as bryophytes become dominant by reducing the direct input of organic matter in the form of roots to soil (Freschet *et al*., 2013; Smith *et al*., 2014).

The effect of P addition on soil C content is somewhat controversial. Soil C content was lower in plots where P was added (in combination with N) (Chapter 3), an effect also observed in other studies considering ecosystem response to P availability (Mack *et al*., 2004). However, this response is potentially habitat specific as the addition of P to a blanket bog suffering chronic N enrichment has been shown to stimulate *Sphagnum* growth (Limpens *et al*., 2004) which can positively influence C storage potential. Whether P addition (probably at low rates) could reduce C losses from blanket bogs suffering from chronic N enrichment (Bragazza *et al*., 2006) would require further research to confirm, but highlights a potentially dissimilar response between upland habitat and soil types to increased P availability.

Lastly, a cautionary note: the sole influence of P on ecosystem processes is impossible to partition in these experiments as pollutant deposition makes the presence of N ubiquitous in this environment. Therefore it would be prudent to consider these results as an effect driven by N and P in combination rather than P alone. In addition, the study site location for the long term experiment has been without grazing livestock since 2007 (~8 years). How this may have influenced vegetation community development is unknown, however there is the potential that the bryophyte dominance observed could be reduced under active livestock grazing.

6.4 THE FUTURE OF THE UPLANDS

Results presented in this thesis have implications for future approaches to land management in the upland ecosystem. The uplands comprise habitats of national and international importance such as blanket bog and heath (Bardgett *et al*., 1995). The stock of these habitats, whilst undeniably important, potentially needs to be considered with greater pragmatism. Current management practices used to maintain the character of upland habitats include grazing and burning, which can influence heath and blanket bog hydrology and C dynamics (Worral *et al*., 2006; Ward *et al*., 2007), negatively affecting existing C stocks with significant implications for C storage. The effect of sheep grazing as management may also threaten current heath habitat resource through landscape simplification as matrix complexity is reduced resulting in a more homogenised, grass dominated system (Ross *et al*., 2012). A more complex mosaic of habitats and transitional zones, which incorporates patches of deciduous tree regeneration, could benefit biodiversity through increased habitat complexity, as well as structure and habitat connectivity (Donald & Evans, 2006; Schneider *et al*., 2015). In addition, there are potential benefits from additional C capture that increased vegetation structure can offer as reducing grazing to allow greater dwarf shrub dominance and tree regeneration can sequester more C than habitats dominated by graminoid species (Bateman & Lovett, 2000; Quin *et al*., 2015).

Both livestock grazing and N deposition are shown to modify upland ecosystem processes and when acting in combination these effects may be amplified (Chapter 2). Rates of total N deposition are unlikely to reduce in the short term to levels below current critical loads, however reductions or total removals of livestock may prove beneficial as the presence and quantity of livestock not only influences ecosystem processes by direct impact, but also relates indirectly to localised reduced N deposition rates which are closely correlated to emissions from livestock wastes (RoTAP, 2012). Hence, reductions in livestock numbers could offer a mechanism for achieving multiple benefits.

Agricultural subsidy reform potentially provides an opportunity to reduce livestock grazing pressures. Sheep grazing in the uplands is heavily dependent on state (EU) subsidy (Monbiot, 2013). Idiosyncrasies in current UK law require that land be maintained in a state ‘fit for agriculture’ (DEFRA, 2014c), which typically means that vegetation is not allowed to regenerate to tree and shrub densities greater than 50 stems ha^{-1}. Changing the focus for how subsidies are currently paid from valuing agricultural productivity to a system which values the services provided by ecosystems could incentivise landowners to manage habitats more sensitively (Farley & Costanza, 2010). However, this would require stringent framework to ensure other upland ecosystem components are not adversely affected in the pursuit to maximise payments (Van Hecken & Bastiaensen, 2010). Current land management approaches, particularly with regard to farming practice, can be harmful to sensitive habitats (Critchley *et al*., 2008; Evans *et al*., 2015), yet stakeholder perceptions of current ecosystem state often remain out of kilter and may lack realism or accuracy (Dallimer *et al*., 2009). A future focus for farmer engagement must be based around education of the benefits ecosystems can provide, if any meaningful landowner engagement is to be expected (Raedeke *et al.*, 2001; McCracken *et al*., 2015).

Future changes in policy must be based on credible evidence rather than individual judgement or opinion if land management approaches are to offer maximum benefit in the long term (Dicks *et al*., 2014). This thesis therefore provides evidence of the ecosystem wide effects of livestock grazing and N enrichment (Chapters 2 – 5) and indicates that reductions in agricultural intensity, with a complete ceasing of agricultural activity in certain sensitive upland habitats, could offer long term benefits in the form of improved provision of ecosystem services such as C storage and flood mitigation (Wheater *et al*., 2008; Klumpp *et al*., 2009). A shift in focus from payments relating to agricultural productivity towards a system recognising and valuing the benefits of ecosystem service provision may also offer greater long term stability.

6.5 FURTHER RESEARCH

This thesis has identified several questions which require further investigation. Phosphorus addition had significant influence on upland ecosystem processes and was shown to exert long term influence from even a single application. There is much inconsistency within the literature with regard to the influence of P availability, which is potentially a consequence of differences in habitat and soil types. It may however also be a factor of addition rate, as studies which have demonstrated a positive influence (i.e. Limpens *et al*., 2004) added P at a lower rate than utilised here. If there is a potential for P addition to reduce the impact of N enrichment in upland habitats, it is potentially with low levels of P enrichment to reduce the initial activity spikes (Chapter 5). Further experiments would be needed with single applications of P at low rates (preferably measured >10 years after application) to fully investigate ecosystem response. Furthermore, the fate of additional N and P and how they are stored and cycled in the long term, could also offer insight on trajectories of community compositional change and the ability of systems to utilise and retain additional N. This could be investigated using isotope labelling or tracer analysis. Curtis *et al.*, (2005) demonstrated the fate of N at different levels of addition in heath and calcifuge grassland with this technique, yet ecosystem responses where P availability is not a limiting factor require further consideration.

Soil organisms contribute significantly to ecosystem biodiversity (Wardle *et al.*, 2004) but are typically overlooked in favour of more obvious or easily surveyed groups such as plants. The influence of nutrient enrichment, particularly N, on soil organism richness and diversity in upland habitats, requires much greater attention, as the below ground response has the potential to affect numerous ecosystem processes (Wagg *et al.*, 2014). Chapter 5 indicated the potential for short term shifts towards more copiotrophic microbial communities, but what influence this had on belowground biodiversity and whether this influence would persist in the long term is unknown. The structure of soil biota may appear reasonably resilient in the short term, however disturbance can initiate community ‘reorganisation’, the effects of which may require decades before fully stabilising due to the oscillatory or feedback effects between group interactions (Bengtsson, 2002).

6.6 SUMMARY

Current environmental change in upland habitats is principally a consequence of anthropogenic activities such as industry and food production. Ecosystem response to such disturbance is often non-linear as the interactions between ecosystem components and their feedback effects, are often highly complex and may require observation durations measured in decades. The research reported in this thesis investigated underlying ecosystem responses to current drivers of environmental change, and also the interaction between drivers, to better understand likely future shifts in biodiversity, species composition and governing ecosystem processes.

Nitrogen enrichment from pollution has been shown in this thesis to influence numerous upland ecosystem processes and has altered the nutrient status of upland habitats, with a shift towards the next least available nutrient, P. The limited availability of P in upland ecosystems is a significant mechanism constraining ecosystem productivity. The release from P limitation can enable organisms to utilise additional N from deposition, altering competitive interactions, however the ecosystem-wide effects demonstrated in this thesis suggest an overall negative influence from augmented P availability. Increasing the availability of P either by deliberate addition as part of a management program (Armitage *et al*., 2012) or by pollutant deposition from anthropogenic activities (Ceulemans *et al*., 2012), is expected to reduce the diversity of vegetation species, but may increase the taxonomic richness and diversity of soil fauna. Therefore, the approach of P addition as a management tool for remediation of habitats adversely affected by N deposition needs careful consideration of potential long term influences.

Environmental change in upland landscapes presents some significant challenges for the coming decades, particularly where future changes in climate and increasing nutrient availability makes the resilience of upland habitats to further perturbation uncertain. If such change is to be effectively managed, then more holistic approaches to land use require implementation. By regarding upland habitats as an opportunity to increase the provision of ecosystem services, particularly with regard to sensitive habitats such as blanket bogs which are major C reservoirs, there are opportunities to effectively manage and, where appropriate, restore these habitats for the benefits of both humans and wildlife combined.

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APPENDIX

Appendix 1 - Chapter 2 vegetation survey results

Blanket bog:

Table A1.1. Vegetation survey data for blanket bog habitat across the Cambrian Mountain range. Values are mean average % cover (± S.E.) for all quadrats taken per site.

	Glaslyn Lake		Foel Fadian		Waun Y Dyffryn		Pumlumon		Y garn		Banc Llechwedd Mawr		Llynoedd Ieuan		Pwllpeiran 1		Pwllpeiran 2	
Agrostis capillaris	0.0	0.0	0.0	0.0	0.0	0.0	9.4	4.6	6.3	2.4	0.0	0.0	0.0	0.0	0.0	0.0	4.7	3.3
Agrostis stolonifera	0.0	0.0	0.0	0.0	4.7	3.3	0.0	0.0	10.9	5.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Aulacomium palustre	0.0	0.0	0.0	0.0	1.6	1.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Calluna vulgaris	59.4	8.1	35.9	9.9	0.0	0.0	0.0	0.0	0.0	0.0	42.2	11.3	23.4	9.0	0.0	0.0	6.3	4.7
Carex binervis	3.1	2.0	3.1	3.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Carex panicea	0.0	0.0	0.0	0.0	3.1	2.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Danthonia decumbens	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	4.7	3.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Deschampsia flexuosa	0.0	0.0	0.0	0.0	0.0	0.0	1.6	1.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Dicranum scoparium	1.6	1.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.6	1.6
Diplophyllum albicans	1.6	1.6	0.0	0.0	0.0	0.0	0.0	0.0	1.6	1.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Empetrum nigrum	14.1	6.4	3.1	3.1	0.0	0.0	0.0	0.0	0.0	0.0	6.3	6.3	1.6	1.6	0.0	0.0	12.5	7.8

Erica tetralix	4.7	2.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	10.9	3.7	0.0	0.0	0.0	0.0
Eriophorum angustifolium	1.6	1.6	3.1	3.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.1	3.1
Eriophorum vaginatum	17.2	9.4	18.8	9.1	0.0	0.0	18.8	6.3	0.0	0.0	4.7	4.7	7.8	4.7	50.0	9.1	64.1	3.7
Galium saxatile	0.0	0.0	0.0	0.0	26.6	7.3	14.1	3.7	25.0	5.8	0.0	0.0	0.0	0.0	4.7	3.3	0.0	0.0
Huperzia selago	0.0	0.0	0.0	0.0	1.6	1.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Hylocomium splendens	0.0	0.0	3.1	3.1	0.0	0.0	0.0	0.0	1.6	1.6	4.7	3.3	0.0	0.0	0.0	0.0	1.6	1.6
Hypnum cupressiforme	3.1	2.0	0.0	0.0	6.3	4.7	0.0	0.0	3.1	3.1	1.6	1.6	0.0	0.0	0.0	0.0	0.0	0.0
Hypnum jutlandicum	21.9	7.4	1.6	1.6	1.6	1.6	4.7	3.3	3.1	2.0	17.2	10.3	7.8	3.3	0.0	0.0	3.1	3.1
Juncus squarrosus	0.0	0.0	0.0	0.0	29.7	9.4	0.0	0.0	32.8	7.1	6.3	4.7	0.0	0.0	0.0	0.0	0.0	0.0
Lophocolea bidentata	0.0	0.0	0.0	0.0	7.8	4.0	0.0	0.0	6.3	2.4	0.0	0.0	4.7	3.3	0.0	0.0	0.0	0.0
Molinia caerulea	0.0	0.0	12.5	5.3	0.0	0.0	0.0	0.0	3.1	3.1	18.8	5.8	35.9	12.1	15.6	7.7	0.0	0.0
Nardus stricta	0.0	0.0	0.0	0.0	23.4	7.6	4.7	3.3	4.7	3.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Narthecium ossifragum	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	15.6	8.4	0.0	0.0	0.0	0.0
Plagiothecium undulatum	0.0	0.0	1.6	1.6	0.0	0.0	0.0	0.0	1.6	1.6	0.0	0.0	0.0	0.0	4.7	2.3	0.0	0.0
Pleurozium schreberi	17.2	9.1	20.3	7.4	6.3	3.3	28.1	8.4	1.6	1.6	20.3	8.5	0.0	0.0	25.0	5.8	18.8	6.3
Polytrichum commune	0.0	0.0	4.7	4.7	23.4	7.6	21.9	7.7	57.8	6.2	10.9	3.7	0.0	0.0	37.5	9.4	48.4	7.6
Polytrichum formosum	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Polytrichum strictum	0.0	0.0	0.0	0.0	4.7	4.7	34.4	11.8	0.0	0.0	0.0	0.0	0.0	0.0	1.6	1.6	6.3	3.3
Potentilla erecta	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.1	3.1	0.0	0.0	0.0	0.0	0.0	0.0
Pseudocleropodium purum	1.6	1.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Ptilidium ciliare	3.1	2.0	0.0	0.0	0.0	0.0	0.0	0.0	7.8	4.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Racomitrium ericoides	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	4.7	4.7	0.0	0.0	0.0	0.0	0.0	0.0
Racomitrium lanuginosum	3.1	3.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.6	1.6	0.0	0.0	0.0	0.0
Rhytidiadelphus loreus	1.6	1.6	3.1	3.1	0.0	0.0	3.1	2.0	1.6	1.6	4.7	3.3	4.7	2.3	1.6	1.6	0.0	0.0
Rhytidiadelphus squarrosus	0.0	0.0	4.7	3.3	3.1	2.0	9.4	5.1	42.2	9.4	15.6	6.6	0.0	0.0	3.1	2.0	3.1	2.0
Sphagnum angustifolium	0.0	0.0	6.3	6.3	0.0	0.0	1.6	1.6	0.0	0.0	1.6	1.6	4.7	3.3	0.0	0.0	0.0	0.0
Sphagnum capillifolium	4.7	4.7	14.1	9.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Sphagnum fallax	1.6	1.6	4.7	3.3	0.0	0.0	10.9	7.6	0.0	0.0	4.7	2.3	3.1	3.1	10.9	7.6	7.8	5.2
Sphagnum papillosum	1.6	1.6	6.3	4.7	0.0	0.0	31.3	10.0	0.0	0.0	12.5	5.3	4.7	4.7	25.0	10.6	25.0	7.5
Sphagnum palustre	0.0	0.0	0.0	0.0	0.0	0.0	1.6	1.6	0.0	0.0	6.3	2.4	0.0	0.0	1.6	1.6	0.0	0.0
Sphagnum squarrosus	0.0	0.0	0.0	0.0	6.3	4.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Sphagnum subnitens	0.0	0.0	32.8	10.8	1.6	1.6	6.3	6.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Trichophorum cespitosum	0.0	0.0	9.4	7.7	0.0	0.0	0.0	0.0	0.0	0.0	29.7	7.4	4.7	4.7	0.0	0.0	0.0	0.0
Vaccinium myrtillus	3.1	2.0	10.9	6.0	10.9	5.0	6.3	3.3	4.7	3.3	4.7	3.3	0.0	0.0	9.4	4.6	17.2	8.8

Calcifuge grassland:

Table A1.2. Vegetation survey data for calcifuge grassland habitat across the Cambrian Mountain range. Values are mean average % cover (± S.E.) for all quadrats taken per site.

	Glaslyn Lake		Foel Fadian		Waun Y Dyffryn		Pumlumon		Y garn		Banc Llechwedd Mawr		Llynoedd Ieuan		Pwllpeiran 1		Pwllpeiran 2	
Agrostis canina	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.6	1.6	0.0	0.0
Agrostis capillaris	0.0	0.0	45.3	9.4	0.0	0.0	0.0	0.0	3.1	2.0	59.4	5.7	0.0	0.0	0.0	0.0	42.2	8.2
Agrostis stolonifera	6.3	3.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Agrostis vinealis	0.0	0.0	0.0	0.0	0.0	0.0	7.8	4.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Aulacomium palustre	0.0	0.0	0.0	0.0	7.8	6.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Calluna vulgaris	3.1	3.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Carex binervis	0.0	0.0	4.7	4.7	0.0	0.0	0.0	0.0	4.7	3.3	0.0	0.0	0.0	0.0	0.0	0.0	1.6	1.6
Carex demissa	0.0	0.0	3.1	2.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Carex panicea	0.0	0.0	0.0	0.0	23.4	7.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Cladonia uncialis	0.0	0.0	0.0	0.0	0.0	0.0	7.8	3.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Deschampsia cespitosa	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	9.4	5.1	0.0	0.0	0.0	0.0
Deschampsia flexuosa	1.6	1.6	0.0	0.0	0.0	0.0	10.9	8.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Diplophyllum albicans	0.0	0.0	0.0	0.0	0.0	0.0	1.6	1.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Empetrum nigrum	0.0	0.0	9.4	5.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Erica cinerea	1.6	1.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Erica tetralix	0.0	0.0	0.0	0.0	1.6	1.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Eriophorum vaginatum	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	6.3	4.7	9.4	7.7	0.0	0.0
Galium saxatile	32.8	5.8	32.8	9.4	10.9	3.7	4.7	2.3	17.2	6.7	48.4	7.6	0.0	0.0	10.9	4.4	29.7	5.8
Huperzia selago	0.0	0.0	0.0	0.0	0.0	0.0	32.8	6.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Hylocomium splendens	32.8	10.5	39.1	9.6	9.4	6.1	0.0	0.0	0.0	0.0	10.9	7.3	0.0	0.0	6.3	4.1	4.7	4.7
Hypnum cupressiforme	3.1	3.1	0.0	0.0	0.0	0.0	0.0	0.0	9.4	6.6	9.4	3.1	0.0	0.0	0.0	0.0	0.0	0.0
Hypnum jutlandicum	6.3	3.3	0.0	0.0	7.8	3.3	7.8	3.3	40.6	8.8	0.0	0.0	0.0	0.0	12.5	4.7	0.0	0.0
Juncus squarrosus	14.1	7.3	1.6	1.6	1.6	1.6	0.0	0.0	3.1	2.0	6.3	3.3	0.0	0.0	0.0	0.0	0.0	0.0
Molinia caerulea	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	90.6	6.1	62.5	10.3	0.0	0.0
Nardus stricta	34.4	7.4	68.8	8.2	42.2	5.8	42.2	11.6	26.6	7.3	29.7	8.5	0.0	0.0	18.8	7.5	56.3	6.7
Plagiothecium undulatum	1.6	1.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Pleurozium schreberi	39.1	11.4	6.3	4.1	0.0	0.0	1.6	1.6	14.1	6.0	0.0	0.0	0.0	0.0	7.8	4.7	1.6	1.6
Polytrichum commune	6.3	4.7	0.0	0.0	3.1	3.1	43.8	10.6	20.3	10.0	4.7	4.7	0.0	0.0	9.4	7.7	0.0	0.0
Polytrichum strictum	0.0	0.0	0.0	0.0	1.6	1.6	0.0	0.0	9.4	4.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Potentilla erecta	0.0	0.0	1.6	1.6	6.3	4.1	0.0	0.0	0.0	0.0	1.6	1.6	6.3	4.7	0.0	0.0	1.6	1.6

Pseudocleropodium purum	7.8	6.2	1.6	1.6	0.0	0.0	0.0	0.0	1.6	1.6	1.6	1.6	0.0	0.0	0.0	0.0	9.4	5.1
Ptilidium ciliare	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	6.3	4.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Racomitrium ericoides	0.0	0.0	0.0	0.0	7.8	6.2	0.0	0.0	23.4	12.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Racomitrium lanuginosum	0.0	0.0	0.0	0.0	0.0	0.0	39.1	12.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Rhytidiadelphus loreus	0.0	0.0	0.0	0.0	3.1	3.1	0.0	0.0	21.9	7.7	1.6	1.6	4.7	4.7	1.6	1.6	7.8	6.2
Rhytidiadelphus squarrosus	25.0	4.7	9.4	3.9	7.8	3.3	0.0	0.0	6.3	4.7	53.1	7.0	4.7	2.3	25.0	9.1	17.2	5.8
Sphagnum compactum	0.0	0.0	0.0	0.0	3.1	3.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Trichophorum cespitosum	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.1	3.1	0.0	0.0
Vaccinium myrtillus	20.3	6.7	0.0	0.0	10.9	5.0	21.9	8.8	23.4	6.9	7.8	3.3	0.0	0.0	3.1	2.0	0.0	0.0
Vaccinium vitis-idaea	0.0	0.0	0.0	0.0	0.0	0.0	7.8	7.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Heath:

Table A1.3. Vegetation survey data for heath habitat across the Cambrian Mountain range. Values are mean average % cover (± S.E.) for all quadrats taken per site.

	Glaslyn Lake		Foel Fadian		Waun Y Dyffryn		Pumlumon		Y garn		Banc Llechwedd Mawr		Llynoedd Ieuan		Pwllpeiran 1		Pwllpeiran 2	
Agrostis capillaris	0.0	0.0	0.0	0.0	10.9	3.7	15.6	7.4	1.6	1.6	0.0	0.0	0.0	0.0	9.4	7.7	9.4	3.1
Agrostis stolonifera	1.6	1.6	4.7	3.3	0.0	0.0	0.0	0.0	0.0	0.0	18.8	6.3	0.0	0.0	0.0	0.0	0.0	0.0
Agrostis vinealis	0.0	0.0	0.0	0.0	0.0	0.0	14.1	8.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.6	1.6
Aulacomium palustre	0.0	0.0	0.0	0.0	0.0	0.0	3.1	2.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Calluna vulgaris	82.8	10.5	71.9	7.4	4.7	4.7	0.0	0.0	12.5	8.5	4.7	3.3	43.8	9.7	21.9	10.2	0.0	0.0
Carex binervis	0.0	0.0	6.3	3.3	4.7	2.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.1	3.1	7.8	4.7
Cladonia arbuscula	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.6	1.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Cladonia portentosa	0.0	0.0	0.0	0.0	0.0	0.0	1.6	1.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Cladonia uncialis	0.0	0.0	1.6	1.6	0.0	0.0	3.1	2.0	6.3	4.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Deschampsia flexuosa	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.6	1.6	0.0	0.0	0.0	0.0
Dicranum scoparium	0.0	0.0	1.6	1.6	15.6	5.7	0.0	0.0	0.0	0.0	0.0	0.0	1.6	1.6	0.0	0.0	0.0	0.0
Diplophyllum albicans	0.0	0.0	0.0	0.0	0.0	0.0	1.6	1.6	0.0	0.0	1.6	1.6	0.0	0.0	0.0	0.0	0.0	0.0
Empetrum nigrum	15.6	8.4	1.6	1.6	1.6	1.6	0.0	0.0	0.0	0.0	6.3	6.3	25.0	7.1	10.9	3.7	4.7	4.7

Erica tetralix	0.0	0.0	1.6	1.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Eriophorum vaginatum	1.6	1.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.6	1.6	0.0	0.0
Galium saxatile	6.3	4.7	0.0	0.0	9.4	5.1	1.6	1.6	0.0	0.0	26.6	6.4	9.4	5.1	3.1	2.0	26.6	5.5
Hylocomium splendens	42.2	12.3	10.9	6.4	1.6	1.6	1.6	1.6	0.0	0.0	28.1	3.9	31.3	9.7	1.6	1.6	18.8	7.5
Hypnum cupressiforme	3.1	2.0	4.7	3.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	7.8	5.2
Hypnum jutlandicum	7.8	6.2	26.6	8.0	12.5	4.7	53.1	10.5	43.8	12.3	6.3	4.7	15.6	6.6	7.8	4.7	6.3	2.4
Juncus squarrosus	0.0	0.0	1.6	1.6	15.6	5.1	3.1	2.0	3.1	2.0	15.6	6.6	0.0	0.0	0.0	0.0	6.3	6.3
Lophocolea bidentata	1.6	1.6	0.0	0.0	1.6	1.6	0.0	0.0	7.8	4.0	1.6	1.6	0.0	0.0	0.0	0.0	0.0	0.0
Molinia caerulea	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	7.8	5.2	7.8	7.8	10.9	7.3	0.0	0.0
Nardus stricta	1.6	1.6	7.8	4.0	31.3	5.8	9.4	4.6	21.9	6.6	12.5	3.3	1.6	1.6	18.8	6.7	42.2	6.2
Plagiothecium undulatum	0.0	0.0	1.6	1.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Pleurozium schreberi	20.3	8.5	3.1	2.0	3.1	2.0	10.9	5.0	20.3	6.2	29.7	7.1	9.4	3.1	43.8	6.7	3.1	3.1
Polygala serpyllifolia	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.6	1.6
Polytrichum commune	4.7	3.3	0.0	0.0	0.0	0.0	18.8	11.1	10.9	4.4	6.3	4.7	0.0	0.0	10.9	3.7	39.1	14.3
Polytrichum strictum	0.0	0.0	0.0	0.0	3.1	3.1	0.0	0.0	14.1	9.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Potentilla erecta	0.0	0.0	0.0	0.0	0.0	0.0	4.7	3.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.6	1.6
Pseudocleropodium purum	10.9	8.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.6	1.6	0.0	0.0	0.0	0.0

Ptilidium ciliare	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.6	1.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Racomitrium ericoides	0.0	0.0	0.0	0.0	3.1	2.0	35.9	11.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Racomitrium lanuginosum	0.0	0.0	7.8	7.8	1.6	1.6	0.0	0.0	6.3	4.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Rhytidiadelphus loreus	1.6	1.6	0.0	0.0	0.0	0.0	3.1	3.1	25.0	4.7	17.2	6.7	3.1	2.0	4.7	3.3	28.1	9.1
Rhytidiadelphus squarrosus	3.1	2.0	1.6	1.6	14.1	3.7	1.6	1.6	4.7	2.3	31.3	7.1	10.9	7.6	15.6	2.0	7.8	3.3
Trichophorum cespitosum	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	4.7	3.3	0.0	0.0	9.4	6.1	0.0	0.0
Vaccinium myrtillus	26.6	8.3	3.1	3.1	18.8	6.3	26.6	7.6	15.6	6.6	29.7	5.2	26.6	8.0	26.6	7.3	20.3	5.8
Vaccinium oxycoccos	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.6	1.6
Vaccinium vitis-idaea	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	17.2	4.7	0.0	0.0	6.3	4.7	1.6	1.6	0.0	0.0

Appendix 2

Chapter 3 vegetation survey results:

Table A2.1. Vegetation survey data for Pen Y Garn (long term nutrient addition experiment established by CEH). Values are mean average % cover (± S.E.) for all quadrats taken per treatment, converted from Domin scale to percentage cover using Currall (1987).

	CONT		AS10		AS20		SN20	
Agrostis capillaris	0.5	0.3	0.8	0.3	0.3	0.2	0.0	0.0
Aulacomnium palustre	0.0	0.0	0.0	0.0	0.3	0.3	0.0	0.0
Calluna vulgaris	1.7	0.9	0.0	0.0	3.7	1.4	2.9	0.9
Carex binervis	0.7	0.7	0.3	0.2	1.5	1.5	0.3	0.2
Carex pilulifera	5.2	2.6	0.7	0.7	15.9	6.0	25.0	5.5
Cladonia uncialis	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Dicranum scoparium	2.2	1.0	0.8	0.7	2.8	1.4	1.2	0.7
Diplophyllum albicans	2.2	0.7	0.0	0.0	2.9	0.9	1.4	0.9
Empetrum nigrum	3.7	1.4	0.8	0.7	3.5	1.3	1.5	0.9
Eriophorum angustifolium	1.5	1.5	0.7	0.7	0.0	0.0	1.6	1.5
Eriophorum vaginatum	3.5	2.7	3.1	1.9	0.1	0.1	0.8	0.7
Festuca ovina	3.7	1.4	5.2	1.4	8.9	3.8	7.3	2.6
Galium saxatile	2.2	1.0	2.7	0.8	1.7	0.9	3.9	0.5
Huperzia selago	0.0	0.0	0.0	0.0	0.0	0.0	1.1	0.7
Hylocomium splendens	11.6	6.1	5.0	2.7	1.4	0.9	5.8	2.8
Hypnum cupressiforme	1.5	0.9	1.0	0.7	2.4	0.9	1.0	0.7
Hypnum jutlandicum	3.2	0.8	1.0	0.7	2.4	0.9	2.2	1.0
Juncus squarrosus	9.6	1.6	14.6	5.2	12.5	3.2	13.5	5.9
Lophocolea bidentata	0.3	0.3	0.0	0.0	0.3	0.3	0.0	0.0
Luzula multiflora	0.3	0.2	0.2	0.1	0.0	0.0	0.0	0.0
Nardus stricta	18.7	4.2	8.6	4.3	33.9	8.6	32.7	6.5
Plagiothecium undulatum	0.3	0.3	0.3	0.3	0.0	0.0	0.3	0.2
Pleurozium schreberi	3.2	0.8	3.2	0.7	1.7	0.9	2.2	0.7
Polygala serpyllifolia	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Polytrichum commune	5.7	2.6	3.6	1.8	2.0	0.8	7.3	3.9
Polytrichum strictum	10.8	6.3	9.9	4.7	10.4	6.0	4.6	2.1
Potentilla erecta	0.7	0.7	0.3	0.3	2.0	0.8	2.0	0.8
Ptilidium ciliare	0.0	0.0	1.0	0.7	0.0	0.0	0.0	0.0
Racomitrium lanuginosum	9.0	6.1	2.9	0.9	8.4	1.9	7.0	1.8
Rhytidiadelphus loreus	17.0	4.9	48.9	11.6	4.0	1.8	8.4	0.8
Rhytidiadelphus squarrosus	3.2	0.8	9.0	6.1	0.7	0.7	1.7	0.9
Sorbus aucuparia	0.0	0.0	0.0	0.0	0.1	0.1	0.0	0.0

Sphagnum capillifolium	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Sphagnum subnitens	0.3	0.3	0.0	0.0	0.7	0.7	0.3	0.3
Trichophorum cespitosum	2.2	1.0	0.0	0.0	0.0	0.0	0.0	0.0
Vaccinium myrtillus	7.2	2.0	6.3	1.4	7.2	2.0	5.2	0.8
Vaccinium vitis-idaea	2.3	1.6	1.7	0.9	3.0	1.5	0.5	0.3

Appendix 3

Chapter 4 vegetation survey results:

Table A3.1. Vegetation survey data for Pwllpeiran (short term nutrient addition experiment). Values are mean average % cover (± S.E.) for all quadrats taken per treatment, converted from Domin scale to percentage cover using Currall (1987).

	CONT		HN		LN		HP		LP		HNHP		LNLP		HNLP		LNHP	
Agrostis capillaris	2.2	1.0	1.4	0.9	2.2	1.0	2.2	1.0	2.9	0.9	2.9	0.9	2.9	0.9	2.9	0.9	2.9	0.9
Agrostis stolonifera	2.2	1.0	2.2	1.0	2.2	1.0	1.4	0.9	2.9	0.9	1.4	0.9	2.9	0.9	2.2	1.0	2.9	0.9
Anthoxanthum odoratum	1.5	0.9	1.6	1.5	1.3	0.7	2.3	1.5	3.0	1.4	3.4	1.9	1.0	0.7	1.8	1.5	3.7	2.6
Aulacomnium palustre	3.3	2.6	0.5	0.3	5.2	4.3	1.3	0.7	2.1	1.5	3.3	2.6	1.7	0.9	3.5	2.6	2.8	1.4
Calluna vulgaris	0.0	0.0	1.5	1.5	1.6	1.5	0.3	0.3	0.0	0.0	4.3	2.9	0.0	0.0	1.5	1.5	0.0	0.0
Carex binervis	1.7	1.5	3.7	2.6	2.7	2.7	1.6	1.5	0.3	0.3	1.0	0.7	5.0	2.7	4.6	4.4	4.5	4.4
Carex pilulifera	0.1	0.1	0.0	0.0	0.3	0.2	0.4	0.2	0.1	0.1	0.3	0.3	0.3	0.2	0.8	0.7	0.3	0.2
Cladonia chlorophaea	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Cladonia portentosa	0.1	0.1	0.4	0.2	0.8	0.7	0.3	0.2	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1
Cladonia uncialis	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.3	0.0	0.0	0.0	0.0	0.0	0.0
Deschampsia flexuosa	7.1	1.4	8.4	0.8	5.2	0.8	7.6	1.0	5.9	2.2	5.8	1.6	8.1	2.2	7.5	2.2	10.4	3.9
Dicranum scoparium	0.3	0.3	1.0	0.7	1.2	0.7	0.3	0.2	1.8	1.5	0.0	0.0	0.3	0.2	0.3	0.3	0.7	0.7
Empetrum nigrum	5.0	2.7	2.7	2.7	6.0	3.3	4.5	2.8	2.3	1.5	1.8	1.5	1.7	0.9	4.0	1.8	1.4	0.9
Eriophorum vaginatum	4.4	4.4	6.8	6.5	3.0	2.7	1.5	1.5	0.0	0.0	0.3	0.3	0.3	0.3	0.7	0.7	1.5	1.5
Festuca ovina	7.5	2.2	13.0	5.6	9.7	5.9	13.3	5.6	11.9	5.7	12.1	5.6	9.9	4.0	12.7	5.9	12.5	5.8
Galium saxatile	10.5	5.9	6.8	1.1	5.9	2.2	6.8	1.1	7.2	2.0	5.2	0.8	6.8	1.1	4.3	0.0	4.3	0.0
Hylocomium splendens	32.6	8.7	33.2	7.0	24.1	8.1	26.2	4.9	30.9	9.0	32.1	8.5	30.9	9.0	32.8	6.6	25.6	7.3

Hypnum cupressiforme	1.6	1.5	2.5	1.5	1.0	0.7	1.0	0.7	1.8	1.5	0.3	0.3	1.8	1.5	0.5	0.3	0.0	0.0
Hypnum jutlandicum	5.6	1.7	6.0	2.5	6.0	2.5	9.4	4.2	10.7	3.9	5.3	2.6	6.3	1.4	4.0	2.6	6.9	4.1
Juncus squarrosus	3.1	1.3	2.8	1.4	9.3	6.2	4.8	1.5	4.5	1.5	5.8	1.6	3.9	1.7	2.7	0.8	4.5	2.4
Lophocolea bidentata	0.1	0.1	0.0	0.0	0.3	0.3	0.3	0.3	0.0	0.0	0.1	0.1	0.7	0.7	0.0	0.0	0.0	0.0
Luzula multiflora	0.3	0.3	0.1	0.1	0.0	0.0	0.1	0.1	0.2	0.1	0.1	0.1	0.0	0.0	0.1	0.1	0.4	0.2
Luzula multiflora congesta	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Molinia caerulea	1.5	1.5	1.8	1.5	2.8	2.7	0.0	0.0	2.7	2.7	0.3	0.3	0.0	0.0	0.0	0.0	0.3	0.3
Nardus stricta	10.0	2.2	14.9	3.1	12.1	3.9	16.1	3.4	10.1	2.5	12.8	1.6	12.5	3.2	14.0	1.5	15.4	4.9
Plagiothecium undulatum	0.3	0.3	0.0	0.0	0.0	0.0	0.7	0.7	0.7	0.7	0.3	0.3	1.5	1.5	0.3	0.3	0.5	0.3
Pleurozium schreberi	24.9	5.9	22.5	6.1	21.6	6.1	21.9	3.9	28.4	6.1	19.5	4.7	29.3	7.3	34.3	11.2	22.8	4.8
Polygala serpyllifolia	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Polytrichum commune	2.2	1.0	10.4	6.0	6.8	2.5	6.9	4.1	4.8	2.7	6.0	1.5	8.9	4.4	7.3	2.6	10.8	4.3
Polytrichum strictum	3.0	2.7	5.9	4.4	1.4	0.9	1.5	1.5	3.1	1.9	1.5	1.5	4.4	4.4	0.0	0.0	0.7	0.7
Potentilla erecta	1.8	0.8	0.9	0.7	1.0	0.7	1.1	0.7	2.1	1.4	1.5	0.9	0.4	0.2	0.8	0.7	1.7	0.9
Ptilidium ciliare	0.0	0.0	0.3	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.8	0.7	0.0	0.0	0.3	0.3
Racomitrium lanuginosum	10.8	6.2	2.3	1.6	11.7	6.9	6.9	3.1	6.2	3.3	2.3	1.6	5.2	2.6	4.0	1.8	5.4	4.3
Rhytidiadelphus loreus	8.5	2.8	8.0	1.9	5.6	2.3	9.6	3.5	7.3	2.3	10.0	6.0	6.7	2.2	10.0	3.8	3.7	1.4
Rhytidiadelphus squarrosus	6.0	2.5	11.8	5.6	10.4	3.3	6.4	2.3	9.6	1.6	8.8	1.8	8.8	1.8	8.0	1.9	17.3	7.8
Scapania scandica	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Sorbus aucuparia	0.0	0.0	0.0	0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Sphagnum capillifolium	0.0	0.0	0.3	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.3	0.0	0.0	0.0	0.0
Trichophorum cespitosum	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Vaccinium myrtillus	46.4	6.6	38.3	5.1	41.6	7.8	35.5	3.8	33.9	5.6	29.6	4.6	35.6	4.8	35.6	4.8	28.3	5.4