Assessing spatiotemporal relationships between atmospheric nitrogen deposition and butterfly species records through statistical modelling

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Declaration

I declare that the work presented in this thesis is my own and that it has not been submitted for any other degree or professional qualification.

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Abstract

Atmospheric nitrogen deposition has been linked with an overall loss of plant species richness and homogenisation of semi-natural habitats both in GB and elsewhere. We expect that nitrogen-induced changes in plant communities will impact invertebrate species through the loss of reproductive habitat, food plants and suitable microclimatic conditions caused by the shifts in composition of plant communities. Prior to this thesis, no quantitative research had been undertaken to assess the potential effects of nitrogen on fauna in GB. Butterflies are often used as indicator species due to their sensitivity to environmental change, our comprehensive understanding of their ecology, and the existence of long-term datasets on their abundance and distribution.

In this study, I analysed butterfly data from the UK Butterfly Monitoring Scheme alongside data on expected driver variables including nitrogen deposition, sulphur deposition, temperature, rainfall, land use intensity, and elevation. I performed a spatio-temporal analysis on the data for each species individually using generalised additive models to understand the complex and expected non-linear relationships between butterfly trends and their drivers. Model results were summarised to provide an overview of the total number of species exhibiting responses to nitrogen. In addition, results were summarised by trait groupings such as voltinism, host plant category, host plant specificity, and breeding habitat to summarise whether any trait groupings may be particularly strongly impacted by nitrogen pollution. In addition, I performed further detailed analysis on *Lasiommata megera*, the Wall Brown butterfly, which has been shown to be negatively impacted by nitrogen in studies undertaken elsewhere in Europe. I ran a similar spatio-temporal analysis to that mentioned above, but with the addition of two variables I hypothesised would be key drivers of *L. megera*: temperature in the previous September and elevation. The results for this additional analysis were presented separately.

I demonstrated that individual butterfly species vary in their relationships with nitrogen deposition and highlighted both species-level and potential trait level responses. Nine butterfly species were

negatively correlated with historic nitrogen deposition, and nine were negatively correlated with percentage change in nitrogen deposition at the site over time. Two species showed significant negative relationships with both historic nitrogen deposition and percentage change in nitrogen deposition over time: *Fabriciana adippe* (High Brown Fritillary) and *Hipparchia semele* (Grayling). These findings suggest that there is a strong correlative relationship between nitrogen deposition and the abundance of many butterfly species in GB. Other key drivers of change identified in this analysis were time, rainfall, and temperature in the current and previous year. I also demonstrated a strong relationship between abundance of *L. megera* and historic nitrogen deposition using the model with more detailed covariates. Initial summaries based on traits were inconclusive, not highlighting any particular trait groupings as being especially susceptible to the effects of nitrogen pollution.

The results of this study present the first correlative link between nitrogen deposition and negative impacts on terrestrial fauna in GB. It reinforces the importance of continued efforts to reduce emissions to protect the natural environment. It also provides a basis for further field and lab-based work to be undertaken to better understand the causal mechanisms behind the observed relationships.

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Literature Review

Introduction

Current research shows that terrestrial insects are experiencing overall global declines (Powney *et al.*, 2019; van Klink *et al.*, 2020). In the United Kingdom, several insect groups are in decline such as carabid beetles (Brooks *et al.*, 2012), hoverflies (Powney *et al.*, 2019), moths (Fox *et al.*, 2014, 2021), and butterflies (Thomas *et al.*, 2004). We are able to produce robust trend estimates for many species in the UK due to the history of biological recording in the country meaning that we are able to compare current population estimates to historic ones. For example, twenty countries in Europe contribute long-term butterfly monitoring data collected following a single standardised methodology to the European Butterfly Monitoring Scheme network. These data allows us to produce robust butterfly trend estimates for the UK which can be compared to trends in wider Europe. Shifts in species trends can be linked to pressures such as changes in land use, habitat loss, climate change, agricultural intensification. Recently, research has shown nitrogen deposition to be a potential factor impacting the abundance and distribution of butterflies (WallisDeVries and van Swaay, 2006; Wallisdevries and Swaay, 2013; Klop *et al.*, 2015; Roth *et al.*, 2021). Identifying these trends and the drivers behind them allows us to understand which species are at risk of decline or extinction, where impacts are most severe, and what factors are behind them. This knowledge allows conservation efforts to be used more efficiently to slow declines and protect species. In this study, we assessed the impact of nitrogen pollution on butterflies in the UK by applying complex modelling techniques to long-term abundance datasets. I did this at an individual species level for those we expected to be most impacted by nitrogen following research elsewhere in Europe, such as the Wall Brown *Lasiommata megera*, but also within trait-based groupings such as the various levels of diet specificity.

Given the importance of butterflies as biodiversity indicators (Thomas *et al.*, 2004; Van Swaay *et al.*, 2008) and current research showing declines in terrestrial insects, it is important to understand the potential link between declines in insect biodiversity and nitrogen pollution so that appropriate measures can be taken to lessen further negative impacts. In this study, I investigated whether atmospheric nitrogen pollution is linked to trends in butterflies in the UK. I start by providing a comprehensive overview of the relevant literature and discuss where research is currently lacking in the UK. Using an existing long-term dataset of butterflies, I modelled the effects of atmospheric nitrogen deposition and various other expected key driver variables, such as temperature, rainfall, and land-use intensity, on individual butterfly species. I used generalised additive mixed models to allow for non-linear responses of the individual butterfly species to individual driver variables.

Butterflies as indicators of change

It is important to have an understanding of the direction and causes of change in the natural environment, so that we can understand what is changing, why, and if we want to mitigate the change, how we do so. For this, we often use indicator species. Indicator species must be well-studied, respond relatively quickly to environmental change, widespread, easy to identify, and there must be a considerable amount of data available on their abundance and occurrence. Butterflies and birds are commonly used as indicators (Thomas, 2005; Van Swaay *et al.*, 2008; Gregory *et al.*, 2008).

Butterflies are considered to be one of the best indicators of environmental change due to their short lifecycle, meaning that they are sensitive to both short-term and long-term changes in their environment (Thomas *et al.*, 2004; Devictor *et al.*, 2012). The 59 species of butterflies present in the UK – composed of 57 residents and 2 migrants – have a rich and well-studied history, as the favoured organism of many collectors and scientists over the past few centuries. Butterfly collecting was historically a popular hobby, which has allowed us to build up extensive historical museum reference collections. We now have a thorough understanding of their ecology, habitat and food requirements, lifecycles, and breeding behaviours. Much is already understood about their occurrences and abundances across the UK, thanks to the work of organisations such as Butterfly Conservation and the Biological Records Centre, and data-collecting schemes such as the UK

Butterfly Monitoring Scheme (UKBMS). Moths, also in the order *Lepidoptera*, share many of the traits that make butterflies good indicator species, however, they are harder to identify, there are around 2500 species present in the UK (1850 of which are micro-moths, which often require dissection for identification), many are nocturnal and thus more difficult to record, and fewer comprehensive datasets exist for their abundance and distribution.

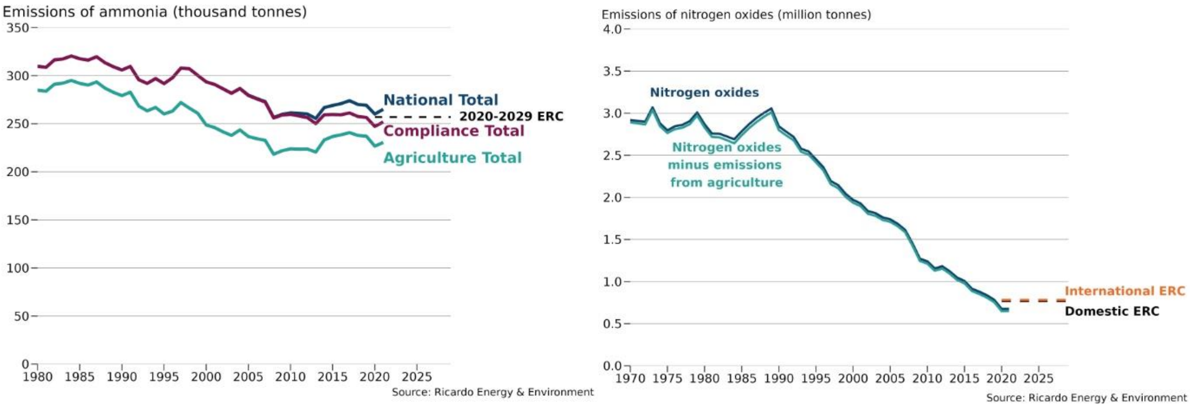
Lepidoptera are often grouped by their traits for analyses. Ecological traits can be broadly grouped into four categories: life cycle ecology and phenology, host plant specificity and characteristics, breeding habitat, and morphological characteristics (Cook *et al.*, 2022). Trait-based methods allow us to investigate responses of functional groups to environmental change and give more generalised, rather than species-specific, narratives. Information on habitat preferences has been used to classify butterfly species as either habitat generalist or specialist for the use in UK-wide butterfly indicators which influence policy (Brereton *et al.*, 2011). Species traits can be important predictors of phenological responses to climate change in UK butterflies, with a recent study finding that the species which have experienced the greatest advancement in their emergent dates were those with advanced overwintering stages, narrow larval diet breadth, and small range sizes (Diamond *et al.*, 2011). More recently, studies have shown that trait-based analyses can sometimes lead to inconsistent results, likely arising from the numerous interacting drivers of population change (Tordoff *et al.*, 2022). Trait-based approaches must therefore be well-considered and used with some caution.

Nitrogen deposition – general overview

Nitrogen is released into the atmosphere from various sources in two main forms: Nitrogen oxides and ammonia. Nitrogen oxides are emitted during the burning of fossil fuels, such as by cars and power stations. Ammonia is released into the environment from natural sources such as from forest fires and the decomposition of organic matter, as well as from manufactured sources. These human-made sources stem mainly from agriculture, including from the application of fertilisers and the storage and spreading of slurry. During slurry spreading and the application of ammonium-containing fertilisers, ammonia is produced as a result of the breakdown and volatilisation of urea. This volatilisation occurs naturally at the soil surface and is more likely to occur in hotter conditions.

Globally, nitrogen emissions rose sharply in the 20th century alongside the rapid industrialisation of the power, transport and farming industries. The invention and widespread adoption of fossil fuel power stations, motor vehicles, and synthetic fertilisers are amongst a few of the factors contributing to these steep rises in the emissions of both oxidised and reduced nitrogen (Fowler *et al.*, 2004; Lamarque *et al.*, 2010).

There has been a long-term fall in emissions of ammonia in the UK of 14 percent in the period 1980-2021, however emissions have remained relatively stable since 2007 (National Statistics, 2021). As the climate warms, increased volatilisation of ammonia is likely to lead to significant future rises in ammonia concentrations (Sutton *et al.*, 2013). Emissions of nitrogen oxides have also fallen, by 77 percent between 1970 and 2021 (DEFRA, 2021). This is because of policy surrounding emissions in nitrogen and sulphur leading to a curb in the emission of nitrogen oxides from transportation, factories and power stations. There was a larger than average yearly decrease of 13% in nitrogen oxide emissions between 2019 and 2020 due to the significant reduction in road traffic during the coronavirus lockdowns, however, it is likely that this will be an outlier.



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Source: Ricardo Energy & Environment

Figure 1a & 1b: trends in emissions of nitrogen oxides (1a) and ammonia (1b) in the UK since 1970 (1a) and 1980 (1b) (DEFRA, 2021; National Statistics, 2021).

This atmospheric nitrogen can be deposited back onto the land and water via dry or wet deposition, summarised clearly in Figure 2 (Plantlife, 2017). Rates of dry deposition from ammonia are highest locally to the source in areas of intensive agriculture, whereas the impacts of wet deposition are seen much further afield, often in remote areas which experience high rainfall. There is also much longer-range transport of nitrogen between countries. Therefore, deposition of pollutants varies to a great extent spatially in the UK (Figures 3a and 3b).

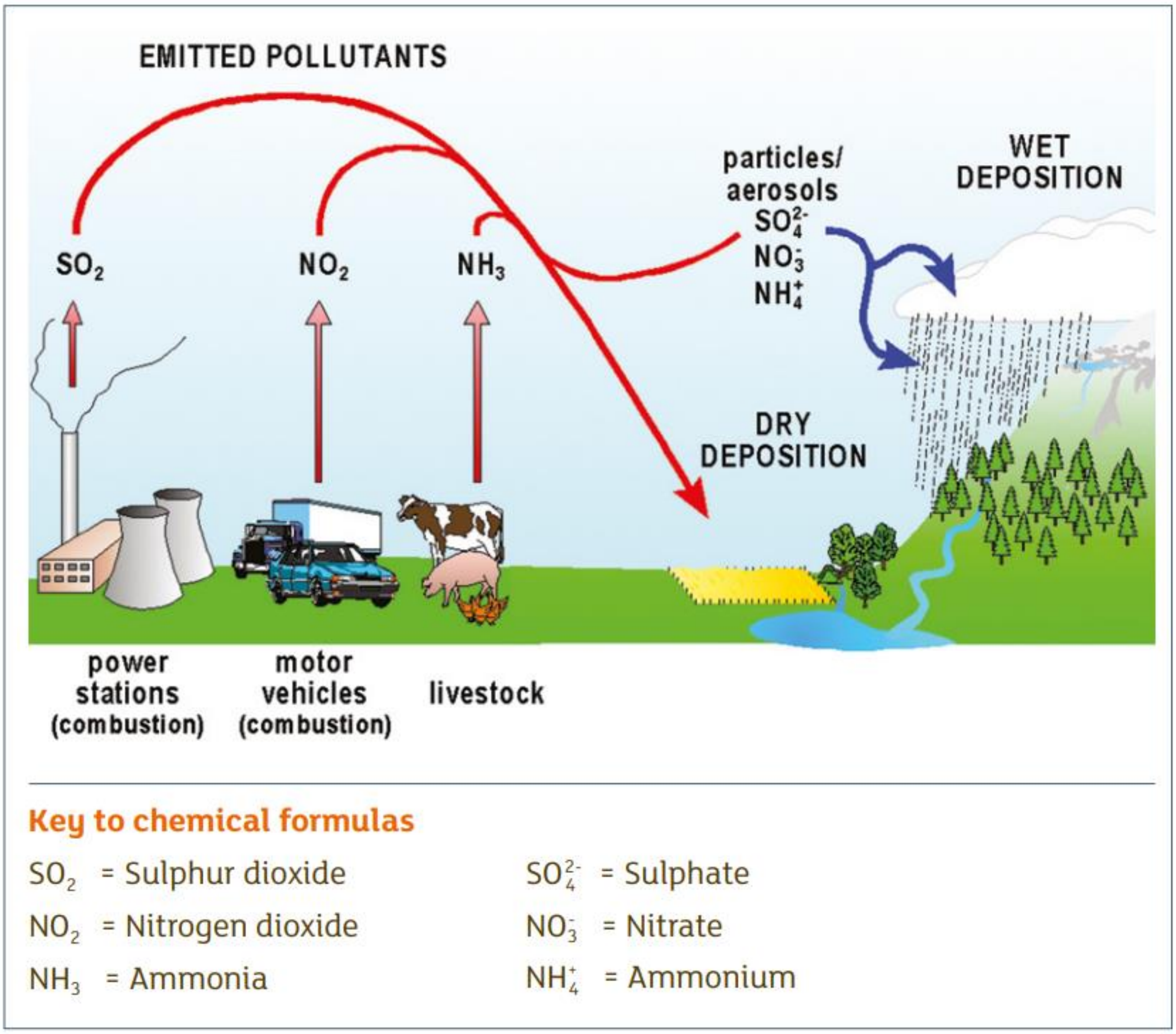
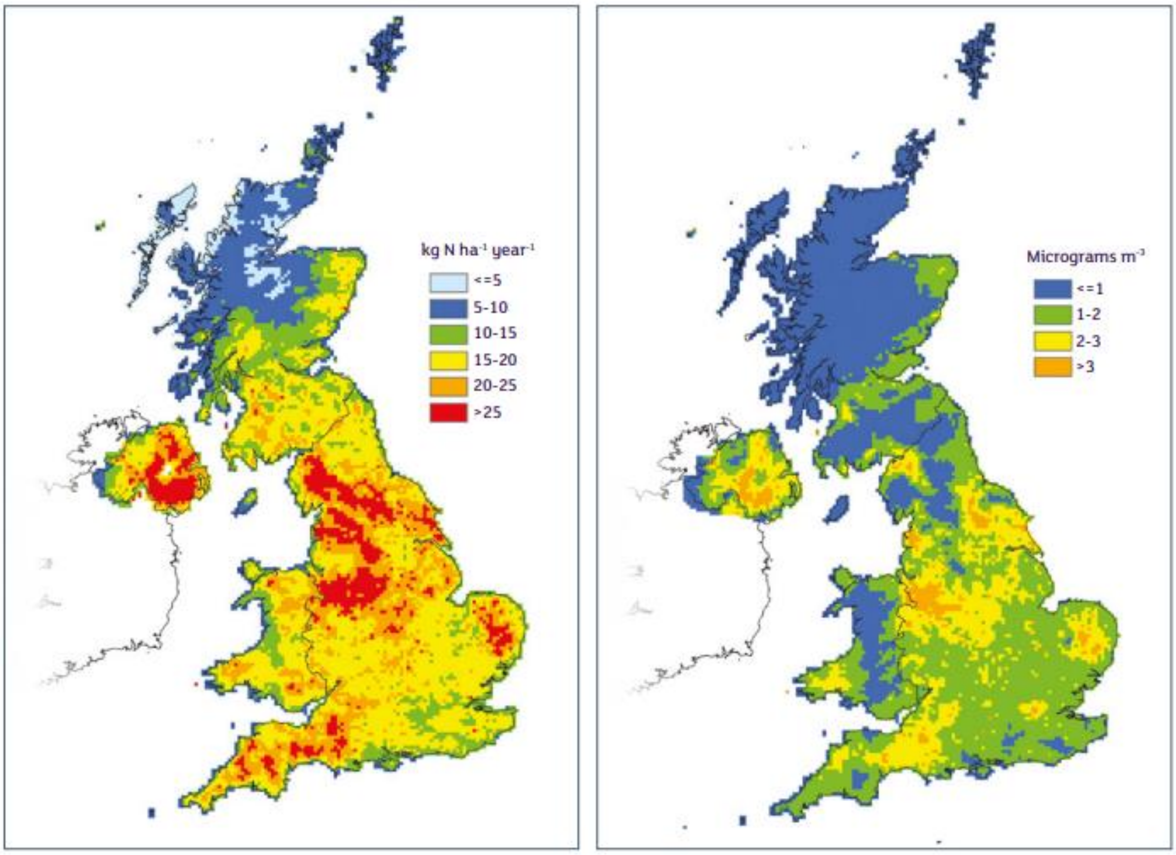


Figure 2: Nitrogen and sulphur emissions processes (Plantlife, 2017).



Figures 3a & 3b: maps of nitrogen deposition (3a) and ammonia concentrations (3b) in the UK (Plantlife, 2017).

Impacts of nitrogen deposition on biodiversity

Nitrogen is a key nutrient needed for the growth and development of plants and animals. Some species have become adapted to living in low-nitrogen environments, whilst others have adapted to high-nitrogen environments. Nitrogen deposition is therefore a key driver of ecosystem change, with highly variable habitat- and species-specific effects.

There are several mechanisms by which nitrogen negatively impacts plants, including: direct toxicity to individual plants; nitrogen enrichment; soil acidification; and plant biochemistry (Stevens *et al.*, 2018; Bobbink *et al.*, 2010). Nitrogen enrichment, the addition of nitrogen to a habitat where it was previously a limiting resource, encourages the growth of tall fast-growing nutrient-demanding species over the growth of smaller slow-growing stress-tolerant species (Hautier *et al.*, 2009). This can lead to the loss of species characteristic for the habitat, and an overall change in function.

Nitrogen deposition has been linked with an overall loss of plant species richness and therefore homogenisation of semi-natural habitats both in the UK and elsewhere through the analysis of long-term field survey data as well as experimental manipulations (Field *et al.*, 2014; Bobbink *et al.*, 2012). Various habitat types are sensitive to nitrogen deposition, including grassland, heathland, peatland/wetland, forest, shrubland and montane ecosystems (Sutton *et al.*, 2011). Stevens *et al.*, (2011) found that nitrogen and sulphur deposition impacted species and community composition in acid grasslands. In a field survey of five air pollution sensitive habitats (acid grassland, bog, upland heath, lowland heath, and sand dune) across Great Britain, high levels of nitrogen deposition were associated with reduced species richness and changed species composition in all habitats (Field *et al.*, 2014). Declines were seen in the diversity of mosses, lichens, forbs, and graminoids (herbaceous grass-like plants), whilst the cover of graminoids increased. This increase in grassiness of habitats has been linked to increases in fertility and decreases in pH by several studies (Maskell *et al.*, 2010; Stevens *et al.*, 2006; Storkey *et al.*, 2015).

Whilst the impacts of nitrogen pollution on plants are relatively well studied, impacts on fauna are less well understood. Many impacts of nitrogen pollution on invertebrates are mediated by plants, such as through the loss of reproductive habitat, food plants and suitable microclimatic conditions caused by the shifts in composition of plant communities (Nijssen *et al.*, 2017a). We can therefore expect that nitrogen deposition has affected many groups of invertebrates, particularly those which inhabit areas vulnerable to the impacts of nitrogen and rely on plants characteristic of these areas as larval food plants, adult nectar sources, and for overwintering habitat. The deposition of nitrogen to sensitive habitats is likely to have a lagged effect on their flora and fauna due to its cumulative nature and tendency to become an issue following critical load exceedance.

Changing butterfly abundance

Since 1976, there has been an overall general significant decline in the abundance and occurrence both habitat-specialist and wider-countryside butterflies (Fox *et al.*, 2015). 76% of butterflies showed long-term decreasing trends in abundance and/or occurrence, whereas only 47% increased in one or both. Butterfly trends in recent years (2005-2014) have slowed somewhat, with 52% decreasing in abundance and 47% decreasing in occurrence. Whilst these downwards trends may have lessened slightly, they are still cause for serious concern and undertaking research to understand the causes of these declines is vital if we are to mitigate them. Declines have also been seen in UK macro-moths with 41% of species exhibiting long-term decreases in abundance, four times as many than were increasing in abundance (Fox *et al.*, 2021). However, the situation is complex and more moths have been seen to increase than decline in distribution in this same period. It is important to note that trends are not produced for micro-moths or the majority of rare macro-moths.

Changing trends in the abundance and occurrence of butterflies in the UK reflect similar widespread global trends. In Europe, several countries have well-established long-term monitoring schemes similar to the UKBMS and have also shown long-term declines (Warren *et al.*, 2021). The European Grassland Butterfly Indicator shows a decline in widespread grassland species of 39% since 1990 in 16 European countries (Swaay *et al.*, 2019). In Flanders, a region of northern Belgium, 19 of their 64 indigenous species went extinct in the 20th century and 50% of the remaining species are currently endangered (Maes and Van Dyck, 2001). Grassland butterflies have decreased in abundance by 12% in Flanders since 1991 (Maes *et al.*, 2020). In the Netherlands, one of the best-studied countries in Europe with respect to butterflies, analysis of opportunistic butterfly records collected between 1890 and 2017, long before detailed monitoring schemes began, showed an estimated decline of 84% in a multi-species Indicator for butterflies (van Strien *et al.*, 2019). An analysis of data collected by the Butterfly Monitoring Scheme of The Netherlands between 1992 and 2007 found that 55% of common, widespread species experienced significant declines in distribution and abundance (Van

Dyck *et al.*, 2009). In the United States, there have been long-term declines in the abundance of butterflies, with a 1.6% annual decline over the past four decades seen in the Western states (Forister *et al.*, 2021) and a 2% annual decline over the past two decades in a Midwestern state (Wepprich *et al.*, 2019). In Japan, changes in land use have caused rapid declines in grassland butterflies, showing a very similar trend to research undertaken in Europe (Nakamura, 2011).

There are numerous mechanisms by which nitrogen and other stressors are likely to be causing changes to the abundance and occurrence of butterflies in the UK. It is vital that these mechanisms are understood and links to changes in butterfly numbers proven, where relevant, so that we can implement policy and ensure mitigation to prevent future declines and extinctions.

Nitrogen deposition impacts on butterflies

There have been various studies investigating the mechanisms by which nitrogen deposition affects butterflies. Nijssen et al. (2017b) provide a comprehensive overview of these mechanisms in their 2017 paper. The main causal mechanisms by which nitrogen is currently considered to impact fauna are chemical stress, changes in prey and host availability for predators and parasitoids, microclimatic cooling, decrease in reproductive habitat for ground-dwelling species, changes in diversity and abundance of host plants, and changes in host plant quality (Nijssen *et al.*, 2017b), as shown in Figure 4. The impacts of nitrogen-related chemical stress are mainly seen in aquatic fauna (Camargo and Alonso, 2006) and the changes in prey and host availability only applies to carnivores which butterflies are not; therefore only the latter four mechanisms will be considered in relation to butterflies. In this section I will cover each of these mechanisms in more detail, providing a summary of where the current research is progressing and where it may be lacking. Given recent research developments in this area, I also suggest two other mechanisms: alteration of floral odours and therefore foraging cues, and shifts in obligate interactions with ants.

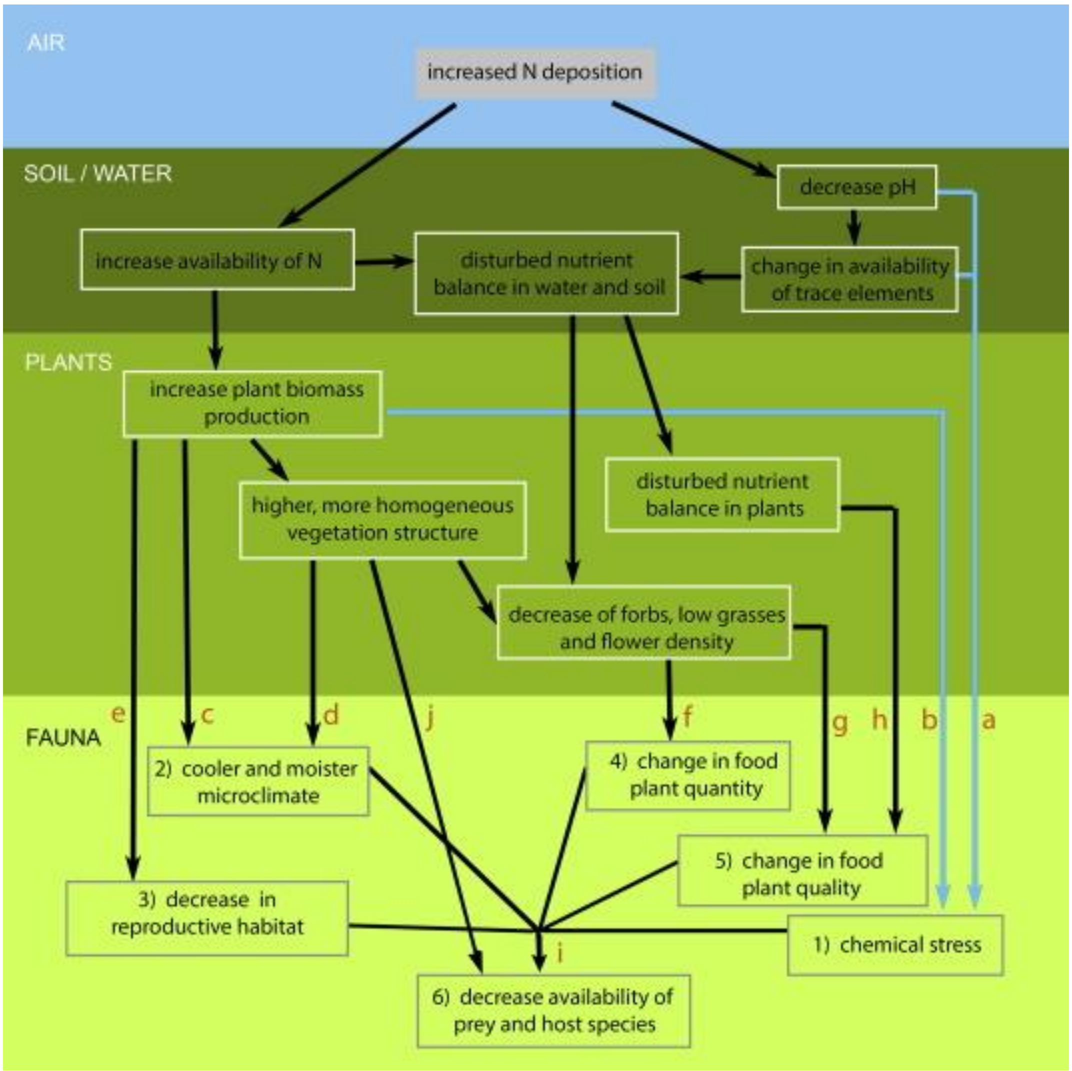


Figure 4: Pathways of nitrogen deposition with direct effects and indirect effects through soil and water, affecting vegetation and fauna (taken from Nijssen et al., 2017b). The different pathways (a to j) and basic effects (1 to 6) are further explained in the main text. Pathway a and b (blue arrows) occur exclusively in aquatic systems or moist soil types; other pathways can occur in aquatic as well as terrestrial habitats.

Buffered microclimates

Buffered microclimates occur as a result of the impact of nitrogen on vegetation, where increased plant growth due to nitrogen enrichment leads to the growth of taller, denser stands of vegetation. These stands block light from reaching the ground causing it to be cooler, and also decrease air circulation due to the density of biomass which increases humidity and soil moisture levels. This leads to cooling of the soil and litter, particularly in the spring. Causal links between changes in microclimate and long-term trends in fauna are lacking, but many correlative links have been drawn.

Some butterfly species overwinter as eggs or larvae and develop into pupa and then adults in spring. This spring larval development stage is highly reliant upon the existence of warm spring microclimates, where the ectothermic larvae rely on this solar irradiation to increase their body temperatures. Microclimatic cooling in spring due to nitrogen-mediated taller and denser plant growth has been shown to threaten thermophilous spring-developing butterfly species in the Netherlands (WallisDeVries and van Swaay, 2006). *Lasiommata megera*, known as the Wall Brown due to its preference for basking on walls, is a species present in the UK and has experienced substantial declines in abundance and occurrence since the 1970s (Fox *et al.*, 2015). Research in the Netherlands suggests that a recent decline in population may be due to microclimatic cooling caused by excess nitrogen deposition (Klop *et al.*, 2015). Butterflies characteristic of early successional habitats with short vegetation heights, such as heathlands dunes, have experienced declines linked with microclimatic cooling due to succession and the consequential increase in vegetation height (Schirmel and Fartmann, 2014). This mechanism may particularly impact species at the colder end of their natural range, therefore the north of the United Kingdom.

Decrease in reproductive habitat

Butterflies such as the Small Copper *Lycaena phlaeas* and Grizzled Skipper *Pyrgus malvae* oviposit on small patches of bare, disturbed ground, created by trampling of vegetation by grazers such as sheep, or through the creation of mounds by species such as the European mole (Streitberger *et al.*, 2014; Merle and Thomas, 2013). These areas of disturbed ground provide the ideal climatic conditions for larval development. As vegetation swards become taller and denser, we can expect that there may be fewer of these patches of bare ground available, and therefore a decrease in reproductive habitat for certain species of butterfly.

Changes in diversity and abundance of host plants

Nitrogen deposition and accumulation can have severe impacts on host-plant species such that they decrease in abundance and/or richness or disappear entirely. This shift in vegetation composition due to atmospheric nitrogen deposition is well-documented in Great Britain (Henrys *et al.*, 2011; Maskell *et al.*, 2010; Mcclean *et al.*, 2011; Stevens *et al.*, 2004). This trend has also been seen elsewhere in Europe (Bobbink, 1991) and further afield such as in North America (Clark and Tilman, 2008). A study in the Netherlands suggested that declines in the abundance of butterfly nectar plants, likely due to eutrophication, was a potential cause of widespread butterfly declines (Wallisdevries *et al.*, 2012). Declining richness and abundance of butterfly host plants will have direct impacts on the success of butterflies, particularly those that are monophagous (only feeding on one type of plant) because they have no alternative food sources should one become less abundant. Conversely, some butterfly species may benefit from this nitrogen-induced change in host plant diversity and abundance. For example, the European Peacock *Aglais io* and Small Tortoiseshell *Aglais urticae* are recognised as benefitting from increased nitrogen availability due to both having one main nitrogen-loving host plant, the Common Nettle *Urtica dioica* (Betzholtz *et al.*, 2013; Kurze *et al.*, 2017; Nilsson *et al.*, 2008; Öckinger *et al.*, 2006). *A. io* is a monophagous species feeding only on the Common Nettle, and *A. urticae* is an oligophagous species feeding only on *U. dioica* and the Small Nettle *U. urens*, an equally eutrophilic species (Cook *et al.*, 2022).

Changes in host plant quality

Excess nitrogen deposition can cause shifts in plant chemistry, changing the total nutrient concentrations but also the ratios between different nutrients. This can affect the nutritional quality of plants. Butterflies may shift to a different food source as one of their food plants becomes suboptimal nutritionally. This may not have a great impact on food-plant generalists, but for monophagous or oligophagous species this could lead to the removal of their main or only food plant as a food source, and subsequently lead to declines in the butterfly. It is understood that there are optimum levels of foliar nitrogen for the success of individual invertebrate species, and once exceeded the consumers will have lower survivorship and reproductive success. For example, an experimental study on the Sooty Copper *Lycaena tityrus* found that when females were given leaves with low or high leaf nitrogen concentration they did not preferentially feed on one over the other, but those that consumed leaves with higher nitrogen content produced offspring with reduced adult size and higher mortality rates (Fischer and Fiedler, 2000). Recent research in a North American prairie ecosystem found that when plants experienced nitrogen enrichment, plant-herbivore interactions are affected through shifts in plant traits that mediate the amount of herbivory, such as leaf hairiness and specific leaf area (Zettlemoyer, 2022). It is therefore possible that nitrogen enrichment in the UK is causing as of yet undiscovered shifts in the food-plant preferences of insects.

Alteration of floral odours

Air pollutants such as nitrogen oxides have been shown to chemically alter floral odours, disrupting the cues that foraging insects such as butterflies use to find and pollinate flowers (Ryalls *et al.*, 2022). This occurs even when pollutants are present at levels below the critical load. These impacts are likely to vary spatially and temporally with peak pollutant emissions, both on a small-scale such as due to impact of the emission of car exhaust diesel on road verges, and on a larger scale where pollutants have been transported far away from their source. We would expect this to impact monophagous and oligophagous butterflies in particular. There is some evidence that Lepidoptera may have the capability of learning to recognise pollutant altered floral odours, however this research was undertaken on a single species of moth and was carried out experimentally which may not reflect the moths natural behaviours (Cook *et al.*, 2020).

Shifts in obligate interactions with ants

In the UK, butterfly species from the family *Lycaenidae* such as the Adonis Blue *Polyommatus bellargus* and Large Blue *Phengaris arion* benefit from interactions with ants and are termed ‘myrmecophilous’ which translates to ant-loving. *P. arion* are a social (brood) parasite of *Myrmeca* ants, exploiting the parental care provided by them within their nests. Fourth instar larvae employ chemical and acoustic mimicry to encourage the ants to adopt them into their nests (Hayes, 2015), where they consume the ants’ brood and obtain the majority of their adult biomass. *P. arion* became extinct in the UK in 1979 due to changes in grazing intensity causing declines in *Myrmica* ants due to the loss of suitable microclimates within the habitat, and thus a loss of larval habitat for the butterfly (Thomas, 1980a). The butterfly has since been reintroduced to the UK from continental Europe, but only exists in a few specially managed reintroduction sites. Whilst this is not an example of nitrogen-related decline, it is clear that the decline in ants and thus the butterfly was caused by increased vegetation height leading to a lack of suitable larval habitat. It therefore seems possible that nitrogen-mediated increases in plant height could cause a similar cascade of events to occur to myrmecophilous butterflies.

These mechanisms are complex and often interlinked, so it is difficult to imply causation from correlation. For example, in a study of two threatened butterfly species in a German coastal heathland, succession occurred in their habitat due to land use change and nitrogen enrichment, which caused a combination of microclimatic cooling as well as grass encroachment and a subsequent loss of suitable host plants (Schirmel and Fartmann, 2014). In addition to the widespread impacts of pollution on butterflies, strong impacts may be seen much closer to the source of the pollution such as chicken farms. A recent study found a positive correlation between diversity of the butterfly and moth community and distance from a copper-nickel smelter, with diversity improving markedly once emissions were reduced (Kozlov *et al.*, 2022).

Other drivers affecting butterflies

There many other mechanisms not related to pollutant deposition by which stressors could be impacting butterflies including climate change, agricultural intensification, pesticide usage, land use change, changes in parasitoid populations, the spread of invasive non-native plant species, and over-collection.

As a result of climate change, the United Kingdom is projected to experience warmer and wetter winters, hotter and drier summers, and more frequent and intense weather extremes. The climate has long been understood to be a key factor influencing the distribution of plant species (Holdridge, 1947), and therefore a shifting climate is expected to have a large impact on plant distributions, especially at their range margins. This will directly impact butterflies (Wilson and Maclean, 2011), especially those with a specific diet and a narrow niche width through the loss of host plants, general degradation of habitat, and disappearance of necessary microclimatic conditions. Climate change has already had a direct impact on many butterfly species. For example, the distribution of the Mountain Ringlet *Erebia epiphron* which is found in mountainous regions of Europe retracted 130–150 metres uphill in Britain between 1970 and 2005 due to climate warming (Franco *et al.*, 2006).

Another impact of the changing climate will be changes in the distribution and abundance of butterfly parasites. The wider countryside Small Tortoiseshell butterfly *Aglais urticae* experienced declines in the UK between 2003 and 2008, correlated with the arrival and climate-change induced spread of a parasitoid, *Sturmia bella* (Gripenberg *et al.*, 2011). *S. bella* is a tachinid fly that commonly parasitizes butterflies in the family Nymphalidae in continental Europe. However, it is important to note that these links are only correlative, and that there are initial indications that *A. urticae* is recovering in recent years with an encouraging, although not statistically significant, 146% rise in abundance in the period 2005-2014 in the UK (Fox *et al.*, 2015). Conversely, the warming climate may reduce the impact of other parasites. The North American Monarch butterfly *Danaus plexippus* is parasitized by environmentally transmitted spores of the protozoan *Ophryocystis elektroscirrha*. Infected butterflies experience a reduction in their survival and reproductive success, shortened lifespans, and decreased flight ability (Sánchez *et al.*, 2021). Warmer temperatures due to climate change have been linked with a reduction in the time that the parasitic spores can persist in the environment, and therefore have led to a reduction in infection probability.

The spread of invasive non-native plant species modifies the composition, dynamics and structure of ecological communities such that they become less suitable for butterflies by replacing native plants and reducing larval survival rates (Tallamy and Shropshire, 2009; Davis and Cipollini, 2014). An analysis of data from the Swiss Biodiversity Monitoring programme showed that invasive plant richness is negatively correlated with butterfly richness, with 24% of butterfly species declining in the presence of invasive plants (Gallien *et al.*, 2017). The species that declined tended to be dispersal-limited.

Countries across Europe have experienced long-term declines in the availability of semi-natural habitats such as heathland and unimproved grassland due to the development of land for construction and farming. Habitat loss directly impacts butterflies, especially those that are habitat specialists, by reducing the amount of available space for vital processes such as breeding and feeding (Warren *et al.*, 2021). Agricultural intensification in the 1900s directly caused habitat loss through conversion of land to arable crop-land. Changing farming practices may have also impacted butterflies through the increased use of pesticides harmful to non-target invertebrates (Gilburn *et al.*, 2015). In addition to the loss of habitats, the remaining habitat patches have become increasingly fragmented. This fragmentation can isolate invertebrate populations with relatively low mobility, as described for the butterfly *Euphydryas aurinia* (Marsh Fritillary) in the UK (Botham *et al.*, 2011).

In the Victorian times, collection of butterfly specimens was a popular hobby, and was thought to have led to considerable disturbance to certain populations of rarer butterflies. Fortunately, specimen collection for display purposes has become much less common in the last century. The availability of comprehensive field identification guides and wealth of free resources available on the internet means that it is no longer necessary to keep a reference collection of specimens in many cases. Sample collection is still needed for many scientific studies, however, these are regulated by ethics reviews to ensure minimal impact on the wild populations. Where butterfly reintroductions have taken place more recently, the exact location of release is often kept secret until the breeding population has properly established, to reduce the potential for disturbance from collectors or photographers.

Data Challenges

Butterfly records can come from many sources, from historical museum specimens, to incidental records submitted by citizen scientists to iRecord, to structured surveys such as the UKBMS. We can combine data from multiple sources for use in analysis through integrated modelling, for example, (Isaac *et al.*, 2020) combined data from three sources, each with a different observation process, to fit integrated species distribution models. However, integrated modelling of data from multiple sources requires the use of complex models and often necessitates higher computing power. Due to the thoroughness of the UKBMS, we can model changes in butterfly populations from 1976 onwards with relative accuracy. Extending further back in time is complex and the simplest option is to use this data individually. For other invertebrate taxa, for example *Diptera* (true flies), similar analysis would not be possible because an official survey schemes does not exist, perhaps due to a lack of taxonomic experts for the "less charismatic" groups such as *Diptera*.

Air quality monitoring and mapping is a rapidly advancing field, with considerable developments in the last decade. Site-based air quality monitoring occurs at sites across the UK as reported by APIENS. Point sampling is costly to set up and requires ongoing maintenance of field equipment. Data obtained from these air quality monitors are used to model the expected air pollution across wider areas, such as the entire UK.

In order to investigate the effects of air pollution on butterflies, we must match the locations of the biological records to air quality data that is representative of the area. To do this, we could decide only to use butterfly data from sites where co-located air quality monitoring takes place, for example at Environmental Change Network or Natural England's Long Term Monitoring Network sites. However, this greatly limits the amount (both spatially and temporally) of butterfly data for inclusion and therefore would necessitate the exclusion of many species from any analysis. Therefore, when doing a UK-scale analysis it makes sense to match biological records to modelled air pollution data, such as from FRAME (Tomlinson *et al.*, 2020), CBED (Levy *et al.*, 2020), or EMEP4UK (Scheffler and Vieno, 2022).

Aims and objectives

This study aims to assess the impacts of atmospheric nitrogen pollution and several other driver variables, such as temperature, rainfall, and land-use intensity, on butterfly species in the UK through spatio-temporal modelling of survey data from the UK Butterfly Monitoring Scheme (UKBMS). In this study, I performed a broad species analysis and a species-specific modelling study for *Lasiommata megera*, the Wall Brown butterfly. The rationale behind these two sections is described below.

Broad species analysis

In this study, I tested the effects of nitrogen deposition and various other drivers on the abundance of individual butterfly species. The direction of significant trends for nitrogen pollution driver variables was summarised across all species and also within trait groupings. Species were grouped by traits and individual species were selected from these groups for in-depth analysis. Three trait-based hypotheses were formed *a priori*:

> Hypothesis 1: Monophagous habitat specialists have experienced declines related to nitrogen deposition.
>
> Hypothesis 2: Butterfly species that emerge very early in the spring have experienced the greatest nitrogen-related declines.
>
> Hypothesis 3: Butterflies with nitrogen-loving hostplants have increased in abundance in relation to nitrogen deposition.

A brief outline of the rationale behind each of these hypotheses is outlined below. In addition to the species mentioned in the hypotheses outlined above, models were fit for all butterfly species in Great Britain wherever possible. Data from Northern Ireland were excluded due to expected differences in the relationships with response variables. Overall results from this wider-species analysis are presented, but ecological mechanisms are only considered in the context of the above hypotheses. A subset of butterfly trends were discussed in further detail with respect to the above hypotheses, with the aim of exploring the potential ecological causes of the trends.

Monophagous habitat specialists

Some butterflies are specialists, feeding on one (monophagous) or few (oligophagous) hostplants as a caterpillar, whereas others are more generalist and feed on several species (polyphagous). Those that feed on a variety of species therefore have greater choice in what they can feed on, and should one food plant not be available for whatever reason, it does not lead to a large loss in fitness because they can feed on a different plant. The choice of food plant(s) is largely controlled by where the parent oviposited, given that food plant preference is limited in the larval stage by dispersal ability. Monophagous species do not experience this ability to choose alternative food sources; if they cannot access their single food plant, the larva simply have nothing to eat and will starve. This is particularly a problem when the single hostplant is scarce itself, for example being specialist to a specific habitat.

The likely mechanism for the link between habitat specialism and declines related to nitrogen deposition is due to nitrogen-mediated shifts in the availability of hostplants. To test this hypothesis, I chose two closely related monophagous habitat specialists with somewhat similar life histories and the same sole larval food plants: *Polyommatus bellargus* (Adonis Blue) and *Polyommatus coridon* (Chalk Hill Blue). Both *P. bellargus* and *P. coridon* have experienced significant declines in distribution in the past half century, however, in this time period the abundance of *P. coridon* has remained stable whilst the abundance of *P. belargus* has increased significantly (Fox *et al.*, 2023). The two species differ in some key life history traits, with *P. belargus* being bivoltine and overwintering as a caterpillar, whereas *P. coridon* is univoltine and overwinters as an egg.

Larva of both butterfly species feed on *Hippocrepis comosa* (Horseshoe Vetch), a perennial flowering plant found on chalk and limestone grasslands which flowers from May to late July. The food plant is present mainly in lowland areas of England and Wales but has declined notably in the last century due to loss and degradation of its host grassland habitat (Stroh *et al.*, 2023). *Hippocrepis comosa* prefers sites that are very light (Ellenberg L of 8), dry (Ellenberg F of 3), with basic (Ellenberg R of 8) and infertile (Ellenberg nitrogen of 2) soil (Hill *et al.*, 2004). At a height of 25 cm tall (Hill *et al.*, 2004) and with a preference for nitrogen-poor infertile sites, nitrogen enrichment due to excess atmospheric nitrogen deposition could lead to declines in the stress-tolerant *H. comosa* as it is outcompeted by other plant species in the newly nutrient-rich habitat. This could then lead to reduction in dependent butterfly species.

Both *P. bellargus* and *P. coridon* are myrmecophilous, meaning that they form mutualistic relationships with ants. *Polyommatus belargus* can benefit from relationships with various species of ants, including with *Myrmica sabuleti* and *Lasius alienus*, whereas *P. coridon* relies on a single species of ant, *Lasius flavus* (Eeles, 2019). Nitrogen-mediated increases in plant height and density may cause microclimatic cooling at the ground level, making it unsuitable for the establishment of ant nests. This has not yet been studied in the field for *P. bellargus* or *coridon*, however, parallels may be drawn with the decline of *Phengaris arion*, the Large Blue butterfly, which became extinct in Great Britain in 1979 (Thomas, 1980b). Many colonies of *P. arion* were lost after the destruction of their habitat through changes in land use in the early 1900s. Efforts were made to protect remaining populations, however, a limited understanding of their ecology led to their ultimate extinction. The key factor that had not been considered during conservation management of the species was that reduced rabbit grazing due to the disease myxmatosis had caused increased grass sward height, thereby drastically reducing microclimatic habitat suitability for the host ant *Myrmica sabuleti*.

Similarly, it may be the case that nitrogen-induced increases in plant height could lead to decreased habitat suitability for the ant hosts of *P. bellargus* and *P. coridon*, ultimately leading to their declines.

Species that emerge as adults early spring

Temperatures fluctuate throughout the year and can have a huge impact on the success of butterfly larvae. Larva of some species awaken from overwintering in the early spring which allows for utilisation of longer season length, however, conditions can be more volatile at this time of year. Often, early spring emergers will rely on basking to increase their body temperatures, as the surrounding air is too cool to heat them sufficiently to allow movement. In order to bask, individuals require areas of bare, open ground or leaf litter. With nutrient enrichment due to nitrogen deposition, increased plant growth may decrease the amount of bare ground available and increase the sward height, thus causing microclimatic cooling and generally reducing the amount of bare ground available. If individuals cannot warm themselves sufficiently, they will be much less likely to survive and consume enough nutrients to pupate and ultimately reach adulthood. Larva that emerge later in the spring or in summer are much less likely to face this issue given that they generally experience much higher average temperatures.

To test hypothesis 2, I chose to look at two closely-related species which emerge as adults in the early spring; *Boloria euphrosyne* (Pearl-bordered Fritillary) and *Boloria selene* (Small Pearl-bordered Fritillary). Both of the *Boloria* species selected overwinter as larva and are single-brooded, although partial second generations can occur in Southern England for both species. *Boloria euphrosyne* is the earliest of the British fritillaries to emerge as an adult, often appearing as early as mid-April. After overwintering in its fourth larval instar, *B. euphrosyne* awakens in March and basks on low-lying leaf litter to warm itself (Eeles, 2019). Similarly, *B. selene* overwinters in its third or fourth larval instar and awakens in March. As adults, both species feed primarily on *Viola riviniana* (Common Dog-violet), although can also feed on other *Viola* species such as *Viola palustris* (Marsh violet) and *Viola canina* (Heath violet). Given that both of these butterfly species emerge very early in the year, we expect the larva to rely on the presence of warm microclimates in order for them to reach sufficient body temperature for movement. To enable some comparison with species which emerge later in the year and are therefore less reliant on warm microclimatic conditions in the spring, I also looked at the effects of nitrogen on *Maniola jurtina* (Meadow Brown) and *Pyronia tithonus* (Gatekeeper), both of which are also in the Nymphalidae (brown) family of butterflies. *Maniola jurtina* typically emerge from early June, whilst *P. tithonus* generally emerge from late June.

Species with nitrophilic host plants

As introduced in hypothesis 1, we expect that the number and characteristics of a butterfly's larval host plant(s) will influence the direction of the effect of nitrogen on individual butterfly species. Where hypothesis 1 discussed the negative effects of nitrogen on monophagous habitat specialists, it is also important to think about the opposite question of whether certain species will benefit from excess nitrogen through changes in the availability of their host plant(s). I hypothesise that butterflies with nitrogen-loving hostplants have increased in abundance due to nitrogen deposition. Of the resident British butterfly species, two were chosen to study for this hypothesis: *Aglias io* (Peacock) and *Aglais urticae* (Small Tortoiseshell). *Aglais io* larvae feed primarily on *Urtica dioica* (Common Nettle) and occasionally on *U. urens* (Small Nettle) and *Humulus lupulus* (Hop). *Aglais urticae* larvae are a genus specialist, feeding only on *U. dioica* and *U. urens*. Whilst *Vanessa atalanta* (Red Admiral) and *V. cardui* (Painted Lady) also feed primarily on *Urtica sp.*, they are primarily migrants from the continent and Northern Africa, unlike *Aglais io* and *A. urticae*. Therefore, the former two are not suitable candidate species on which to investigate this hypothesis because the earlier stages of their life cycle take place mostly in other countries and will potentially be impacted by different drivers. *V. atalanta* is considered resident in Southern England, but these resident individuals make up a small proportion compared to the migrants, which is indistinguishable for the purposes of this analysis.

A possible mechanistic cause behind this proposed hypothesis is that changes in species composition due to nitrogen deposition has favoured fast-growing, competitive, nitrogen-loving species such as *Urtica spp.*, ultimately leading to increases in their abundance and occurrence. In turn, this increased availability of nitrogen-loving plants that act as butterfly larval host plants may lead to an increase in the abundance and/or occurrence of the adult butterflies. A trait-based analysis of Swedish butterflies found that range expansion was greatest in habitat and diet generalists, species typically active during warm conditions, and forest specialists (Betzholtz *et al.*, 2013). They demonstrated a positive relationship between range expansion in monophagous species and the extent to which the larval diet favoured nitrogen.

Lasiommata megera (Wall Brown)

Lasiommata megera (Wall Brown) has undergone continuous declines in the UK in the past half century, with abundance and distribution falling by 86% and 87% between 1976-2019 and 1970-2019 respectively (Fox *et al.*, 2023). It has recently been classified as endangered according to IUCN Red List criteria (Fox *et al.*, 2022). Understanding the drivers of change in the species' abundance and distribution are important if the UK aims to mitigate further decline. I hypothesise that nitrogen deposition and rising autumn temperatures have contributed to observed declines in *L. megera* in Great Britain. In this section of the analysis, I assess whether atmospheric nitrogen pollution is a key driver of decline in *L. megera* in Great Britain by modelling the effect of nitrogen deposition and other driver variables, including climatic drivers, on the species' abundance using long-term data from the UK Butterfly Monitoring Scheme. The explanatory variables in the modelled differed to those used in the broad species analysis to better represent the ecological needs of *L. megera*.

In the Netherlands, correlative research has shown that declines of *L. megera* are greatest in regions with the highest nitrogen deposition (Klop *et al.*, 2015). As discussed in detail in Chapter 1, there are four main pathways for effects of nitrogen on butterflies; changes in host plant quality, shifts in abundance and diversity of host plants, reduced reproductive habitat, and microclimatic cooling due to shading from increased growth of taller plants (Nijssen *et al.*, 2017a). An experimental study found only beneficial effects of nitrogen addition on larval performance of *L. megera*, thus it is unlikely that changes in the nutritional quality of host plants is behind their decline (Klop *et al.*, 2015). However, it is worth noting that the effects of nitrogen addition on adult performance and fecundity are unstudied. *Lasiommata megera* is a generalist that feeds on a variety of grasses (Cook *et al.*, 2022) with a range of Ellenberg nitrogen values (Hill *et al.*, 2004) which are shown in brackets, including *Agrostis* spp., *Avenella flexuosa* (3), *Brachypodium rupestre, Brachypodium sylvaticum* (5), *Dactylis glomerata* (6), *Elymus repens* (7), *Holcus lanatus* (5), and *Poa annua* (7). Given the relatively high Ellenberg nitrogen values of most host plants (with the exception of *A. flexuosa*), we expect that they may be favoured by nitrogen addition, and it is therefore unlikely that declines in *L. megera* are caused by it experiencing a loss of host plants. Therefore, reduced reproductive possibilities due to a loss of suitable areas for oviposition, and microclimatic cooling are the mechanisms most likely to be affecting *L. megera*. These mechanisms are difficult to disentangle because they are both caused by nitrogen-mediated shifts in plant community composition and structure. Field evidence suggests that the main pathway by which nitrogen affects *L. megera* is through microclimatic cooling during larval development of its first early-spring emerging generation (WallisDeVries and van Swaay, 2006; Klop *et al.*, 2015).

Climate warming has also been linked to declines in *L. megera*. European butterfly species follow general gradients in voltinism, whereby the number of generations in a single year is positively correlated with the length of the summer growing season. Individual species differ in their voltinism depending on their spatial location, life history, host plants, and thermoregulation strategies. A species may evolve to have more or fewer generations per year depending on the pressures they face and the impact this has on their fitness. Warming autumn temperatures can encourage the mostly bivoltine *L. megera* to pursue a third adult generation late in the year, as opposed to going directly into larval diapause for the winter. Environmental conditions are often unsuitable for the third generation *L. megera*, leading to a high mortality rate and a ‘lost generation’ which has potential negative impacts on future population persistence (Van Dyck *et al.*, 2015).

In this section of the study, I tested the effects of nitrogen deposition and several other drivers on the abundance of *L. megera*. I hypothesised that nitrogen deposition and autumn temperature in the previous year were key drivers of change in *L. megera* abundance. The analysis done in this section differs from the broad species analysis through the use of one additional and one changed driver variable. Average temperature in the previous September was used in place of average temperature in the previous June to account for the potential negative effects of the third 'lost generation’ of adults arising from warm autumn temperatures. Elevation was also added as an explanatory variable in the model. As in the broad species analysis, butterfly data from Great Britain were used.

Methods

Data

This section provides an overview of the butterfly, plant, and driver datasets used throughout the study.

Butterflies

The United Kingdom Butterfly Monitoring Scheme (UKBMS) began recording data in 1976 and now records information on 71 species at over 2,000 sites per year using a combination of fixed transects, the Wider Countryside Butterfly Survey (WCBS), timed counts, and egg and larval nest monitoring. Long-term temporal trends were created for 56 of the 59 UK butterflies in 2015 (Fox et al., 2015), not including the Chequered Skipper *Carterocephalus palaemon*, Cryptic Wood White *Leptidea juvernica* and Mountain Ringlet *Erebia epiphron* due to insufficient data being available for those species. In this study, I used the Generalised Abundance Index (GAI) which takes into account all butterflies across the whole season to create an overall index of maximum species abundance at a site in a particular year, whilst accounting for seasonal variation and missing data (Dennis *et al.*, 2016). In the UKBMS GAI dataset, values of '-2' are given where the species was present but insufficient data were available to calculate the GAI.

Prior to my analysis, I transformed all values of '-2' to 'NA'. In addition, for the broad species analysis the data were filtered on a species-by-species basis to only include sites where the particular species had been observed at least once within the time series. Similarly for the further *Lasiommata megera* analysis, the data were filtered to only include sites where *L. megera* had been observed greater than or equal to two times within the time series in an attempt to be representative of sites where the species is resident.

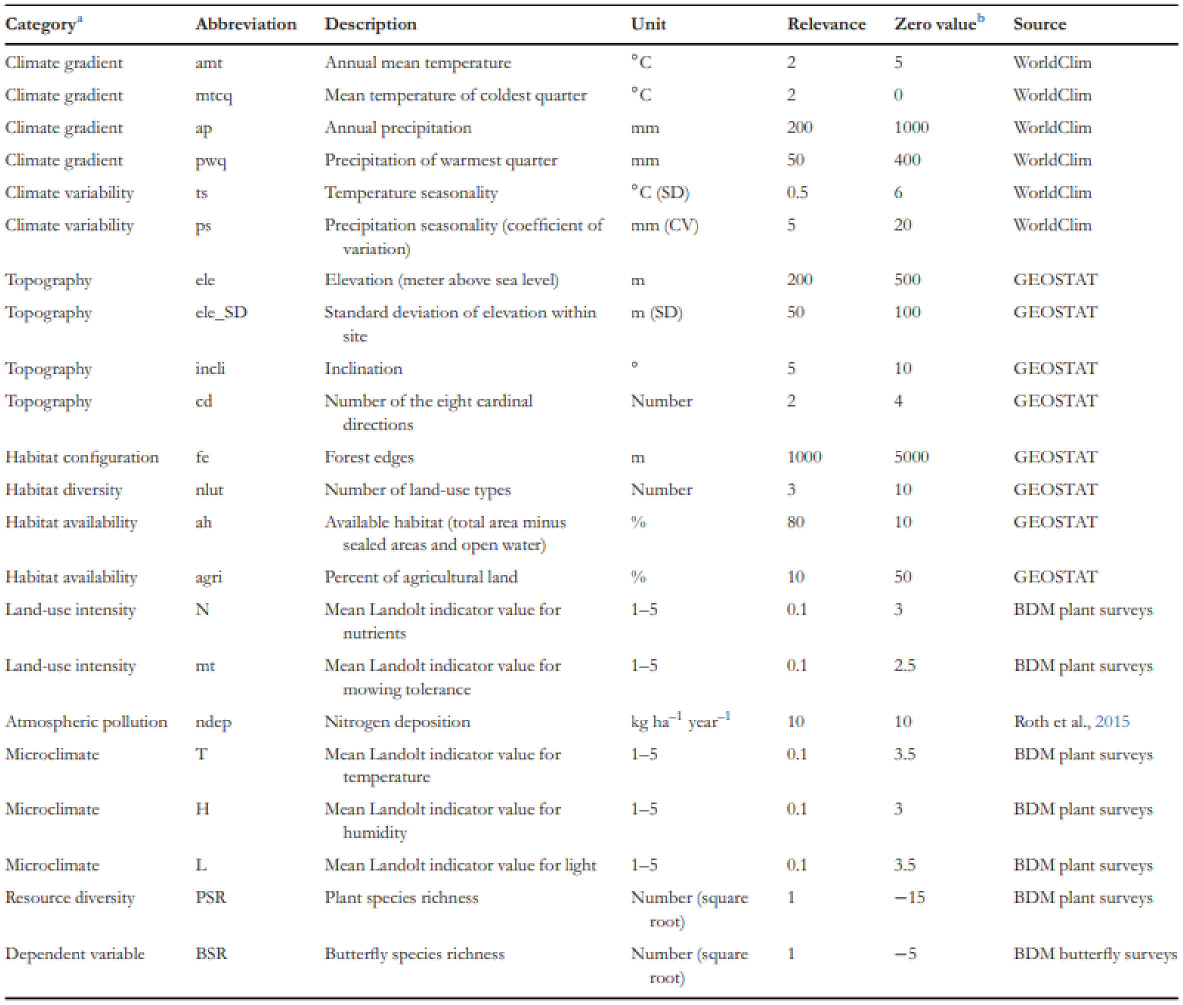
Butterfly traits

Butterfly trait data were obtained from a recent database (Cook *et al.*, 2022) which includes data on each butterfly species' life cycle, host plants, habitat, and trends.

Driver variables

Butterfly abundance and richness are affected by many species-specific driver variables, which vary depending on their life history, phenology, geographic location, and habitat preference. A comprehensive review of studies that applied multivariate regression models with several predictor variables and used variation in butterfly species richness as the response variable (Roth *et al.*, 2021) identified 22 key driver variables (Table 1) representing the key categories of: climate gradient, climate variability, topography, habitat configuration, habitat diversity, habitat availability, land use intensity, atmospheric pollution, microclimate, and resource diversity. We used these categories as a guide and collated similar data wherever possible, as summarised in Table 2.

Table 1: Predictor variables used to explain butterfly species richness in Roth et al., 2021.



Category[a]	Abbreviation	Description	Unit	Relevance	Zero value[b]	Source
Climate gradient	amt	Annual mean temperature	°C	2	5	WorldClim
Climate gradient	mtcq	Mean temperature of coldest quarter	°C	2	0	WorldClim
Climate gradient	ap	Annual precipitation	mm	200	1000	WorldClim
Climate gradient	pwq	Precipitation of warmest quarter	mm	50	400	WorldClim
Climate variability	ts	Temperature seasonality	°C (SD)	0.5	6	WorldClim
Climate variability	ps	Precipitation seasonality (coefficient of variation)	mm (CV)	5	20	WorldClim
Topography	ele	Elevation (meter above sea level)	m	200	500	GEOSTAT
Topography	ele_SD	Standard deviation of elevation within site	m (SD)	50	100	GEOSTAT
Topography	incli	Inclination	°	5	10	GEOSTAT
Topography	cd	Number of the eight cardinal directions	Number	2	4	GEOSTAT
Habitat configuration	fe	Forest edges	m	1000	5000	GEOSTAT
Habitat diversity	nlut	Number of land-use types	Number	3	10	GEOSTAT
Habitat availability	ah	Available habitat (total area minus sealed areas and open water)	%	80	10	GEOSTAT
Habitat availability	agri	Percent of agricultural land	%	10	50	GEOSTAT
Land-use intensity	N	Mean Landolt indicator value for nutrients	1–5	0.1	3	BDM plant surveys
Land-use intensity	mt	Mean Landolt indicator value for mowing tolerance	1–5	0.1	2.5	BDM plant surveys
Atmospheric pollution	ndep	Nitrogen deposition	kg ha^{-1} year^{-1}	10	10	Roth et al., 2015
Microclimate	T	Mean Landolt indicator value for temperature	1–5	0.1	3.5	BDM plant surveys
Microclimate	H	Mean Landolt indicator value for humidity	1–5	0.1	3	BDM plant surveys
Microclimate	L	Mean Landolt indicator value for light	1–5	0.1	3.5	BDM plant surveys
Resource diversity	PSR	Plant species richness	Number (square root)	1	−15	BDM plant surveys
Dependent variable	BSR	Butterfly species richness	Number (square root)	1	−5	BDM butterfly surveys

Table 2: Predictor variables used to explain butterfly species abundance in this analysis.

Category	Description	Unit	Source
Atmospheric pollution	Historic nitrogen deposition	kg/ha	Levy *et al.* (2020)
Atmospheric pollution	Change in nitrogen deposition at a site over time (1986-2012)	%	Levy *et al.* (2020)
Atmospheric pollution	Historic sulphur deposition	Kg/ha	Levy *et al.* (2020)
Climate gradient	June temperature in year of survey	°C	Met Office *et al.* (2023)
Climate gradient	June temperature in year preceding survey	°C	Met Office *et al.* (2023)
Climate gradient	June rainfall in year of survey	mm	Met Office *et al.* (2023)
Land use intensity	Land use intensity	%	Rowland *et al.* (2020)
Topography	Elevation	m	Ordinance Survey (2023)

Climate

The impact of weather variables on butterflies is complex and species dependent (Roy *et al.*, 2001), with species responding differently to changes in weather depending on the species' voltinism, overwintering stage and timing of their flight period, amongst other factors. The analysis by Roy *et al.* (2001) showed associations between monthly rainfall and temperature with most UK butterfly species. The strongest associations for both univoltine and bivoltine species were with the summer (June) temperature of the current year. The success of several species was also strongly correlated with the summer temperature of the previous year. The direction of this correlation was species-dependent, with species that overwinter as adults benefitting from warm summers in the previous year, whereas some satyrids show negative associations with warm and dry previous summers due to desiccation of their food plants. Given this, it is reasonable to use different driver variables for each species, dependent upon their life history. I represent summer temperature from the current and previous years using the average June temperature from each year, and summer rainfall as the June rainfall from each year. Whilst these may not be ideal for all species, particularly those for which climate associations are still untested, they will be used in this analysis to provide a generalised approach.

Atmospheric deposition

Following analysis undertaken by Henrys *et al.* (2011), we calculated a measure of historic total nitrogen deposition as the mean of the estimates for 1996, 1997 and 1998 (the approximate mid-point of the butterfly data time series) from the Concentration Based Estimated Deposition (CBED) model for deposition to moorland. Despite looking at species characteristic of all habitats, I decided to use nitrogen deposition to moorland as the data source, given that the grid average nitrogen includes agricultural ammonia emissions which strongly skew them. I do not take into account cumulative deposition but recognise its importance. Cumulative deposition does not give a picture of change over time in deposition at a particular location, which is an important factor to consider. We therefore use a measure of change over time in nitrogen deposition at a site, measured as the percentage change between deposition in 1986 and 2012. Sulphur deposition to moorland was calculated similarly to historic nitrogen from CBED, as the mean of the estimates for 1996, 1997 and 1998. CBED modelled data were available at 5x5 km resolution.

Land use intensity

The UKCEH Land Cover Map (LCM) provides information on land cover in the UK at 1km resolution in 21 target habitat classes. To calculate land use intensity, we calculated the sum of arable land and improved grassland in each 1km square in 1990 to give us a representation of land use intensity which is equal to the proportion of intensive habitats within the square. For example, if 5% of a square was classified as arable land and 12% as improved grassland, the square would have a value of 17 out of 100 for land use intensity. This gives an idea of intensity within the square that each UKBMS transect is placed but does not necessarily represent the land use intensity of the surrounding area. Because butterflies are mobile species, it is important to include a measure of this. We therefore calculated the land use intensity of the surrounding $9km^2$ for each transect, as represented in figure 5.

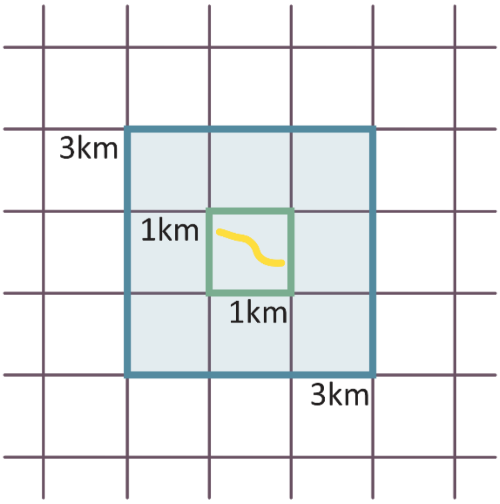


Figure 5: Representation of mean land use intensity in the 3km by 3km square (highlighted in blue) surrounding the UKBMS transect line (yellow).

Ideally, we would include pesticide application rates in our model as a covariate; however, this data is not available as a time series for the UK. Modelled pesticide application rates for Great Britain are available for the static time point of 2012-2017 (Jarvis *et al.*, 2020), but we do not expect this to be representative of pesticide applications from the 1970s as the use of specific ingredients changes over time, for example due to changes in pesticide regulation and changes in farmers preferences. We therefore use a simplified proxy for land use intensity in this study.

Topography

Elevation data were obtained from the OS Terrain 50 Digital Terrain Map (DTM) (Ordinance Survey, 2023).

Analysis

Broad species analysis

Data

Following the scoping of available datasets undertaken during chapter 1, butterfly abundance data from the United Kingdom Butterfly Monitoring Scheme (UKBMS) was used in all further modelling. As outlined in detail in table 2, driver variable data were collated for each UKBMS site and year resulting in the creation of seven key covariates for inclusion in subsequent broad species models; historic nitrogen deposition, percentage change in nitrogen at a site over time, historic sulphur deposition, June temperature in the year of survey, June temperature in the year preceding the survey, June rainfall in the year of survey, and land use intensity.

Modelling

For the trait-based models, the same model with the same inputs was run for 52 of the 58 species recorded by the UKBMS (table A1). *Carterocephalus palaemon* (Chequered Skipper), *Leptidea juvernica* (Cryptic Wood White), *Melitaea cinxia* (Glanville Fritillary), and *Phengaris arion* (Large Blue) were excluded due to their rarity and the subsequent lack of data points available from UKBMS data. *Papilio Machaon* (Swallowtail) and *Colias croceus* (Clouded Yellow) were excluded due to them both being primarily migrants and therefore facing potentially very different driver pressures. For each species, data were only included from transects where the species had been recorded in at least one year. This was done to filter out sites where the species has never been seen during the transect monitoring.

I expected that some of the driver variables will have a non-linear relationship with butterfly abundance. For example, the covariate representing historic nitrogen deposition may be beneficial to many species up to a certain point as all butterflies and their food plants require nitrogen to survive. However, above a certain point we expect the relationship to change and become negative as the site experiences eutrophication and acidification due to excess nitrogen availability, as following the idea of critical loads. Given this, I chose to fit generalized additive models (GAMs) to the butterfly data, allowing for this flexible non-linearity in the response variables.

The following GAM was run for each species individually:

Model 1.1:

$$\begin{aligned} Butterfly\ abundance \sim\ & Year + Historic\ N\ deposition + Change\ in\ N\ deposition\ over\ time \\ & + Historic\ S\ deposition + June\ temperature + Previous\ June\ temperature \\ & + Land\ Use\ Intensity + June\ rainfall + Location + Site\ number \end{aligned}$$

Model 1.1 was fit using the bam function from the mgcv R package (Wood, 2017) with a negative binomial distribution given that the response variable was count data. A negative binomial distribution was used rather than a Poisson distribution in order to account for observed overdispersion in the response data. The location variable in model 1.1 was fit as a fixed-effect tensor product smooth of easting and northing. All other variables were defined as spline based smooth terms with fixed effects, with the exception of site number, which was defined as a random effect. Site number was included as a random effect to attempt to account for any residual spatial autocorrelation, in other words, to account for the fact that observations from the same site are more likely to be similar than observations from different sites. I was not able to include a term to account for temporal autocorrelation because this increased model complexity to the point that it resulted in convergence errors for many species. Model fit was determined by the percentage deviance explained value. For each variable, I calculated the predicted butterfly abundance at an average site for all values in the range of the observed data. For example, when predicting the butterfly abundance at differing levels of historic nitrogen deposition, mean values were used for all other driver variables.

Variable selection

Drivers of change will vary depending on which butterfly species is being modelled. Many driver variables were computed, but we do not expect that all of these will have an impact on each butterfly species. The R mgcv packages provides an automated shrinkage approach for smooth selection that removes covariates which have little or no effect on the response variable (Marra and Wood, 2011). In theory, this could improve the accuracy of our models, however, the removal of covariates would prevent us from being able to make direct comparisons between the results for different species, as different variables may be removed. Additionally, this automated variable selection vastly increases the computing time and power required and often leads to convergence failures. Thus, I chose to include all variables in all models for comparability and ease of running.

Data visualisation

For ease of interpretation, plots were produced to show the strength and direction of each significant relationship for each species. To produce these plots, all predictors other than the one of interest were held at mean levels.

Lasiommata megera analysis

Modelling

For this hypothesis, I used a similar generalised additive model to that used in the broad species analysis (model 1.1), but included slightly different variables thought to impact *L. megera* specifically based on previous research. Prior to fitting the models, I tested the collinearity between variables to ensure that variables showing pairwise collinearity were not used in the models. I found that the variables differed enough to all be included in subsequent modelling (figure A2). Given that data on pesticide applications to crops in the UK is not currently available at a suitable spatial and temporal scale for this study, it was not included as a term in this modelling. Instead, land use intensity term is included as a proxy for nearby agricultural intensity, on the basic assumption that pesticide usage is highest in areas with the most arable crop-land. This does not take into account organic farming or information on land management practices such as Agri-Environment Schemes. See Table 2 for full descriptions of the data sources. Model 2.1 was fit using the mgcv R package (Wood, 2017) as follows with a negative binomial distribution:

Model 2.1

$$\begin{aligned} Butterfly\ abundance \sim Year + N\ deposition + Change\ in\ N + S\ deposition \\ + June\ temperature + Previous\ September\ temperature \\ + \ Land\ Use\ Intensity + June\ rainfall + Location + Site\ number \end{aligned}$$

The location variable for model 2.1 was fit as a fixed-effect tensor product smooth of easting and northing. All other variables were defined as spline based smooth terms with fixed effects, with the exception of site number, which was defined as a spline based smooth term with a random effect. Model 2.1 fit fairly well with a deviance explained of 66%.

Following this, I tested whether the fit of Model 2.1 was improved with automatic variable selection using the ‘select = TRUE’ argument within the mgcv bam function. The rationale behind doing this was to attempt to understand whether the model fit changed with the removal of the sulphur deposition variable, which showed fairly strong (0.68) pairwise collinearity with the variable for historic peak nitrogen deposition, but not enough to remove it from the model based on the commonly used cut-off point of 0.7. This model (2.2) did not converge. As an alternative, I tested whether stepwise variable selection, in other words manually removing non-significant covariates based on their P-value, improved model fit. Stepwise variable selection is not considered the most statistically robust method given its tendency to cause overfitting of models (Smith, 2018), but it was used given that the model using mgcv automatic variable selection based on penalised splines did not converge. To do this, I first removed the variable with the highest P value and continued until all variables were significant. Model 2.3 was the same as model 2.1 but with the change in nitrogen deposition variable removed because it had the highest P-value. This resulted in a model with a deviance explained of 66%. The other non-significant variable of sulphur deposition was then removed in model 2.4, resulting in a model fit with a deviance explained of 66%. Fitting models with stepwise removal of variables did not significantly improve the fit of the model, therefore model 2.1 was judged to be the best and used in all further results for this chapter.

Results

Broad species analysis

The shape and significance of responses grouped by driver variables and also trait categories are presented in table 3 and table 4. Following Henrys *et al.* (2011) directions of the significant relationships are grouped into three categories for ease of comparison: positive, negative, U-shaped.

An extensive summary of the results from the broad species analysis is presented in table A1, highlighting the significance and direction of relationships between each variable and the abundance of that butterfly species, as well as indicating the strength of the model fit as measured by the percentage deviance explained. Most of the model fits were reasonable at above 60%, with only 6 of the 52 species falling below this. Models for *Vanessa cardui* (Painted Lady) and *Apatura iris* (Purple Emperor) fit particularly poorly (Deviance explained: 41.5 and 40.6%). The significance of variables differed by species, however, year, spatial location, and site number were significant for most species.

Table 3: Summary of the number of species from the broad species analysis with significant or non-significant responses to each individual driver variable. Significant responses are split into three pre-defined shapes: positive, negative, and U-shaped.

	Positive	Negative	U-shaped	No relationship
Year	11	28	9	4
Historic nitrogen deposition	12	9	3	28
Change in nitrogen deposition over time	11	9	1	31
Historic S deposition	6	15	3	28
June temperature	20	8	14	10
Previous June temperature	21	4	17	10
June rainfall	5	24	11	12
Land Use Intensity	1	5	6	40

Table 4: Summary of the number of species from the broad species analysis with significant or non-significant responses to historic nitrogen deposition and/or change in nitrogen deposition, grouped by trait. Significant responses are categorised into three pre-defined shapes: positive (↑), negative (↓), and U-shaped (∩). Non-significant responses are represented by the – icon.

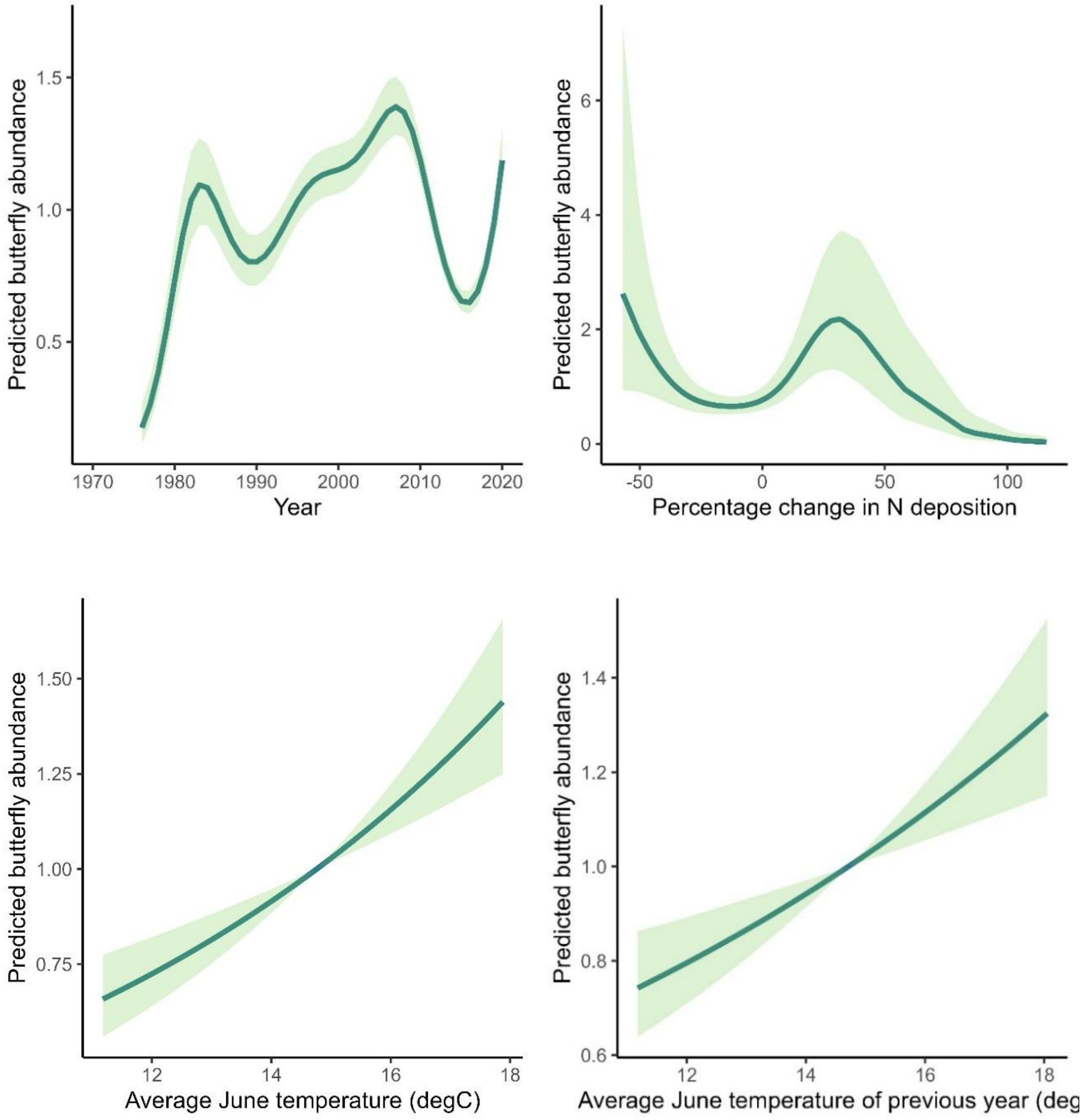
		Relationship with Historic nitrogen deposition				Relationship with change in nitrogen deposition			
Trait grouping	Trait	↑	↓	∩	–	↑	↓	∩	–
Voltinism	Obligate univoltine	10	6	2	26	9	8	0	27
	Obligate multivoltine	5	4	2	3	6	3	1	4
	Partial generation	5	1	1	8	1	3	0	11
Host plant specificity	Monophagous	3	0	0	8	1	1	0	9
	Oligophagous (family)	7	6	1	7	5	5	0	11
	Oligophagous (genus)	1	1	0	10	1	3	0	8
	Polyphagous	1	2	2	3	4	1	0	3
Host plant category	Broadleaf trees	0	1	0	4	1	0	0	4
	Forbs	8	3	2	15	8	5	1	14
	Grasses	3	5	1	5	2	3	0	9
	Sedges	0	0	0	1	0	0	0	1
	Shrubs	1	1	0	5	1	1	0	5
Breeding habitat	Acid grassland	4	4	1	5	2	4	0	8
	Bogs, mosses and mires	0	0	0	4	0	0	0	4
	Broadleaf woodland	4	2	1	13	1	4	0	15
	Calcareous grassland	4	3	1	16	2	5	1	16
	Coastal shingle	1	0	0	1	0	1	0	1
	Coniferous woodland	4	1	1	9	1	3	0	11
	Fens and marshes	2	0	0	1	1	1	0	1
	Grassland	9	5	3	20	6	8	1	22
	Heathland	4	2	1	4	0	4	0	7
	Moorland	2	0	0	6	0	2	0	6
	Sand dunes	4	3	0	5	0	4	0	8
	Scrubs and hedgerows	5	2	1	10	2	3	0	13
	Wet grassland	1	0	0	3	1	0	0	3
	Wet woodland	0	0	0	1	0	0	0	1
	Wetlands	2	0	0	4	1	1	0	4
	Woodland	6	5	1	17	4	5	0	20

Monophagous habitat specialists

The statistical significance of each explanatory term in model 1.1 is summarised in table A1.

Significant trends in the target species are plotted below in figure 6 and 7.

The abundance of *Polyommatus bellargus* was positively correlated with year (fig 6a, $P < 0.001$), June temperature in the year of survey (fig 6c, $P = 0.0099$), June temperature in the previous year (fig 6d, P = 0.046), and June rainfall in the year of survey (fig 6f, P = 0.0406). Abundance showed a hump-backed relationship with percentage change in nitrogen at the site (fig 6b, P= 0.0249) and land use intensity (fig 6e, P = 0.0025). No statistically significant relationship was observed between *P. bellargus* abundance and either historic nitrogen deposition or historic sulphur deposition. The spatial term and the random effect of site also significantly correlated with abundance (P = 0.0121, P < 0.001).



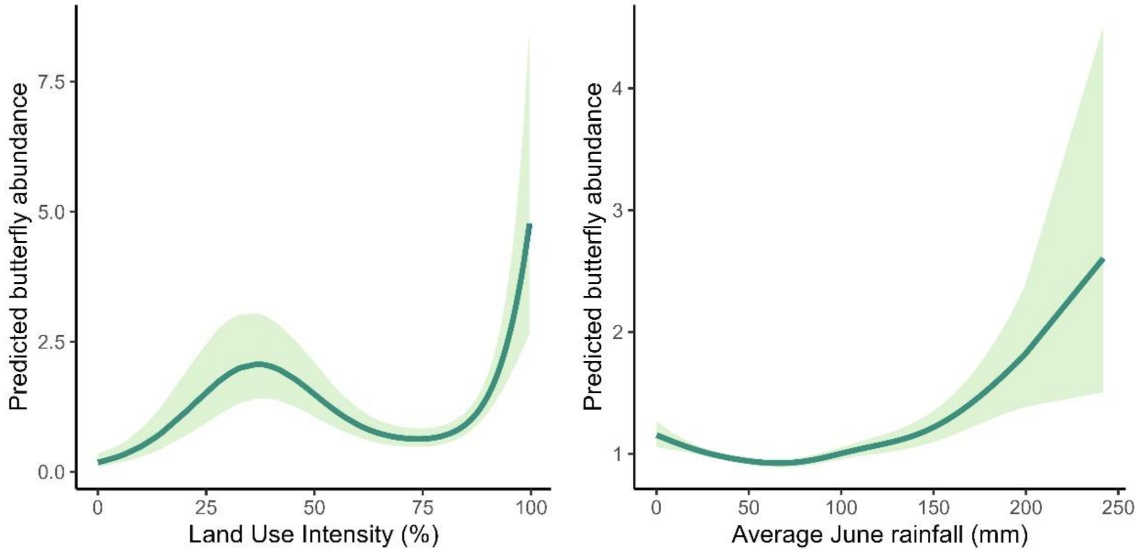


Figure 6a, 6b, 6c, 6d, 6e & 6f: Predicted abundance from model 1.1 of Polyommatus bellargus at an average site with year (6a), percentage change in nitrogen deposition (6b), temperature in the current June (6c), temperature in the previous June (6d), land use intensity in 1990 (6e), and average June rainfall in current year (6f).

The observed relationships between abundance and drivers of *P. coridon* differed greatly from those of *P. bellargus.* Of the ten variables modelled, only five were found to be statistically significant drivers of abundance. Abundance of *P. coridon* was negatively correlated with year (fig 7a, $P < 0.001$), June temperature in the previous year (fig 7c, $P = 0.0052$), and June rainfall (fig 7d, $P = 0.001$). The negative relationship between abundance and June temperature in the current year was marginally statistically significant (fig 7b, $P = 0.0965$). No statistically significant relationship was observed between *P. coridon* abundance and historic nitrogen deposition, percentage change in nitrogen at a site over time, historic sulphur deposition, or land use intensity. The spatial term and the random effect of site also significantly correlated with abundance ($P = 0.0071$, $P < 0.001$).

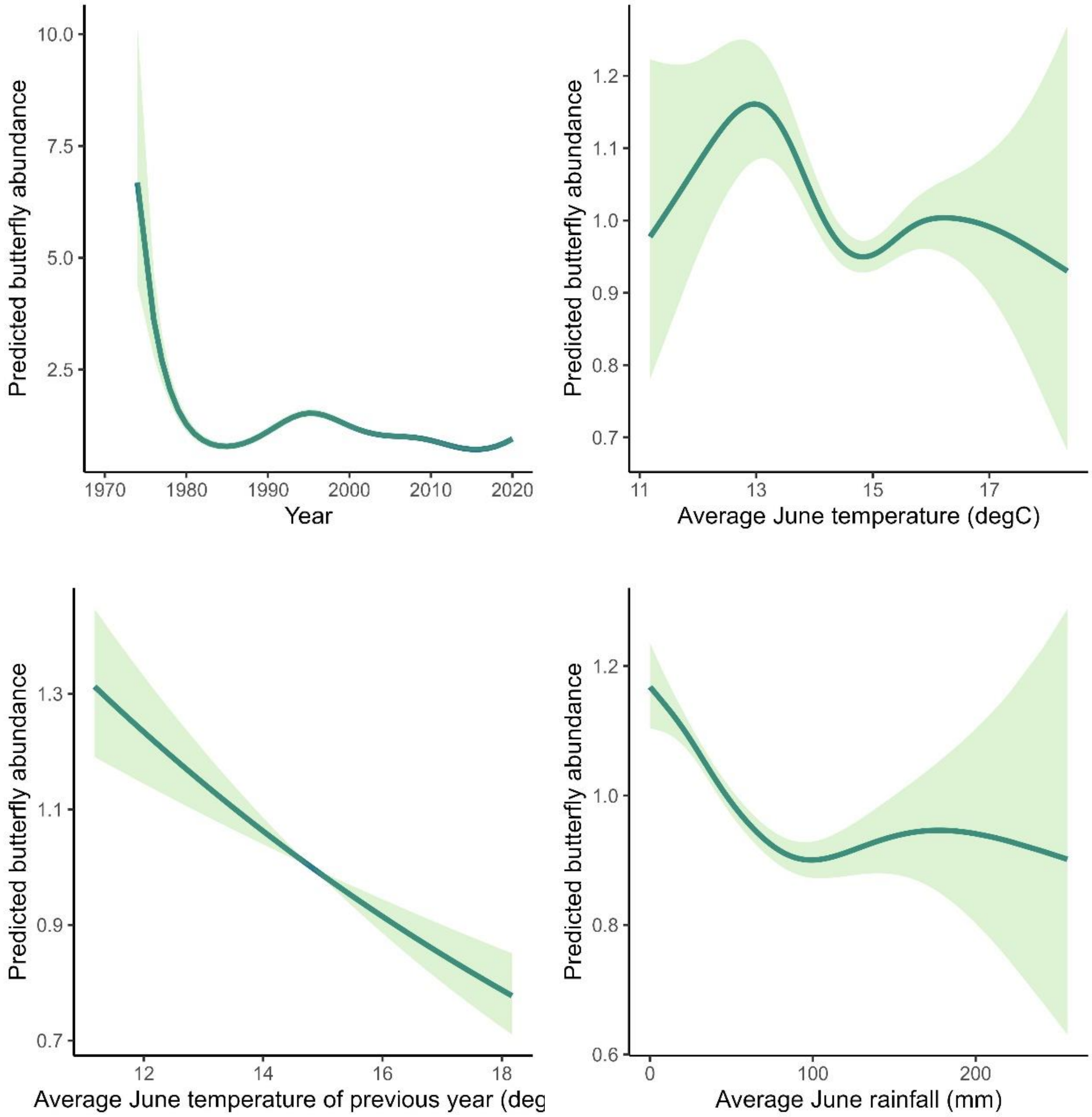


Figure 7a, 7b, 7c & 7d: Predicted abundance from model 1.1 of Polyommatus coridon at an average site with year (7a), temperature in the current June (7b), temperature in the previous June (7c), and average June rainfall in the current year (7d).

Species that emerge as adults early in spring

The statistical significance of each explanatory term in model 1.1 is summarised in table A1. For *Boloria euphrosyne,* seven of the ten covariates were statistically significant; year, change in nitrogen deposition over time, June temperature of current year, June temperature of previous year, June rainfall, spatial location, and site number. Only three variables were not found to be statistically significant; historic nitrogen deposition, sulphur deposition and land use intensity. For *Boloria* *selene,* only five of the ten covariates were statistically significant; year, June temperature of the current year, June temperature of previous year, spatial location, and site number. Five variables were not found to be statistically significant; historic nitrogen deposition, percentage change in nitrogen, sulphur deposition, June rainfall, and land use intensity. Both models showed a high value for deviance explained, indicating that the model performance was good.

The model run for *Maniola jurtina* fit very well (80.1% deviance explained). Eight variables were found to be statistically significant; year, historic nitrogen deposition, sulphur deposition, June temperature in the current year, June temperature in the previous year, June rainfall, spatial location, and site number. The remaining two variables, percentage change in nitrogen and land use intensity, were both found to be marginally statistically significant with P values of < 0.1. The model fit for *Pyronia tithonus* was slightly poorer (67.6% deviance explained). For *P. tithonus*, nine variables were statistically significant; year, historic nitrogen deposition, sulphur deposition, June temperature in the current year, June temperature in the previous year, June rainfall, land use intensity, spatial location, and site number. The only non-significant variable was percentage change in nitrogen.

The abundance of *Boloria euphrosyne* was positively correlated with June temperature in the year of survey (fig 8c, P = 0.0005) and June temperature in the previous year (fig 8d, P = 0.0097), and showed a negative relationship with year (fig 8a, P < 0.001). The hump-backed relationship between *B. euphroysne* abundance and June rainfall was also significant (fig 8e, P < 0.001). A significant relationship was observed between abundance and percentage change in nitrogen at the site (fig 8b, P = 0.0188), however, the confidence intervals on the predictions are so high that I consider there to be no real trend present. Observed trends for *B. selene* were somewhat similar, showing a negative relationship with year (fig 9a, P < 0.001), a positive relationship with June temperature in the year of survey (fig 9b, P < 0.001), and a hump-backed relationship with June temperature in the previous year (fix 9c, P < 0.001). For both *B. euphrosyne* and *B. selene*, spatial location and the random effect of site were strongly statistically significant. Neither species abundance was strongly correlated with historic nitrogen deposition, percentage change in nitrogen at the site over time, or historic sulphur deposition.

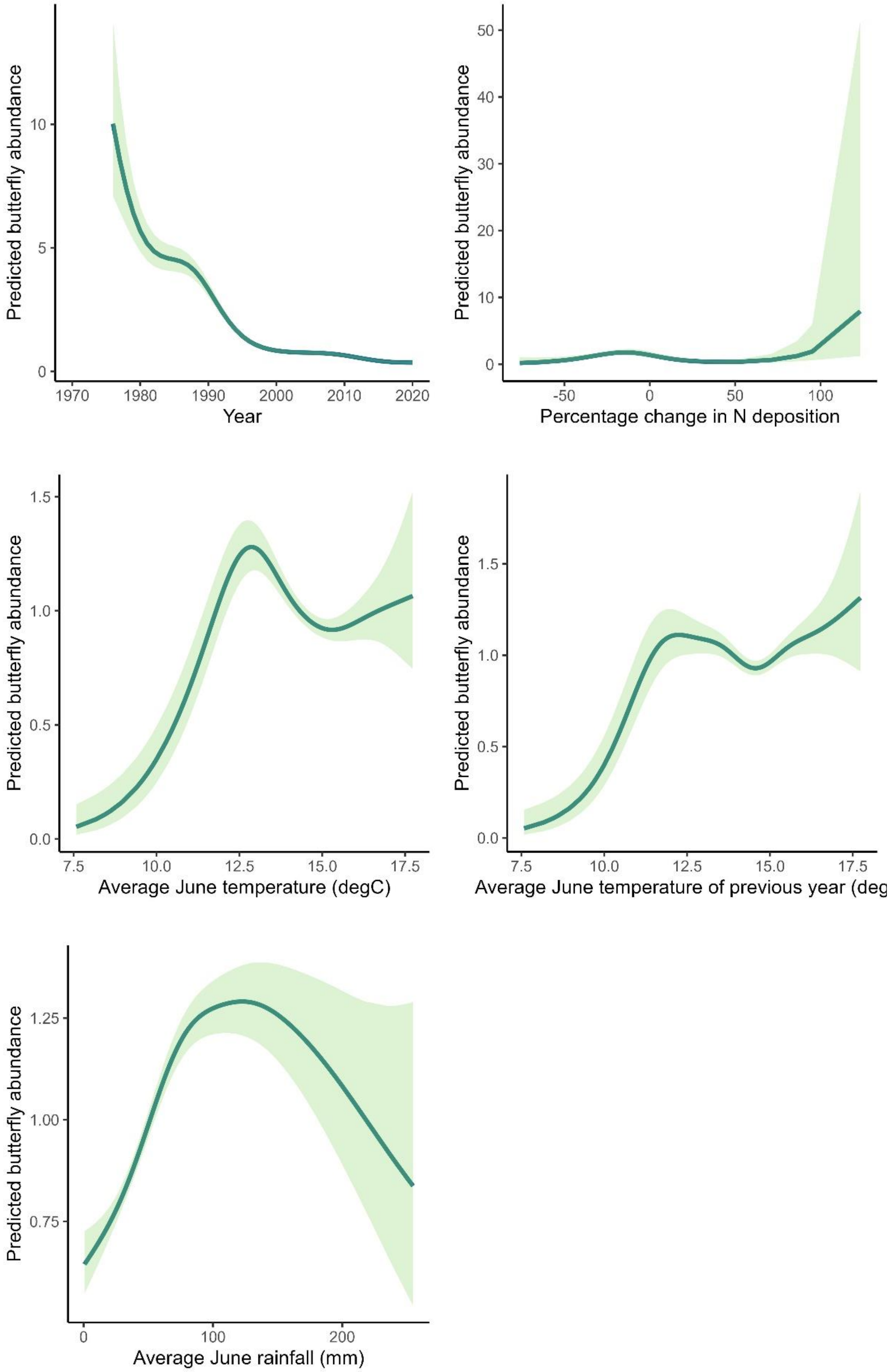

Predicted butterfly abundance
0
5
10
1970
1980
1990
2000
2010
2020
Year
Predicted butterfly abundance
0
10
20
30
40
50
-50
0
50
100
Percentage change in N deposition
Predicted butterfly abundance
0.0
0.5
1.0
1.5
7.5
10.0
12.5
15.0
17.5
Average June temperature (degC)
Predicted butterfly abundance
0.0
0.5
1.0
1.5
7.5
10.0
12.5
15.0
17.5
Average June temperature of previous year (deg
Predicted butterfly abundance
0.75
1.00
1.25
0
100
200
Average June rainfall (mm)

Figure 8a, 8b, 8c, 8d & 8e: Predicted abundance from model 1.1 of Boloria euphrosyne at an average site with year (8a), percentage change in nitrogen deposition (8b), temperature in the current June (8c), temperature in the previous June (8d), and average June rainfall in current year (8e).

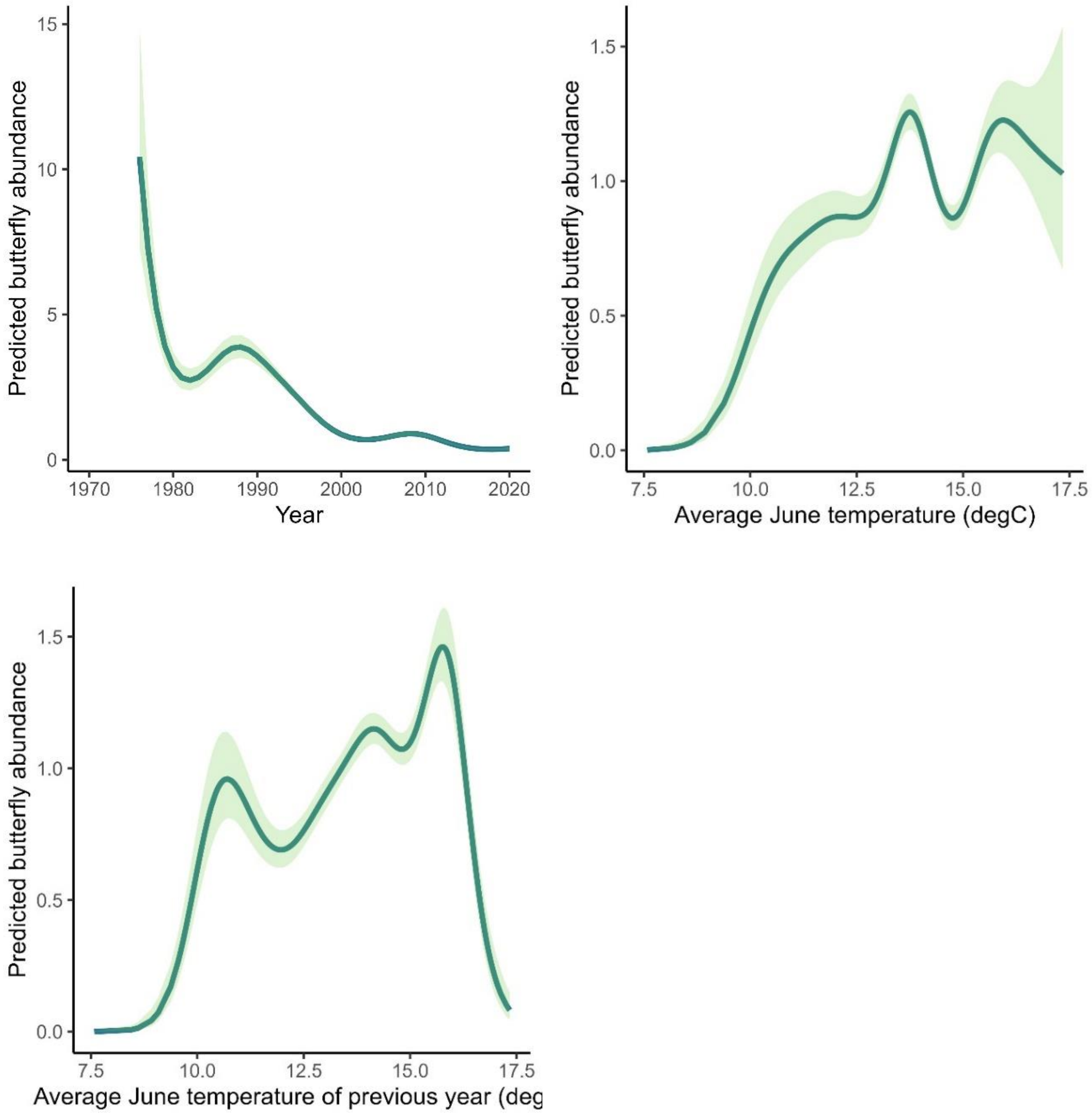
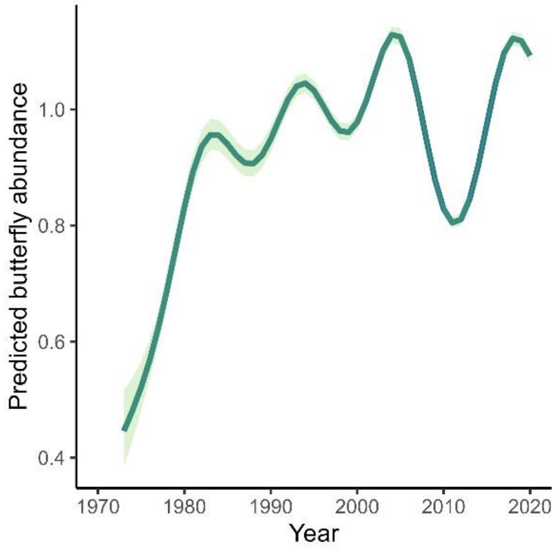
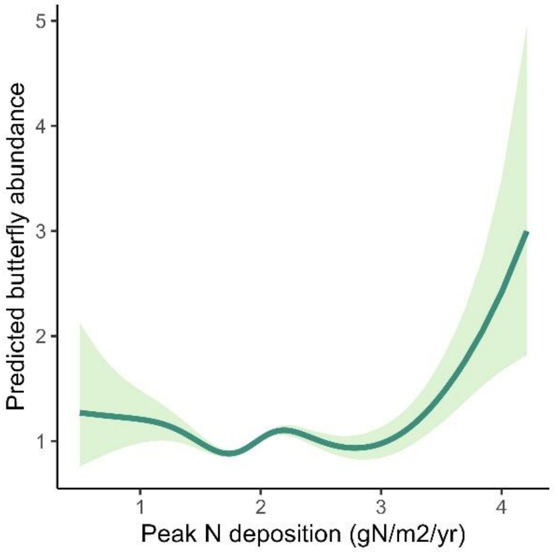
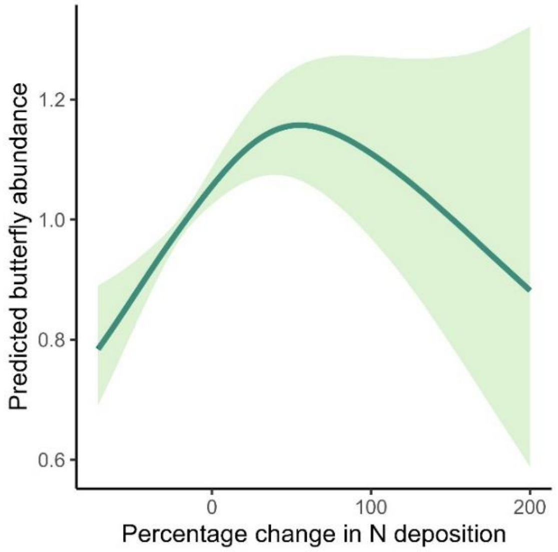


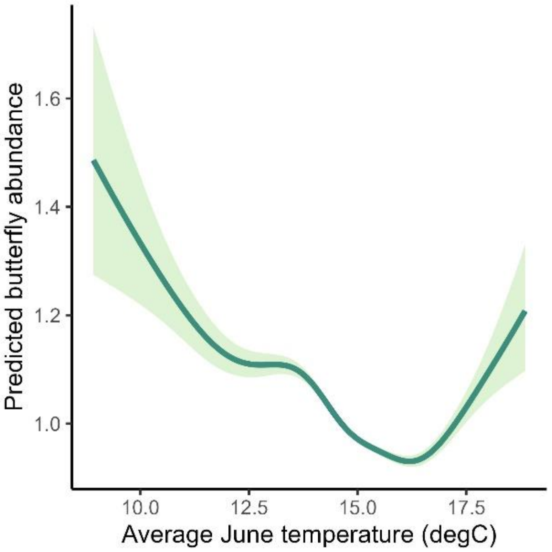
Figure 9a, 9b & 9c: Predicted abundance from model 1.1 of Boloria selene at an average site with year (9a), temperature in the current June (9b), and temperature in the previous June (9c).

Maniola jurtina abundance was positively correlated with year (fig 10a, P < 0.001) and historic nitrogen deposition (fig 10b, P = 0.0059), but negatively correlated with sulphur deposition (fig 10h, P < 0.001). Abundance of *M. jurtina* showed a hump-backed relationship with June temperature in the previous year (fig 10e, P < 0.001) and June rainfall in the year of survey (fig 10g, P < 0.001), but an inverse hump-backed relationship with June temperature in the year of survey (fig 10d, P < 0.001). Marginally statistically significant relationships were seen with percentage change in nitrogen at the site (fig 10c, P = 0.0807, hump-back) and land use intensity (fig 10f, P = 0.0583, inverse hump-back). *Pyronia tithonus* abundance was negatively correlated with year (fig 11a, P < 0.001), historic nitrogen deposition (fig 11b, P = 0.0001), June temperature in the year of survey (fig 11c, P < 0.001), sulphur deposition (fig 11g, P = 0.0006), and land use intensity (fig 11e, P < 0.001). A hump-backed relationship was observed between *P. tithonus* abundance and June rainfall (fig 11f, P < 0.001), whilst a slight inverse hump-backed relationship was observed with June temperature in the previous year (fig 11d, P < 0.001). Both *M. jurtina* and *P. tithonus* abundances were strongly correlated with spatial location and the random effect of site.

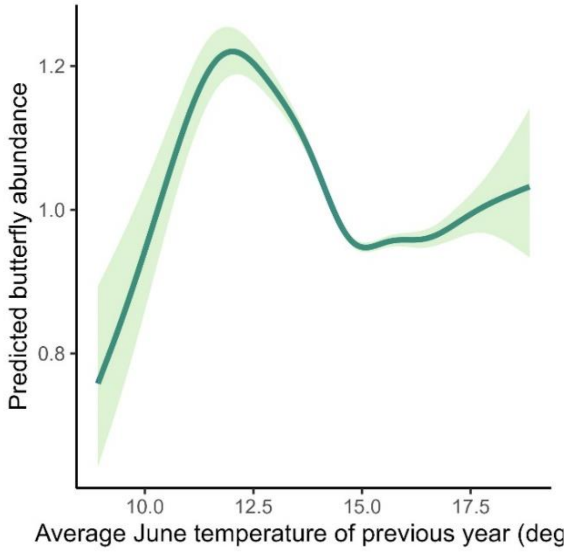

Predicted butterfly abundance
0.4
0.6
0.8
1.0
1970
1980
1990
2000
2010
2020
Year

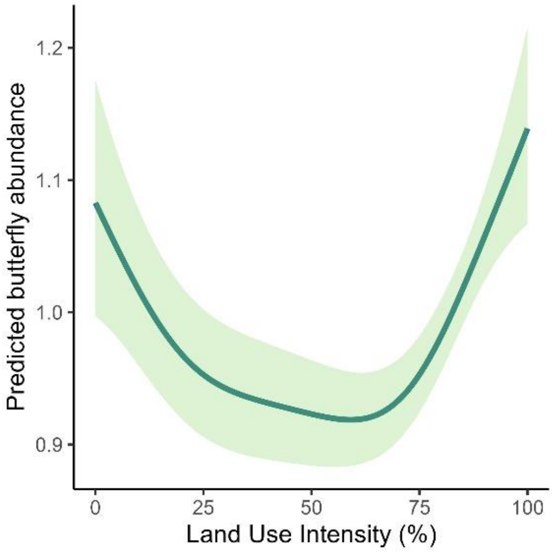

Predicted butterfly abundance
1
2
3
4
5
1
2
3
4
Peak N deposition (gN/m2/yr)


Predicted butterfly abundance
0.6
0.8
1.0
1.2
0
100
200
Percentage change in N deposition


Predicted butterfly abundance
1.0
1.2
1.4
1.6
10.0
12.5
15.0
17.5
Average June temperature (degC)

7.5


Predicted butterfly abundance
0.8
1.0
1.2
10.0
12.5
15.0
17.5
Average June temperature of previous year (deg


Predicted butterfly abundance
0.9
1.0
1.1
1.2
0
25
50
75
100
Land Use Intensity (%)

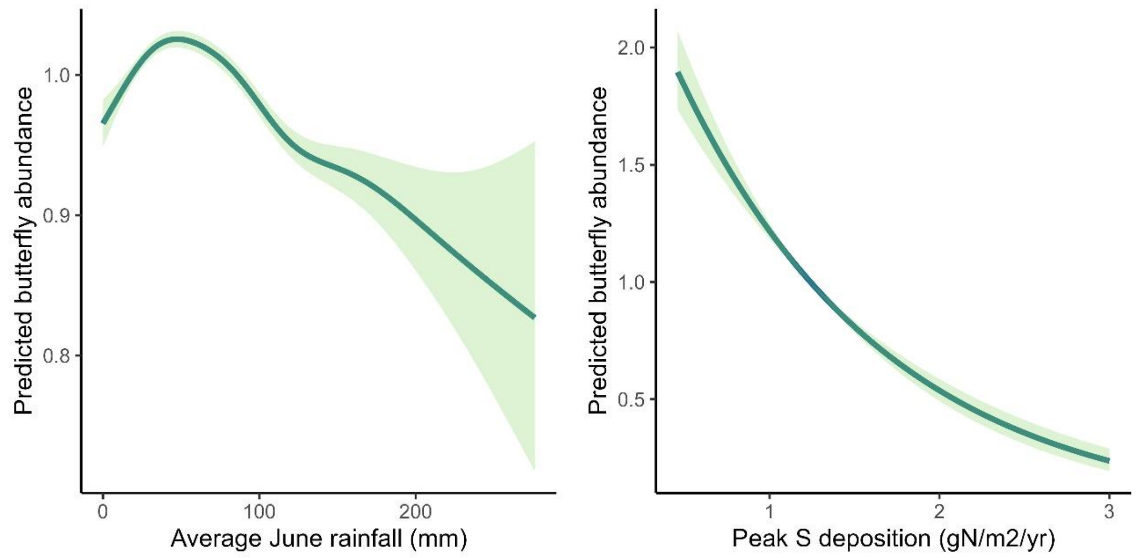
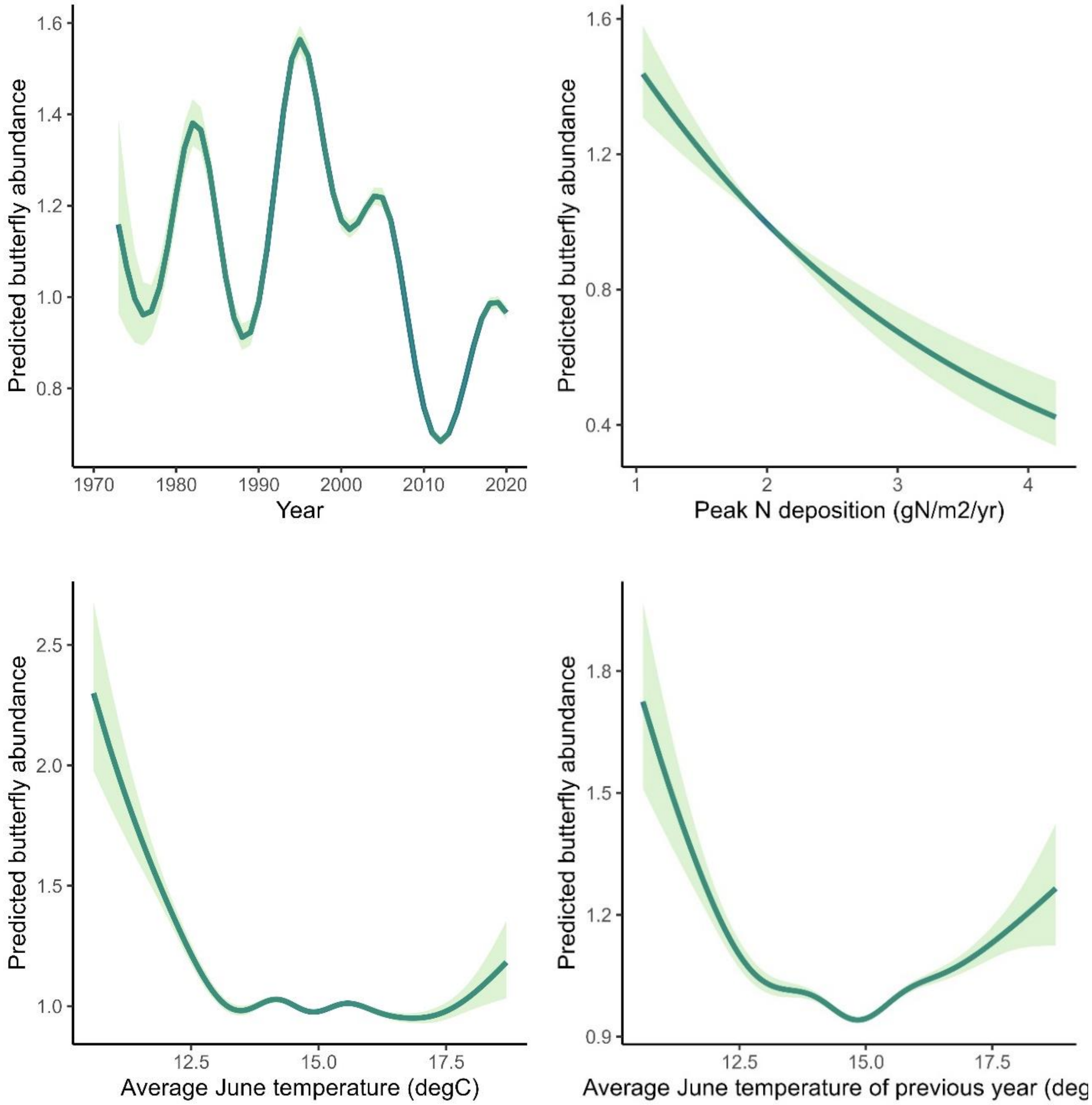


Figure 10a, 10b, 10c, 10d, 10e, 10f, 10g & 10h: Predicted abundance from model 1.1 of Maniola jurtina at an average site with year (10a), historic peak in nitrogen deposition (10b), percentage change in nitrogen (10c), temperature in the current June (10d), temperature in the previous June (10e), land use intensity in 1990 (10f), average June rainfall in current year (10g), and historic peak sulphur deposition (10h).


Predicted butterfly abundance
1.6
1.4
1.2
1.0
0.8
1970
1980
1990
2000
2010
2020
Year
Predicted butterfly abundance
1.6
1.2
0.8
0.4
1
2
3
4
Peak N deposition (gN/m2/yr)
Predicted butterfly abundance
2.5
2.0
1.5
1.0
12.5
15.0
17.5
Average June temperature (degC)
Predicted butterfly abundance
1.8
1.5
1.2
0.9
12.5
15.0
17.5
Average June temperature of previous year (deg

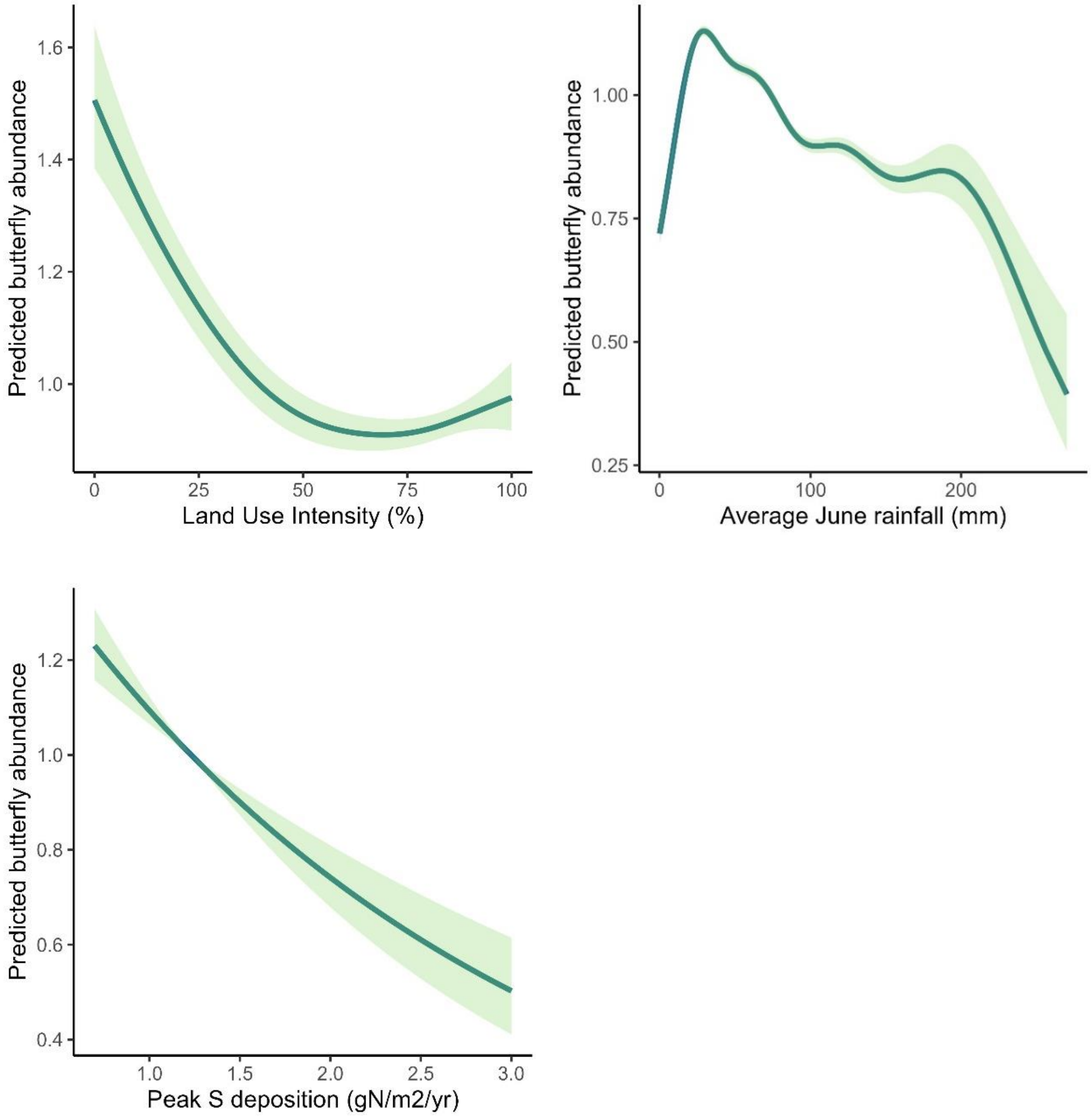
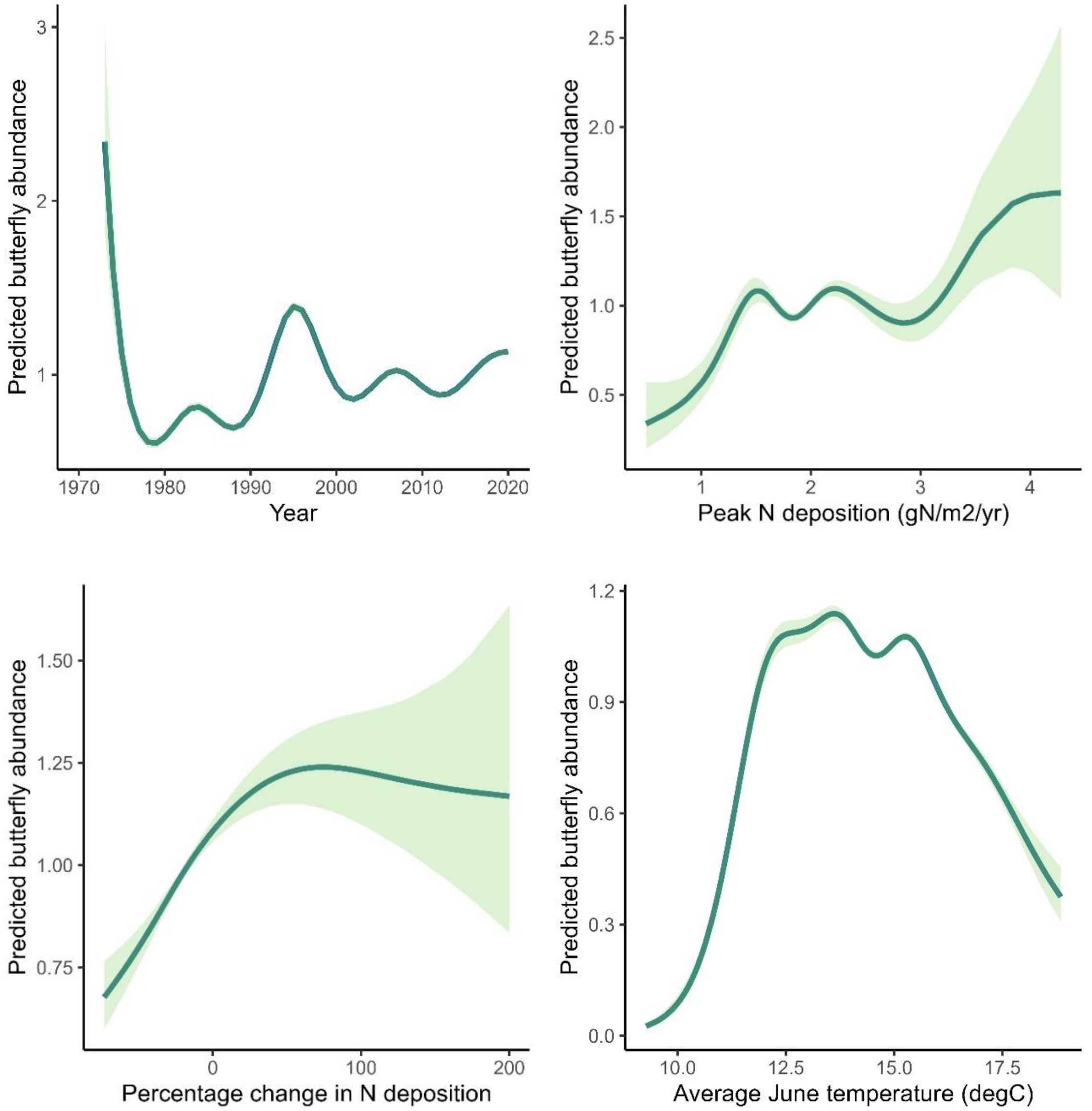


Figure 11a, 11b, 11c, 11d, 11e, 11f & 11g: Predicted abundance from model 1.1 of Pyronia tithonus at an average site with year (11a), historic peak in nitrogen deposition (11b), temperature in the current June (11c), temperature in the previous June (11d), land use intensity in 1990 (11e), average June rainfall in current year (11f), and historic peak sulphur deposition (11g).

Species with Nitrophilic host plants

The model run for *Aglais io* fit reasonably well (66.3% deviance explained). Nine variables were found to be statistically significant: year, historic nitrogen deposition, percentage change in nitrogen, sulphur deposition, June temperature in the current year, June temperature in the previous year, June rainfall, spatial location, and site number. Land use intensity was not found to be statistically significant. The model fit for *Aglais urticae* was similar (66.1% deviance explained). Eight variables were statistically significant for *A. urticae*: year, percentage change in nitrogen, sulphur deposition, June temperature in the current year, June temperature in the previous year, June rainfall, spatial location, and site number. Historic nitrogen deposition was marginally statistically significant ($P = 0.0556$). The only non-significant variable was land use intensity.

Many of the relationships between butterfly abundance and driver variables were similar in both *A. io* and *A. urticae,* including positive relationships with historic nitrogen deposition (fig 12b, $P = 0.006$; fig 13b, $P = 0.0556$, marginally significant) and percentage change in nitrogen at the site over time (fig 12c, $P = 0.0005$; fig 13c, $P < 0.001$) and hump-backed relationships with June temperature in the year of survey (fig 12d, $P < 0.001$; fig 13d, $P < 0.001$) and June temperature in the previous year (fig 12e, $P < 0.001$; fig 13e, $P < 0.001$). *Aglais io* abundance was negatively correlated with June rainfall (fig 12f, $P < 0.001$) and sulphur deposition (fig 12g, $P < 0.001$), whereas *A. urticae* abundance showed a hump-backed relationship with both variables (fig 13f, $P < 0.001$; fig 13g, $P = 0.0309$). The species also differed in their response with year, with *A. io* showing a wiggly and slightly inverse hump-backed but overall fairly stable trend (fig 12a, $P < 0.001$) whilst *A. urticae* showed a hump-backed relationship (fig 13a, $P < 0.001$). Spatial location and the random effect of site number were strongly statistically significant drivers of abundance for both *Aglais* species ($P < 0.001$).


Predicted butterfly abundance
3
2
1
1970
1980
1990
2000
2010
2020
Year
Predicted butterfly abundance
2.5
2.0
1.5
1.0
0.5
1
2
3
4
Peak N deposition (gN/m2/yr)
Predicted butterfly abundance
1.50
1.25
1.00
0.75
0
100
200
Percentage change in N deposition
Predicted butterfly abundance
1.2
0.9
0.6
0.3
0.0
10.0
12.5
15.0
17.5
Average June temperature (degC)

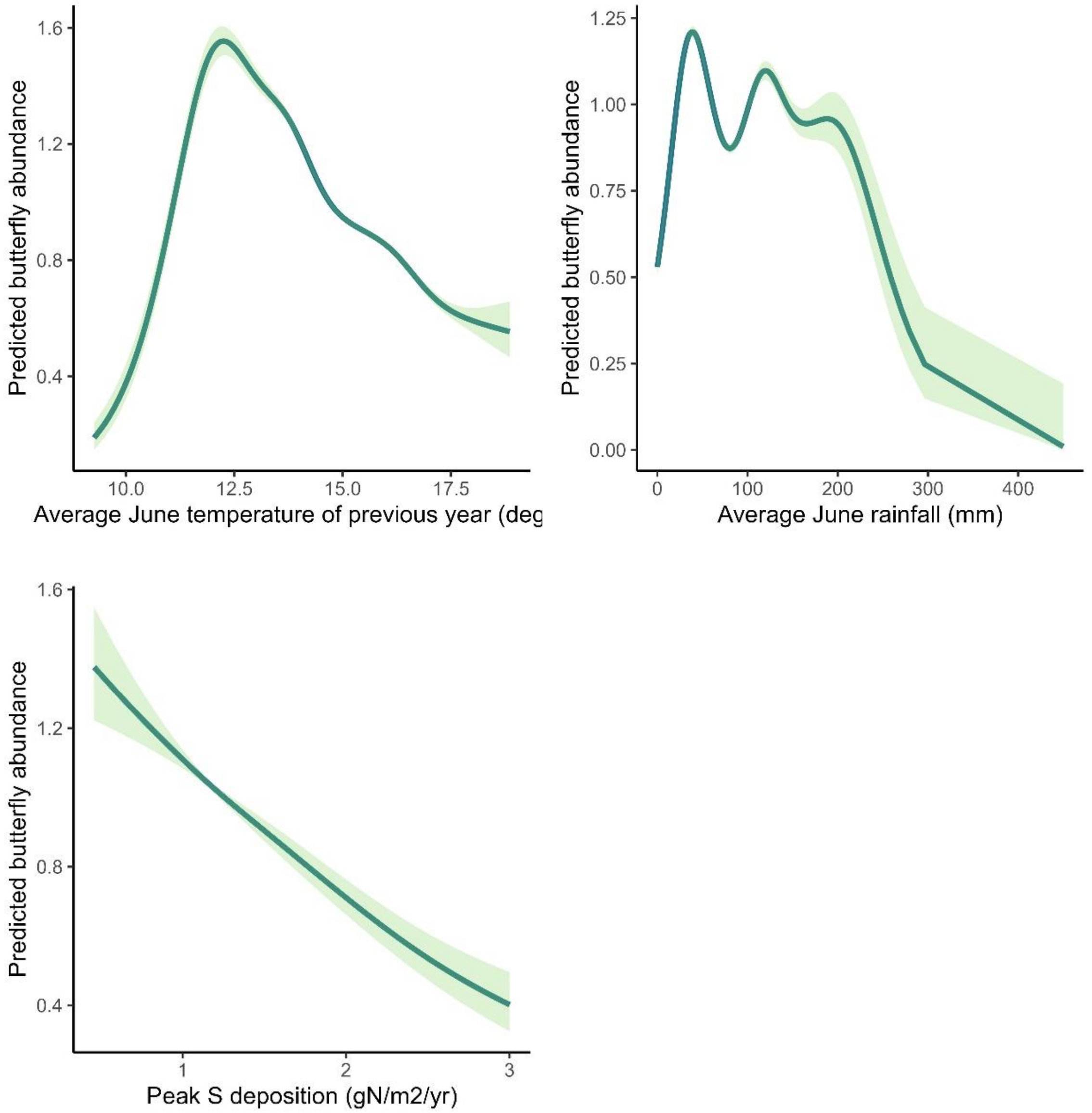
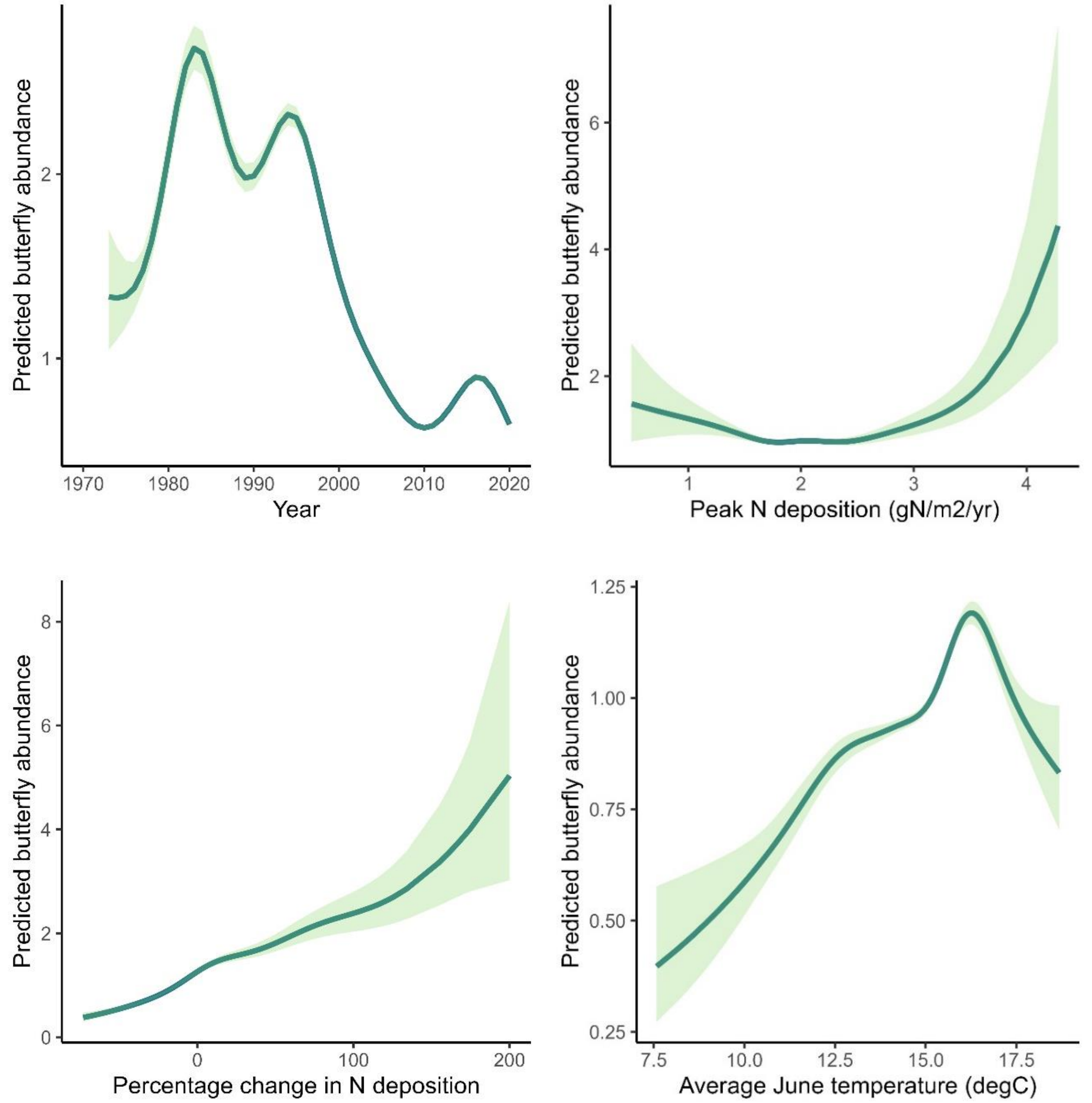


Figure 12a, 12b, 12c, 12d, 12e, 12f & 12g: Predicted abundance from model 1.1 of Aglais io at an average site with year (12a), historic peak in nitrogen deposition (12b), percentage change in nitrogen (12c), temperature in the current June (12d), temperature in the previous June (12e), average June rainfall in current year (12f), and historic peak sulphur deposition (12g).


Predicted butterfly abundance
2
1
1970
1980
1990
2000
2010
2020
Year
Predicted butterfly abundance
6
4
2
1
2
3
4
Peak N deposition (gN/m2/yr)
Predicted butterfly abundance
8
6
4
2
0
0
100
200
Percentage change in N deposition
Predicted butterfly abundance
1.25
1.00
0.75
0.50
0.25
7.5
10.0
12.5
15.0
17.5
Average June temperature (degC)

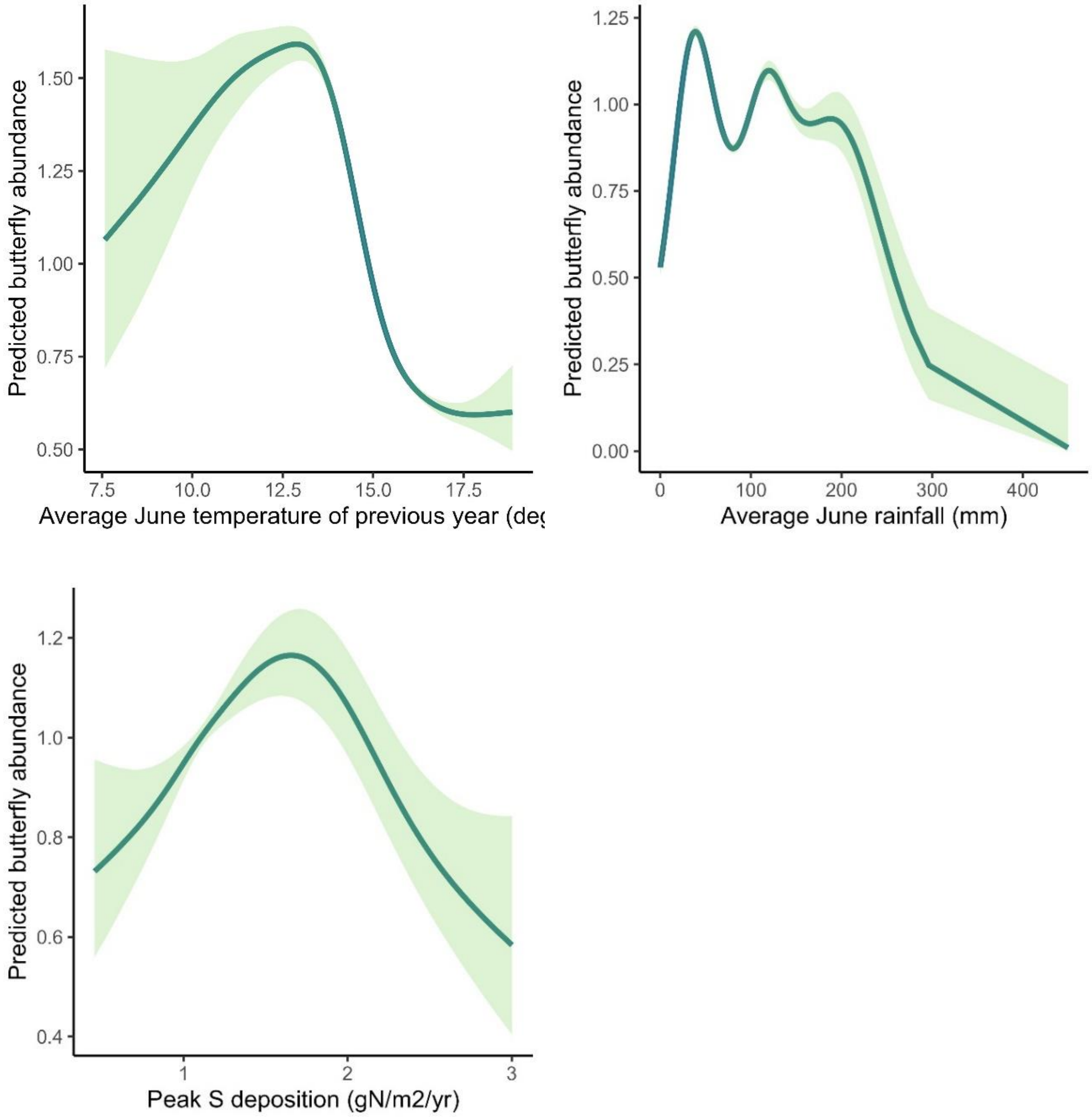


Figure 13a, 13b, 13c, 13d, 13e, 13f & 13g: Predicted abundance from model 1.1 of Aglais urticae at an average site with year (13a), historic peak in nitrogen deposition (13b), percentage change in nitrogen (13c), temperature in the current June (13d), temperature in the previous June (13e), average June rainfall in current year (13f), and historic peak sulphur deposition (13g).

Lasiommata megera analysis

Lasiommata megera has clear limits to its distribution, as shown in figure 14 which shows all UK Butterfly Monitoring Scheme transect locations that the butterfly occurred at in the period 1973-2021. It is fairly widespread in England with the exception of some Northern areas such as the Lake District. In Wales it occurs somewhat less frequently, and mostly in close proximity to the coast. *L. megera* only occurs in two areas of Scotland, near Edinburgh and Dumfries, which are both coastal and relatively far south. There is a large area with no occurrences of *L. megera* between northern England and the Scottish border, and also a large distance between the two Scottish populations.

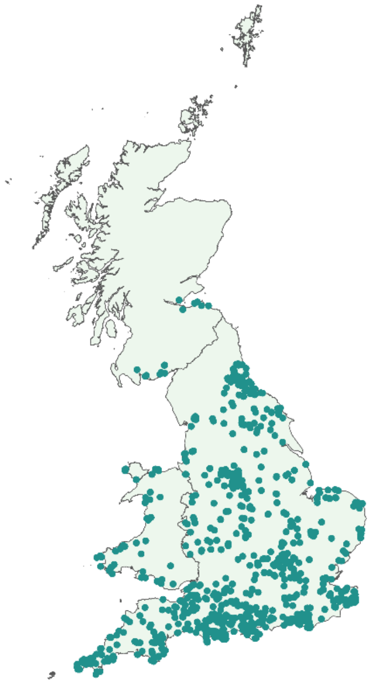
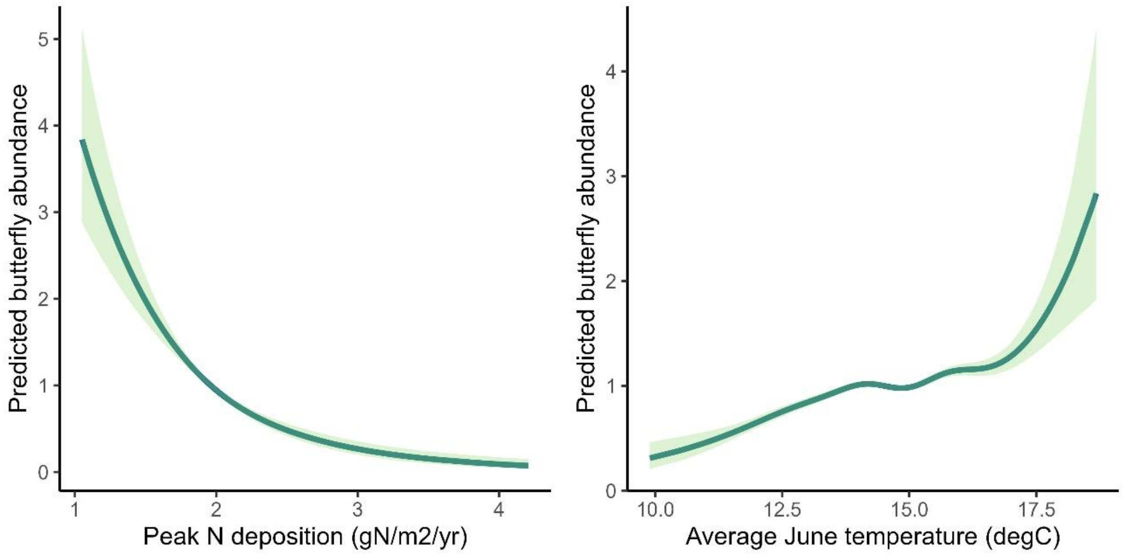


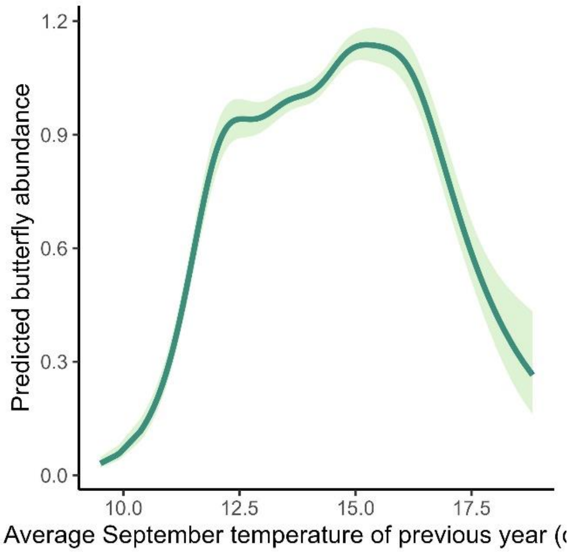
Figure 14: Spatial occurrence of Lasiommata megera across Great Britain 1973-2021.

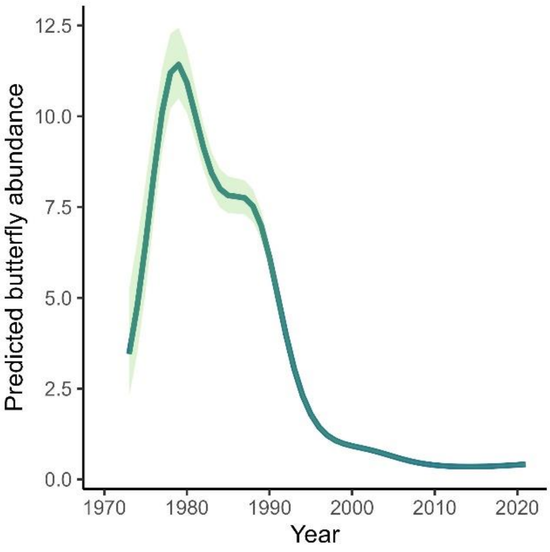
The statistical significance of each explanatory term in model 2.1 is summarised in table 5. Nine of the eleven covariates were statistically significant: year, nitrogen deposition, June temperature of current year, September temperature of previous year, land use intensity, June rainfall, elevation, spatial location, and site number. Only two variables were not found to be statistically significant: sulphur deposition and percentage change in nitrogen.

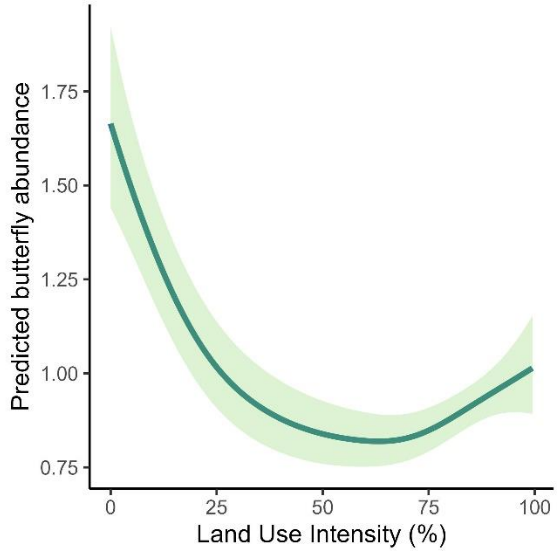
Table 5: Statistical significance of covariates in model 2.1.

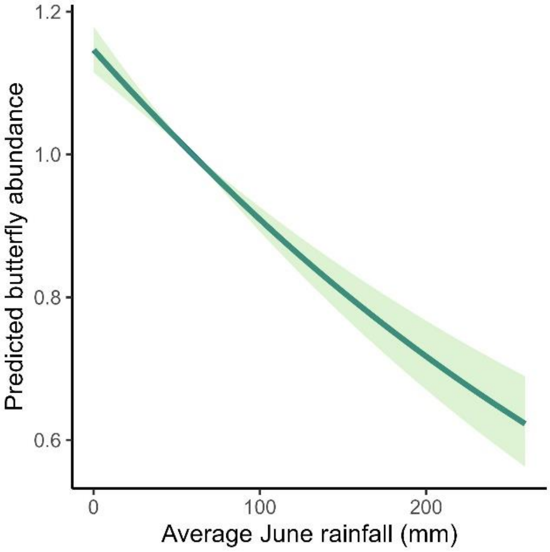
Covariate	P-value
Year	< 0.001 ***
Historic peak nitrogen deposition	< 0.001 ***
Nitrogen % change	0.1784
Sulphur deposition	0.5931
June temperature of current year	< 0.001 ***
September temperature of previous year	< 0.001 ***
Land use intensity	0.0023 **
June rainfall	< 0.001 ***
Elevation	< 0.001 ***
Spatial location	< 0.001 ***
Site number	< 0.001 ***




Predicted butterfly abundance
1.2
0.9
0.6
0.3
0.0
10.0
12.5
15.0
17.5
Average September temperature of previous year (


Predicted butterfly abundance
12.5
10.0
7.5
5.0
2.5
0.0
1970
1980
1990
2000
2010
2020
Year


Predicted butterfly abundance
1.75
1.50
1.25
1.00
0.75
0
25
50
75
100
Land Use Intensity (%)


Predicted butterfly abundance
1.2
1.0
0.8
0.6
0
100
200
Average June rainfall (mm)

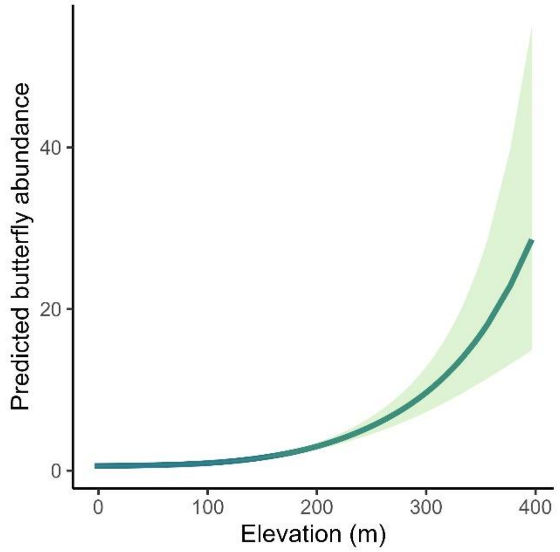

Predicted butterfly abundance
40
20
0
0
100
200
300
400
Elevation (m)

Figure 15a, 15b, 15c, 15d, 15e, 15f & 15g: Predicted abundance from model 2.1 of Lasiommata megera at an average site with changing historic peak in nitrogen deposition (15a), temperature in the current June (15b), temperature in the previous September (15c), year (15d), land use intensity in 1990 (15e), average June rainfall in current year (15f), and elevation (15g).

Discussion

Broad species analysis

Overall results

The strength and direction of responses of individual species to the different drivers were varied and complex, as seen in Table 3. Consistently significant drivers of change across most species studied were year, June temperature in the year of survey, June temperature in the year preceding the survey, and June rainfall in the year of survey. The abundance of most species showed strongly significant relationships with all four of these driver variables, indicating that these are the key drivers of change across all taxa. As hypothesised, nitrogen pollution was also an important driver of change in many of the species, with 24 showing a significant relationship with historic nitrogen deposition, and 21 with percentage change in nitrogen deposition over time (Table 3). Of these significant relationships, around half were positive and half negative. 9 species were negatively correlated with historic nitrogen deposition, and 9 were negatively correlated with percentage change in nitrogen deposition over time. Two species showed significant negative relationships with both historic nitrogen deposition and percentage change in nitrogen deposition over time: *Fabriciana adippe* (High Brown Fritillary) and *Hipparchia semele* (Grayling). Both *F. adippe* and *H. semele* are listed as species of high conservation priority to Butterfly Conservation, Section 41 species under the NERC Act, and are Biodiversity Action Plan (BAP) Priority Species. Both species rely to some extent on the presence of bare ground to bask in order to increase their body temperatures. It is possible that nitrogen deposition has caused these observed negative relationships through changing the height, type, and extent of vegetation in their habitats and therefore decreasing the amount of bare ground or short vegetation. Changing woodland management practices may also have impacted the availability of the warm ground microclimates, especially through declines in coppicing of woodlands. Coppicing can increase the species richness and densities of butterflies in woodlands because it encourages early and mid-successional plant growth rather than late successional high tree cover (Fartmann *et al.*, 2013). Management intervention and intensity was not included in the model due to insufficient data being available.

The abundance of 12 species showed a positive correlation with historic nitrogen deposition, whilst the abundance of 11 species showed a positive correlation with percentage change in nitrogen deposition. Three species showed significant positive relationships with both historic nitrogen deposition and percentage change in nitrogen deposition over time: *Aglais io* (Peacock), *Anthocharis cardamines* (Orange-tip), and *Pieris napi* (Green-veined White). All three are fairly ubiquitous species and feed on commonly occurring plants, most of which have high Ellenberg nitrogen values (Hill *et al.*, 2004).

In order to understand the potential effect of different trait groupings on the strength and direction of relationships with the two nitrogen deposition variables, grouped results were summarised in table 4. The relationships between trait groupings and each of the two nitrogen deposition variables were complicated and inconsistent, with species from none of the individual trait groupings standing out as a having a particularly strong relationship with both nitrogen deposition drivers. This is unsurprising, given the complexity of the requirements of each butterfly species. Other studies of Lepidopteran population change have found inconclusive results, suggesting that trait-based approaches are of limited usefulness in predicting generalised species declines (Tordoff *et al.*, 2022).

Monophagous habitat specialists

I hypothesised that monophagous habitat specialists with nitrophobic host plants have experienced declines due to nitrogen deposition, whilst monophagous habitat generalist with nitrophilic host plants have experienced increases due to nitrogen deposition. Across all monophagous species, 8 of the 11 species showed no relationship with historic nitrogen deposition and 9 of the 11 species showed no relationship with change in nitrogen deposition. Of the two significant relationships with change in nitrogen deposition, one was positive and one negative. All three species with significant relationships between monophagous butterflies and historic nitrogen deposition were positive.

These species, *Vanessa atalanta* (Red Admiral), *Aglais io* (Peacock), and *Polyommatus icarus* (Common Blue), are, however, all widespread across many habitats. Thus, it seems that some monophagous species have not experienced decline if they are also habitat generalists.

Two closely related butterfly species were chosen *a priori* to investigate the potential negative effects of nitrogen deposition on monophagous habitat specialists in more detail: *Polyommatus bellargus* (Adonis Blue) and *P. coridon* (Chalk Hill Blue). Both species are monophagous, feeding on *Hippocrepis comosa* (Horseshoe Vetch), and are myrmecophilous as larva. After modelling the effects of nitrogen deposition and several other drivers on each species individually, I found that the responses of the two species to the various drivers differed. Historic nitrogen deposition was not a significant driver of abundance for either species. Abundance of *P. bellargus* was negatively correlated with percentage change in nitrogen deposition at the site over time. This suggests that abundance is lowest at sites that continue to receive high levels of nutrient input through nitrogen deposition.

Average June temperature in the year of survey and the year preceding the survey showed strong relationships with abundance in both species, but the directions of the relationships differed. *Polyommatus bellargus* abundance was positively correlated with both measures of temperature, suggesting that the species only benefits from increased temperature. It is worth noting that the model would not be able to predict accurately beyond the levels of temperature surveyed by the UKBMS. Thus, we are unable to test the impact of potential future climate change induced rises in temperature on the species. Conversely, the abundance of *P. coridon* was negatively correlated with both measures of temperature. There was evidence of a slight hump-backed relationship with average June temperature in the year of survey, however, the confidence intervals were fairly wide.

The two *Polyommatus* species differed in their responses to average June rainfall in the year of survey, with *P. bellargus* showing a positive relationship whilst *P. coridon* showed a negative relationship. Interestingly, the scale of this effect was also much greater for *P. bellargus*. This could suggest that rainfall is a key limiting factor in the environments that *P. bellargus* inhabits, whereas for the habitat of *P. coridon*, low rainfall or drought-like conditions are perhaps preferable.

Perhaps surprisingly, *P. bellargus* abundance was also strongly positively correlated with land use intensity. In this study, I calculated land use intensity as a percentage of agricultural land in the surrounding area. We would have expected both of these species to be somewhat negatively correlated with land use intensity, as many of the practices used in modern farming are harmful for butterflies. *P. bellargus* is a species characteristic of lowland chalk or limestone grassland, and thrives in warm, sheltered ex-industrial quarries and chalk pits. It is possible that these former quarries and chalk pits are often surrounded by intensively farmed land, thus leading to this observed increase in abundance with land use intensity.

Overall, we can conclude that the drivers of change in populations of *P. bellargus* and *P. coridon* are complex. Nitrogen deposition may contribute to declines in *P. bellargus*, as discussed above, but requires some further research. Future work could look at the effects of different nitrogen deposition variables on the two species. Given that *P. bellargus* abundance was negatively correlated with percentage change in nitrogen deposition, it appears that current levels of nitrogen deposition are perhaps more important for the species than historic loading. It would therefore be interesting to test the effect of a rolling average of nitrogen deposition on both species to allow us to better understand the potential contribution of recent nitrogen deposition as an important driver of change. Given that both species occur in lowland areas often surrounded by farmland, it would also be interesting to test whether the form of nitrogen deposition is important. It is possible that ammonia deposition is a particularly strong driver of these species given the proximity to farmed areas.

Species that emerge as adults early in spring

I hypothesised that butterfly species that emerge very early in the spring have experienced nitrogen-related declines. Two closely related species with other similar life-history traits were selected to explore this hypothesis: *Boloria euphrosyne* and *B. selene*. Both of these species have experienced severe declines in the last half century. Despite some similarities in traits, many of the modelled trends differed between the two species. The model run showed a significant relationship between percentage change in nitrogen deposition and *B. euphrosyne* abundance, however, upon inspecting the shape of the trend and the associated confidence intervals (fig 8b), it is clear that this model has overfit to a few data points at the high end of the nitrogen measure which correspond to high butterfly abundance. It is possible that nitrogen has not been shown as a significant contributor to spatio-temporal change in these species due to their relatively restricted ranges, and subsequently the inability to detect influences of small-scale changes in nitrogen deposition due to the use of 5 x 5 km deposition data. Habitat management to create sites suitable for fritillary butterflies is often undertaken with the aim of improving their numbers. Management was not considered in this analysis due to there not being a sufficient dataset on management interventions at specific sites.

For consistency and comparability, we used the same models as the other trait-based approaches. One potential drawback of this is that the butterfly data used in the model is the Generalised Abundance Index (GAI) which takes into account all butterflies across the whole season to create an overall index of maximum species abundance at a site in a particular year. Despite both *Boloria* species being primarily univoltine, there will inherently be variation in emergence dates due to geographic and other variables. It is possible that the effects of nitrogen are most pronounced on populations of this first generation that emerge earliest, but we are unable to detect this when using GAI data. It would be beneficial to revisit this analysis with the use of the weekly UKBMS count data rather than the GAI data to investigate this further.

As a comparison, I investigated whether two closely related members of the Nymphalidae family, *Maniola jurtina* and *Pyronia tithonus*, which both emerge later in the year, show differing relationships with nitrogen pollution than the *Boloria spp.* tested. In *M. jurtina*, these opposing trends were observed; butterfly abundance was positively correlated with both historic peak nitrogen levels and percentage change in nitrogen deposition. Conversely, *P. tithonus* abundance was negatively correlated with historic peak in nitrogen deposition. These mixed results show the complexity of trait-based analysis and suggest that other factors are likely to be contributing to declines.

Species with Nitrophilic host plants

I hypothesised that butterflies with nitrogen-loving hostplants have increased in abundance due to nitrogen deposition. This study revealed strong positive effects of nitrogen on both *Aglais io* and *A. urticae* abundance, as measured by both the historic nitrogen deposition variable and the measure of change in nitrogen deposition over time at a site. Abundance of both species was highest in sites where the nitrogen loading had increased significantly over the last half century. *A. io* abundance was also positively correlated with increasing historic nitrogen loading. Interestingly, this effect of nitrogen was strong in both species despite them having different responses to year. *A. io* abundance has remained relatively stable in the last half century, whereas *A. urticae* abundance has decreased significantly. These trends over time are reflected in official trends produced using the same dataset (Fox *et al.*, 2023).

Both species showed similar trends with the two temperature variables. Hump-backed relationships were observed between abundance and June temperature in the year of survey and June temperature in the year preceding the survey for both species, suggesting that warm temperatures are beneficial up to a point, after which they become detrimental. A similar trend is observed with rainfall. These results suggest that meteorological variables are key drivers of change in both species. Given the directions of these correlative trends, we may expect to observe future declines in both species as a result of warming temperatures and increased rainfall due to climate change. The abundance of both species was negatively correlated with historic sulphur deposition.

These results suggest that atmospheric nitrogen pollution may have beneficial effects on certain species of butterfly, possibly due to their traits of being ubiquitous species feeding on nitrogen-loving hostplants. Despite this potentially beneficial relationship demonstrated between nitrogen enrichment and British *Aglais spp.* abundance, other drivers of change such as climate and sulphur deposition are clearly important factors to consider. Although there are possible beneficial effects of nitrogen on *A. urticae*, the species has still undergone considerable decline in the last half century.

Lasiommata megera analysis

This study highlights the likelihood that peak atmospheric nitrogen deposition and climate change are significantly correlated with recent declines in *L. megera* in Great Britain, as seen elsewhere in Europe but not yet studied in Great Britain until now. I hypothesised that nitrogen deposition has contributed to observed declines in *L. megera* in Great Britain. This hypothesis was strongly supported by the results, with peak historic nitrogen deposition shown to be strongly negatively correlated with the abundance of *L. megera*. This complements the findings of quantitative analysis on the impacts of peak nitrogen on *L. megera* using data from the Dutch Butterfly Monitoring Scheme (Klop *et al.*, 2015; Wallisdevries and Swaay, 2013). Microclimatic cooling is likely the main mechanism by which nitrogen impacts *L. megera* (WallisDeVries and van Swaay, 2006; Klop *et al.*, 2015). Excessive nitrogen availability increases the biomass and height of vegetation which enhances shading and green to dead matter ratio of the vegetation, both of which contribute to microclimatic cooling at the ground level (Stoutjesdijk and Barkman, 2015). An additional mechanism could be shifts in the nutritional quality of host plants, given that excess nitrogen enrichment can negatively impact butterflies once it exceeds their physiological tolerance. An experimental study showed that nitrogen enrichment of host plants decreased the survival rate of the grass-feeding species *Pararge aegeria* (which is closely related to *L. megera*), amongst several other butterfly species (Kurze *et al.*, 2017). However, a different experimental study showed that nitrogen does not cause a deterioration of host plant quality for *L. megera* (Klop *et al.*, 2015). Further laboratory and field-based studies may be needed to fully understand this potential association in Great Britain.

I also hypothesised that rising autumn temperatures due to climate change would be a key driver of declines in *L. megera*, which was again supported by the results. As hypothesised by Van Dyck *et al.* (2015), warmer autumn temperatures can encourage the progression of second generation *L. megera* larvae to pupate and progress into adulthood, giving rise to a third generation of adults that fly in autumn. In many cases, we expect survival of this third generation to be poor due to unsuitable climatic conditions and a lack of food resources. We would expect autumn temperature in the preceding year to have a positive effect on *L. megera* abundance up to a point, because an unusually cold autumn may negatively impact dormant second generation larvae, in addition to having general negative impacts on the habitat and future host or nectar plants. However, once the temperature reaches a certain point, it may start to encourage the development of the 'lost' third generation, and therefore correlate with a lower abundance in the following year. My results followed this expectation precisely, with average September temperature in the year preceding the survey having a bell-shaped effect, correlating with increases in butterfly abundance up to around 16^{o}C, followed by a decline.

Several other drivers also showed strong relationships with *L. megera* abundance, highlighting the complexity of the drivers of change in *L. megera* populations. Average June temperature during the year of survey had a strong positive effect on abundance as expected (Roy *et al.*, 2001). Elevation also showed a statistically significant relationship with abundance, staying linear up to about 100m, above which abundance increases with elevation up to around 400m which is approximately the maximum elevation at which *L. megera* has been recorded by the UKBMS. Elevation is negatively associated with temperature, with increasing elevation correlating with decreasing average temperatures. Therefore, a potential causal explanation for the observed trend could be that the third autumn generation are less likely to develop at higher elevation sites due to temperature limitations. Nitrogen deposition is often greater at high elevation locations because of increased nitrogen loading through wet deposition as a result of higher rainfall. Critical load exceedance is also likely at high elevations because the habitats present tend to be nutrient-poor. Thus, it is interesting that elevation showed a positive relationship with *L. megera* abundance, and furthers the evidence for the importance of the lost generation hypothesis.

Overall, *L. megera* abundance was negatively correlated with year, as expected due to reports of decline in the species during this time period under other analyses using the same dataset (Fox *et al.*, 2023). However, in figure 15a, we see a very steep increase in abundance from approximately 1974-1979, perhaps due to the record-breaking drought of 1976 (Marsh *et al.*, 2007) leading to low butterfly abundance in the following two years, followed by a rise back up to average levels in around 1979. Abundance was also negatively correlated with land use intensity, which was used as a proxy for the potential impacts of farming such as pesticide usage and lack of suitable habitat. Modern farming practices are associated with declines in many butterfly species, so this finding is unsurprising. Butterfly abundance also showed a negative relationship with rainfall which could have several potential causal mechanisms. High rainfall can be positively associated with total nitrogen deposition, a driver that I found to be negatively correlated with *L. megera* abundance in this study. High rainfall could also lead to unsuitable habitat conditions for *L. megera* development. Its larvae feed on a variety of grasses which generally have Ellenberg moisture values of 5 or lower (Hill *et al.*, 2004), indicating that they prefer moist but not wet or aquatic soil and therefore are unlikely to be present in areas of very high rainfall.

Further work

This work presents the first study of the effects of atmospheric nitrogen pollution on GB butterflies. Future modelling work could focus on testing the importance of each driver of change on the abundance of individual butterfly species, perhaps by undertaking a TITAN (Threshold Indicator Taxa Analysis) analysis (Baker and King, 2010). Further statistical analysis could be undertaken to better understand the impacts of additional drivers. It would be beneficial to revisit this analysis upon the future publication of a highly temporally and spatially resolved dataset of pesticide applications across the UK in order to test the potential effects of pesticides on this species. Another likely important factor to consider is management interventions and their intensity, for example by looking at the cover and intensity of Agri-Environment Scheme (AES) interventions in the area surrounding each butterfly transect. Additionally, consideration of the cumulative deposition of nitrogen and sulphur would be interesting to examine, as these likely influence soil chemistry and therefore plant nutrient uptake and overall productivity. Analysis to test whether the effects of nitrogen pollution on butterflies are immediate or delayed, for example only occurring after a certain time delay, would also be valuable. Further work could also consider whether there are any interactions between the covariates used in the model, such as temperature in the previous year and nitrogen deposition. This would allow us to understand whether, for example, negative effects are greatest in areas experiencing both high nitrogen deposition and temperature anomalies. Further work could also consider whether driver effects vary for different within-year butterfly generations by using the weekly UKBMS count data rather than the smoothed Generalised Abundance Index (GAI).

In this analysis, the potential effect of nitrogen pollution on butterfly larval foodplants were not considered quantitatively but were discussed with respect to the results. Future work could focus on attempting to understand the effects of nitrogen pollution on butterfly larval hostplants and floral resources and how these could be linked causally to changes in butterfly abundance. Additionally, further work could be undertaken to test the effects of nitrogen on summary butterfly measures such as species richness and diversity.

This research also highlights the need for further investigation into the causal mechanisms of decline through laboratory and field based studies. For example, larval and adult success could be measured under varying combinations of nitrogen pollution and temperatures to help assess the mechanisms of change in survival and fecundity, as well as the extent to which combined stressors lead to potentially additive negative effects. Field-based studies could also include long-term management manipulation such as bracken removal.

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Appendices

Table 6/A1: Statistical significance of covariates in model 1.1. Covariates with a P-value lower than 0.05 are considered statistically significant because at this level there is less than 5% chance that the observed data could have occurred under the null hypothesis.

Species Latin name	**Species common name**	**Year**	**Historic nitrogen deposition**	**Nitrogen % change**	**June temp**	**Previous June Temp**	**June Rain**	**Sulphur deposition**	**Land Use Intensity**	**Coordinates**	**Site**	**Model fit - deviance explained (%)**
Aglais io	Peacock	< 0.001 *** Stable wiggly	0.006 ** Positive	0.0005 *** Positive	< 0.001 *** Hump-back	< 0.001 *** Hump-back	< 0.001 *** Negative	< 0.001 *** Negative	0.4825	< 0.001 ***	< 0.001 ***	66.3
Aglais urticae	Small Tortoiseshell	< 0.001 *** Hump-back	0.0556 . Positive	< 0.001 *** Positive	< 0.001 *** Hump-back	< 0.001 *** Hump-back	< 0.001 *** Hump-back	0.0309 * Hump-back	0.1716	< 0.001 ***	< 0.001 ***	66.1
Anthocharis cardamines	Orange-tip	< 0.001 *** Positive	< 0.001 *** Positive	< 0.001 *** Positive	0.0552 . Positive	< 0.001 *** Positive	< 0.001 *** Hump-back	0.0068 ** Negative	0.1805	< 0.001 ***	< 0.001 ***	66.8
Apatura iris	Purple Emperor	< 0.001 *** Negative	0.1954	0.4156	0.0332 * Hump-back	0.1291 Positive	0.0913 . Hump-back	0.1963	0.0898 . Negative	< 0.001 ***	< 0.001 ***	40.6
Aphantopus hyperantus	Ringlet	< 0.001 *** Positive	< 0.001 *** Positive	0.006 ** Negative	< 0.001 *** Positive	< 0.001 *** Positive	< 0.001 *** Hump-back	< 0.001 *** Negative	0.0143 * Positive	< 0.001 ***	< 0.001 ***	71.5
Argynnis paphia	Silver-washed Fritillary	< 0.001 *** Positive	< 0.001 *** Positive	0.0001 *** Negative	< 0.001 *** Positive	0.0528 . Inverse Hump-back	< 0.001 *** Negative	< 0.001 *** Negative	0.2367	< 0.001 ***	< 0.001 ***	79.9
Aricia agestis	Brown Argus	< 0.001 *** Positive	< 0.001 *** Positive	0.0005 *** Negative	< 0.001 *** Positive	< 0.001 *** Positive	< 0.001 *** Negative	< 0.001 *** Negative	0.1179	< 0.001 ***	< 0.001 ***	66.9

Species Latin name	**Species common name**	**Year**	**Historic N deposition**	**N % change**	**June temp**	**Previous June Temp**	**June Rain**	**Sulphur deposition**	**Land Use Intensity**	**Coordinates**	**Site**	**Model fit - deviance explained (%)**
Aricia artaxerxes	Northern Brown Argus	< 0.001 *** Hump-back	0.783	0.0188 * Stable	0.0001 *** Positive	0.001 *** Hump-back	0.0709 . Negative	0.1751	0.1662	0.0103 *	< 0.001 ***	80.3
Boloria euphrosyne	Pearl-bordered Fritillary	< 0.001 *** Negative	0.9649	0.0419 * Stable	0.0005 *** Positive	0.0097 ** Positive	< 0.001 *** Hump-back	0.8955	0.4522	0.0155 *	< 0.001 ***	75.1
Boloria selene	Small Pearl-bordered Fritillary	< 0.001 *** Negative	0.21	0.166	< 0.001 *** Positive	< 0.001 *** Hump-back	0.97	0.223	0.689	< 0.001 ***	< 0.001 ***	73
Callophyrs rubi	Green Hairstreak	< 0.001 *** Negative	0.1829	0.0718 . Negative	< 0.001 *** Hump-back	< 0.001 *** Positive	0.0022 ** Hump-back	0.573	0.4057	0.0018 **	< 0.001 ***	63.9
Celastrina argiolus	Holly Blue	< 0.001 *** Stable Wiggly	< 0.001 *** Negative	< 0.001 *** Positive	< 0.001 *** Negative	< 0.001 *** Inverse Hump-back	< 0.001 *** Hump-back	< 0.001 *** Positive	0.493	< 0.001 ***	< 0.001 ***	51.5
Coenonympha pamphilus	Small Heath	< 0.001 *** Negative	< 0.001 *** Positive	0.0257 * Negative	< 0.001 *** Increase	< 0.001 *** Increase	< 0.001 *** Increase	0.0008 *** Negative	0.0001 *** Inverse Hump-back	< 0.001 ***	< 0.001 ***	74.7

Species Latin name	**Species common name**	**Year**	**Historic N deposition**	**N % change**	**June temp**	**Previous June Temp**	**June Rain**	**Sulphur deposition**	**Land Use Intensity**	**Coordinates**	**Site**	**Model fit - deviance explained (%)**
Coenonympha tullia	Large Heath	0.0008 *** Negative	0.2018	0.8279	0.1789	0.001 ** Positive	0.5076	0.2832	0.8689	0.0105 *	< 0.001 ***	81.3
Cupido minimus	Small Blue	< 0.001 *** Negative	0.3295	0.2193	0.0556 . Positive	0.0082 ** Positive	0.2152	0.1381	0.8897	0.4519	< 0.001 ***	68
Erebia aethiops	Scotch Argus	< 0.001 *** Hump-back	0.0037 ** Stable	0.0127 * Stable	0.8191	0.5193	0.0535 . Negative	0.0141 * Stable	0.7709	0.0002 ***	< 0.001 ***	89.2
Erynnis tages	Dingy Skipper	< 0.001 *** Negative	0.9829	0.9707	< 0.001 *** Hump-back	0.0234 * Positive	< 0.001 *** Positive	0.1334	0.0476 * Inverse Hump-back	0.2113	< 0.001 ***	69.3
Euphydryas aurinia	Marsh Fritillary	< 0.001 *** Negative	0.2214	0.605	< 0.001 *** Positive	< 0.001 *** Positive	0.2149	0.0170 * Hump-back	0.2191	0.0322 *	< 0.001 ***	69.1
Fabriciana adippe	High Brown Fritillary	< 0.001 *** Negative	0.0201 * Negative	0.003 ** Negative	0.3545	0.6175	0.006 ** Negative	0.0124 * Stable	0.1412	0.0002 ***	< 0.001 ***	83.6
Favonius quercus	Purple hairstreak	< 0.001 *** Negative	0.771	0.149	0.0115 * Positive	0.0002 *** Positive	< 2e-16 *** Negative	0.6156	0.3944	0.0279 *	< 0.001 ***	68.1
Gonepteryx rhamni	Brimstone	< 0.001 *** Positive	< 0.001 *** Positive	0.0006 *** Negative	< 0.001 *** Negative	< 0.001 *** Inverse hump-back	< 0.001 *** Negative	< 2e-16 *** Negative	0.7093	< 0.001 ***	< 0.001 ***	82

Species Latin name	**Species common name**	**Year**	**Historic N deposition**	**N % change**	**June temp**	**Previous June Temp**	**June Rain**	**Sulphur deposition**	**Land Use Intensity**	**Coordinates**	**Site**	**Model fit - deviance explained (%)**
Hamearis lucina	Duke of Burgundy	< 0.001 *** Negative	0.1858	0.7188	0.491	0.1511	0.1541	0.3099	0.0987 . Positive	0.8268	< 0.001 ***	73.1
Hesperia comma	Silver-spotted Skipper	< 0.001 *** Hump-back	0.1078	0.0784 . Stable	0.1066	0.1854	0.695	0.1225	0.3453	0.1365	< 0.001 ***	70
Hipparchia semele	Grayling	< 0.001 *** Negative	0.0135 * Negative	< 0.001 *** Negative	0.495	0.0633 . Negative	0.0008 *** Negative	0.1094	0.6009	< 0.001 ***	< 0.001 ***	82.5
Lasiommata megera	Wall	< 0.001 *** Negative	0.044 * Negative	0.6431	< 0.001 *** Positive	< 0.001 *** Hump-back	< 0.001 *** Negative	0.9674	0.0016 ** Negative	< 0.001 ***	< 0.001 ***	70.4
Leptidea sinapis	Wood White	< 0.001 *** Negative	0.2154	0.0855 . Negative	0.8152	0.1563	0.5726	0.8733	0.0093 ** Hump-back	< 0.001 ***	< 0.001 ***	77.3
Limenitis camilla	White Admiral	< 0.001 *** Negative	0.1257	0.2458	< 0.001 *** Positive	< 0.001 *** Positive	< 0.001 *** Negative	0.0005 *** Negative	0.0191 * Negative	< 0.001 ***	< 0.001 ***	70.1
Lycaena phlaes	Small Copper	< 0.001 *** Negative	0.0105 * Positive	0.0016 ** Negative	< 0.001 *** Positive	< 0.001 *** Positive	< 0.001 *** Negative	0.0354 * Negative	0.2986	< 0.001 ***	< 0.001 ***	67.7

Species Latin name	**Species common name**	**Year**	**Historic N deposition**	**N % change**	**June temp**	**Previous June Temp**	**June Rain**	**Sulphur deposition**	**Land Use Intensity**	**Coordinates**	**Site**	**Model fit - deviance explained (%)**
Maniola jurtina	Meadow Brown	< 0.001 *** Positive	0.0059 ** Positive	0.0807 . Hump-back	< 0.001 *** Inverse Hump-back	< 0.001 *** Hump-back	< 0.001 *** Hump-back	< 0.001 *** Negative	0.0583 . Inverse Hump-back	< 0.001 ***	< 0.001 ***	80.1
Melanargia galathea	Marbled White	< 0.001 *** Positive	< 0.001 *** Negative	0.0512 . Hump-back	0.0011 ** Negative	< 0.001 *** Negative	< 0.001 *** Hump-back	< 0.001 *** Negative	0.423	< 0.001 ***	< 0.001 ***	76.5
Melitaea athalia	Heath Fritillary	< 0.001 *** Stable	0.0296 * Stable	0.2744	0.0191 * Hump-back	0.9510	0.3767	0.001 *** Stable	0.0711 . Hump-back	0.0115 *	< 0.001 ***	62
Ochlodes sylvanus	Large Skipper	< 0.001 *** Hump-back	0.0124 * Hump-back	0.7658	< 0.001 *** Positive	0.0005 *** Positive	< 0.001 *** Negative	0.0006 *** Inverse Hump-back	0.0025 ** Inverse Hump-back	0.006 **	< 0.001 ***	61.7
Pararge aegeria	Speckled Wood	< 0.001 *** Hump-back	< 0.001 *** Negative	0.0874 . Hump-back	< 0.001 *** Positive	0.0019 ** Hump-back	< 0.001 *** Negative	< 0.001 *** Positive	0.1528	< 0.001 ***	< 0.001 ***	73.3
Pieris brassicae	Large White	< 0.001 *** Stable	< 0.001 *** Hump-back	< 0.001 *** Positive	< 0.001 *** Hump-back	< 0.001 *** Hump-back	< 0.001 *** Negative	0.127	0.626	< 0.001 ***	< 0.001 ***	60.1

Species Latin name	**Species common name**	**Year**	**Historic N deposition**	**N % change**	**June temp**	**Previous June Temp**	**June Rain**	**Sulphur deposition**	**Land Use Intensity**	**Coordinates**	**Site**	**Model fit - deviance explained (%)**
Pieris napi	Green-veined White	< 0.001 *** Negative	0.0009 *** Positive	< 0.001 *** Positive	< 0.001 *** Positive	< 0.001 *** Positive	< 0.001 *** Negative	0.0516 . Negative	0.0869 . Positive	< 2e-16 ***	< 0.001 ***	72.3
Pieris rapae	Small White	< 0.001 *** Negative	< 0.001 *** Hump-back	< 0.001 *** Positive	< 0.001 *** Hump-back	< 0.001 *** Hump-back	< 0.001 *** Negative	0.0003 *** Positive	0.1457	< 2e-16 ***	< 0.001 ***	63.5
Plebejus argus	Silver-studdied Blue	< 0.001 *** Negative	0.6577	0.0299 * Stable	0.0212 * Positive	0.1707	< 0.001 *** Negative	0.2502	0.0552 . Positive	0.0109 *	< 0.001 ***	88.3
Polygonia c-album	Comma	< 0.001 *** Positive	0.0039 ** Negative	0.0001 *** Positive	< 0.001 *** Inverse Hump-back	< 0.001 *** Hump-back	< 0.001 *** Negative	0.2609	0.9162	< 0.001 ***	< 0.001 ***	64.9
Polyommatus bellargus	Adonis Blue	< 0.001 *** Positive	0.8573	0.0249 * Negative	0.0099 ** Positive	0.0460 * Positive	0.0406 * Positive	0.8772	0.0025 ** Hump-back	0.0121 * Positive	< 0.001 ***	73.2
Polyommatus coridon	Chalk Hill Blue	< 0.001 *** Negative	0.3077	0.2306	0.0965 . Negative	0.0052 ** Negative	< 0.001 *** Negative	0.1347	0.3717	0.0071 **	< 0.001 ***	80.7
Polyommatus icarus	Common Blue	< 0.001 *** Hump-back	< 0.001 *** Positive	0.2283	< 0.001 *** Positive	< 0.001 *** Positive	< 0.001 *** Negative	< 0.001 *** Negative	0.0004 *** Negative	< 0.001 ***	< 0.001 ***	72

Species Latin name	**Species common name**	**Year**	**Historic N deposition**	**N % change**	**June temp**	**Previous June Temp**	**June Rain**	**Sulphur deposition**	**Land Use Intensity**	**Coordinates**	**Site**	**Model fit - deviance explained (%)**
Pyrgus malvae	Grizzled Skipper	< 0.001 *** Negative	0.3224	0.0731 .	0.0056 ** Negative	0.0171 * Positive	< 0.001 *** Positive	0.1302	0.2596	0.0551 .	< 0.001 ***	69.1
Pyronia tithonus	Gatekeeper	< 0.001 *** Negative	0.0001 *** Negative	0.893	< 0.001 *** Negative	< 0.001 *** Inverse Hump-back	< 0.001 *** Hump-back	0.0006 *** Negative	< 0.001 *** Negative	< 0.001 ***	< 0.001 ***	67.6
Satyrium pruni	Black Hairstreak	0.0005 *** Inverse Hump-back	0.7646	0.3252	< 0.001 *** Hump-back	0.0003 *** Positive	0.001 ** Negative	0.0372 * Positive	0.0811 . Hump-back	0.2534	< 0.001 ***	60.4
Satyrium w-album	White-letter hairstreak	< 0.001 *** Negative	0.7537	0.1453	0.0228 * Positive	0.0068 ** Hump-back	< 0.001 *** Negative	0.0218 * Positive	0.0861 . Positive	0.01 *	< 0.001 ***	65.8
Speyeria aglaja	Dark Green Fritillary	< 0.001 *** Positive	0.3237	0.0006 *** Negative	< 0.001 *** Hump-back	0.0183 * Negative	0.069 . Negative	0.0028 ** Negative	0.6113	0.0391 *	< 0.001 ***	72.2
Thecla betulae	Brown Hairstreak	< 0.001 *** Hump-back	0.5639	0.2701	0.0099 ** Negative	0.0311 * Positive	0.035 * Negative	0.9587	0.7022	0.0326 *	< 0.001 ***	58.6
Thymelicus acteon	Lulworth Skipper	< 0.001 *** Negative	0.0774 . Stable	0.0143 * Stable	0.0854 . Hump-back	0.0419 * Negative	0.0213 * Negative	0.2104	0.0615 . Stable	0.0795 .	< 0.001 ***	81.8

Species Latin name	**Species common name**	**Year**	**Historic N deposition**	**N % change**	**June temp**	**Previous June Temp**	**June Rain**	**Sulphur deposition**	**Land Use Intensity**	**Coordinates**	**Site**	**Model fit - deviance explained (%)**
Thymelicus lineola	Essex Skipper	< 0.001 *** Negative	0.3016	0.0013 ** Positive	0.0367 * Hump-back	0.0368 * Positive	< 0.001 *** Hump-back	0.2834	0.6238	< 0.001 ***	< 0.001 ***	59.1
Thymelicus sylvestris	Small Skipper	< 0.001 *** Negative	0.457	0.0013 ** Positive	< 0.001 *** Hump-back	< 0.001 *** Hump-back	< 0.001 *** Hump-back	0.6886	0.0586 . Negative	< 0.001 ***	< 0.001 ***	58.3
Vanessa atalanta	Red Admiral	< 0.001 *** Positive	0.0016 ** Positive	0.0808 . Positive	< 0.001 *** Positive	< 0.001 *** Hump-back	< 0.001 *** Positive	< 0.001 *** Negative	0.002 ** Inverse Hump-back	< 0.001 ***	< 0.001 ***	62.1
Vanessa cardui	Painted Lady	< 0.001 *** Negative	< 0.001 *** Negative	< 0.001 *** Positive	< 0.001 *** Hump-back	< 0.001 *** Hump-back	< 0.001 *** Negative	0.0084 ** Positive	0.0245 * Negative	< 0.001 ***	< 0.001 ***	41.5

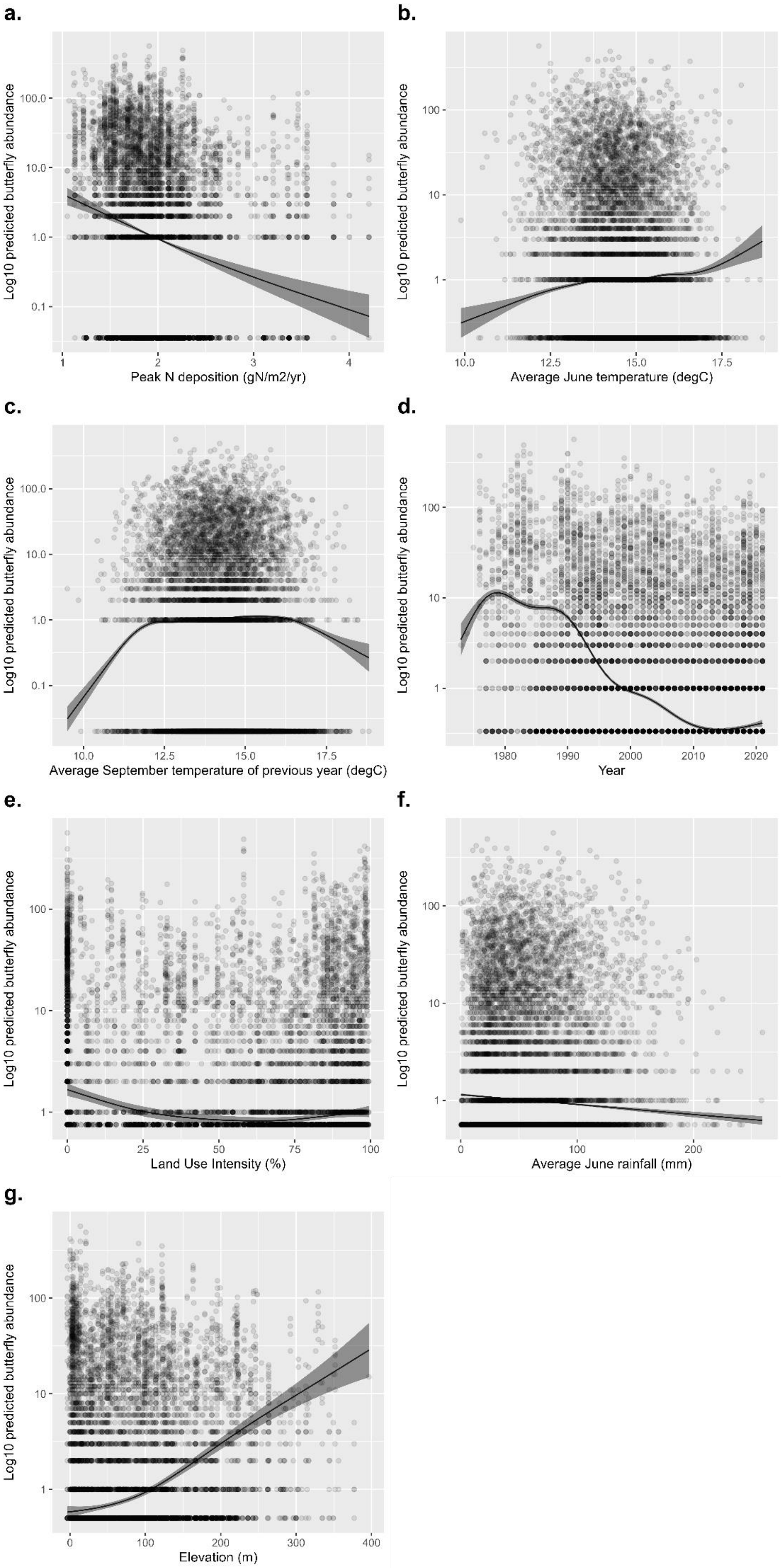
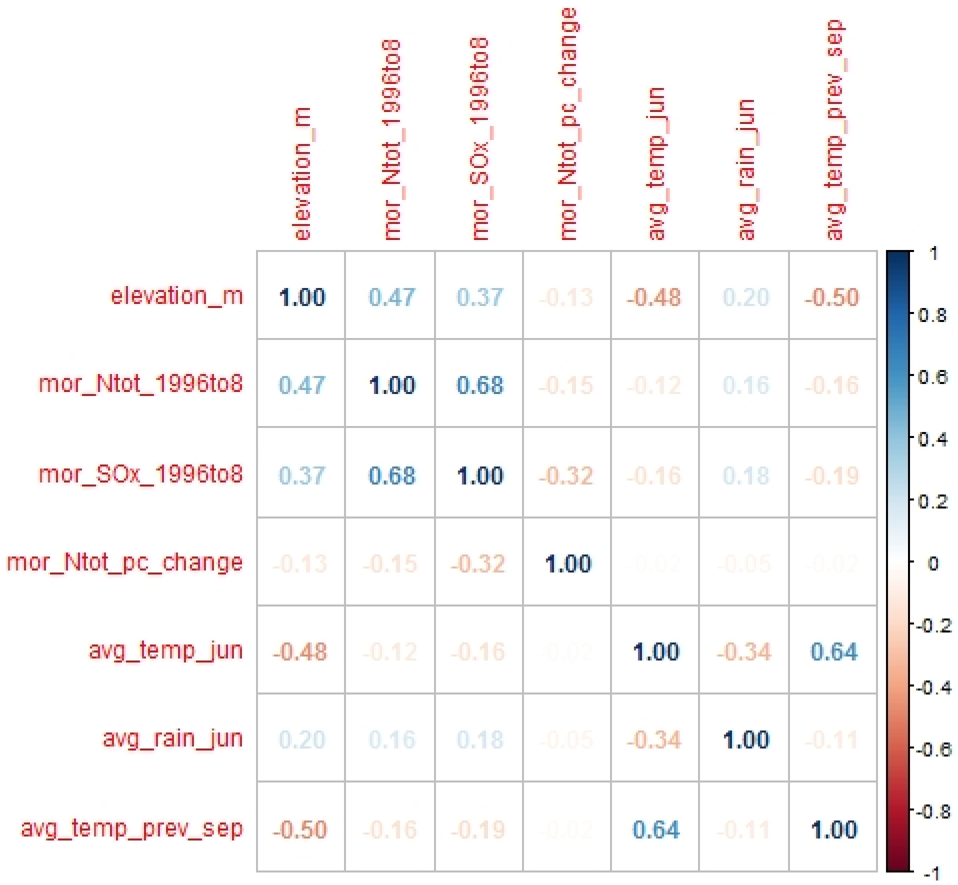

a.
Log10 predicted butterfly abundance
100.0
10.0
1.0
0.1
1
2
3
4
Peak N deposition (gN/m2/yr)
b.
Log10 predicted butterfly abundance
100
10
1
10.0
12.5
15.0
17.5
Average June temperature (degC)
c.
Log10 predicted butterfly abundance
100.0
10.0
1.0
0.1
10.0
12.5
15.0
17.5
Average September temperature of previous year (degC)
d.
Log10 predicted butterfly abundance
100
10
1
1980
1990
2000
2010
2020
Year
e.
Log10 predicted butterfly abundance
100
10
1
0
25
50
75
100
Land Use Intensity (%)
f.
Log10 predicted butterfly abundance
100
10
1
0
100
200
Average June rainfall (mm)
g.
Log10 predicted butterfly abundance
100
10
1
0
100
200
300
400
Elevation (m)

Figure 16/A1: Log10 predicted abundance of Lasiommata megera from model 2.1 at an average site with historic peak in nitrogen deposition (A1a), temperature in the current June (A1b), temperature in the previous September (A1c), year (A1d), land use intensity in 1990 (A1e), average June rainfall in current year (A1f), and elevation (A1g). The underlying butterfly abundance point data has been overlaid following a Log10 transformation.



	elevation_m	mor_Ntot_1996to8	mor_SOx_1996to8	mor_Ntot_pc_change	avg_temp_jun	avg_rain_jun	avg_temp_prev_sep
elevation_m	1.00	0.47	0.37	-0.13	-0.48	0.20	-0.50
mor_Ntot_1996to8	0.47	1.00	0.68	-0.15	-0.12	0.16	-0.16
mor_SOx_1996to8	0.37	0.68	1.00	-0.32	-0.16	0.18	-0.19
mor_Ntot_pc_change	-0.13	-0.15	-0.32	1.00	-0.02	-0.05	-0.02
avg_temp_jun	-0.48	-0.12	-0.16	-0.02	1.00	-0.34	0.64
avg_rain_jun	0.20	0.16	0.18	-0.05	-0.34	1.00	-0.11
avg_temp_prev_sep	-0.50	-0.16	-0.19	-0.02	0.64	-0.11	1.00

Figure 17/A2: Collinearity between variables used in Lasiommata megera modelling.